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COMPARISON OF BACTERIAL CORONAL LEAKAGE BETWEEN DIFFERENT OBTRUCTION MATERIALS
(AN IN VITRO STUDY)

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Abstract:
Introduction: Bacteria and their by-products are the main cause of pulpal and periapical diseases. That is why all routes between root canal system and peridontium should be sealed to prevent bacterial leakage.

Objectives: Was to compare the coronal bacterial leakage between different obturation materials.

Materials and methods: Sixty single-canaled lower premolars were used in this study. Teeth were decoronated to standardize root length to 12 mm. Instrumentation was done using Protaper universal rotary system to file size F4, then teeth were divided into six groups, four experimental and two control (n=10). Group I: obturation was done using CPoint and Endosequence bioceramic sealer using single-cone technique; Group II: obturation was done using Protaper gutta percha and Endosequence bioceramic sealer; Group III: obturation was done using Protaper gutta percha and Adseal resin sealer; Group IV: obturation was done using Protaper gutta percha and MTA Fillapex sealer; Group V: used as positive control; Group VI: used as negative control. Groups II, III, IV, VI were obturated using lateral condensation technique. A split-chamber microbial leakage model was used to detect bacterial leakage. Turbidity in the lower chamber was observed daily for 60 days. Data were analyzed using Kruskal–Wallis and Monte-Carlo test.

Results: all experimental groups showed leakage throughout the study period, Group I showed the best results with a mean of (37.30±8.21) days and was found to be statistically significant than the other groups (P=0.048).

Conclusions: None of the tested materials were able to provide a complete hermetic seal. CPoint with endosequence bioceramic sealer provided the best coronal seal while MTA Fillapex was the worst. CPoint obturation system can be an alternative to lateral condensation technique.

Keywords: Endodontics, Bacterial leakage, CPoint, Endosequence bioceramic sealer, Adseal, MTA Fillapex.

INTRODUCTION

Complete obturation and hermetic seal of the root canal system is a critical step in successful root canal therapy, providing a bacterial-tight seal to inhibit bacterial penetration and their by-products into the cleaned and disinfected root canal system. For this reason, different endodontic materials and obturation techniques have been developed for decreasing microleakage and improvement of the seal of the prepared root canals.

The most common obturation technique is the cold lateral condensation of gutta percha using different type of sealers. The sealer is very important for long-term seal of the root canal filling because it adheres gutta percha to the root canal dentin and fills irregularities and spaces among gutta percha cones and between the root canal walls and fillings (1).

Endosequence BC sealer (Brasseler USA, Savannah, GA) is a new bioceramic sealer used with gutta percha. Endosequence BC Sealer is a premixed and injectable endodontic sealer, and its nanoparticle size allowed it to flow into canal irregularities and dentinal tubules. It is hydrophilic and uses moisture in dentinal tubules to initiate and complete its setting reaction. In addition, no shrinkage occurs on setting, resulting in a gap-free interface between the gutta-percha, sealer, and dentin (2). BC sealer has the ability to form hydroxyapatite forming chemical bond with the dentin wall (3). It is composed of calcium silicates, calcium phosphate monobasic, calcium hydroxide and zirconium oxide.

MTA Fillapex (Angelus, PR, Brasil) is a MTA-based sealer. Its composition after mixing is basically MTA, salicylate cement, natural resin, bismuth and silica (4). MTA Fillapex is the first two paste MTA-based root canal sealer. According to the manufacturers its MTA-based composition provides a perfect sealing integrity and high biological regeneration (5).

Adseal sealer (Meta Biomed Co, Korea) is a resin-based sealer. It contains epoxy resin, zirconium oxide, Ethylene glycol salycilate, and bismuth subcarbonate. Available in two paste-containing tubes (6), it has good adaptation to canal walls, low solubility, good flow and good radiopacity (7).

CPoint obturation system (EndoTechnologies, Shrewsbury, USA), also known as Propoint which is a part of the Smartsel obturation system (8) is a novel single-cone hydrophilic system, according to the manufacturer, which provides a three-dimensional seal as it had greater efficiency in filling simulated lateral canals and a comparable homogeneity of obturation (9). CPoint cones contain a central polymide core to provide good handling with an outer bonded hydrophilic polymer coating, which expands laterally without expanding axially by absorbing residual water from the instrumented root canal space and the naturally present moisture in the dentinal tubules (8). The lateral expansion of CPoints is claimed to occur nonuniformly with the expandability depending on the extent to which the hydrophilic polymer is pre-stressed (i.e., contact with a canal wall will reduce the rate or extent of polymer expansion) (10).

The sealing ability is a basic feature that needs to be tested for every root canal filling material or technique. Many experimental tests have been developed to detect the leakage that occurs along root fillings. Despite the limitation of the in vitro leakage tests, they supply an appropriate initial framework of new filling materials and techniques (11, 12). These methods include dye penetration, glucose leakage, fluid transport, electrochemical, radioisotopes and bacterial leakage. The bacterial leakage model was used in this study as it is more clinically relevant, producing more precise and reproducible data (13), using the etiologic agent of apical periodontitis (14).

The aim of the present in vitro study was to compare the coronal bacterial leakage through different obturation material and to study the effectiveness of the single-cone CPoint system to resist bacterial leakage compared to that of laterally condensed gutta percha with different sealers.

MATERIALS AND METHODS
Sixty single-canaled lower premolar teeth with fully developed root apices were used in this study. Teeth were thoroughly cleaned from any soft tissue or calculus deposition using curettes, with care not to damage the root surface.

To ensure the same length for all specimens, the crowns of all teeth were removed using diamond disks keeping the length of all roots standardized at 12 mm. Apical patency was checked using #10 K-files. #15 K-file was inserted into each root canal for determination of the working length.

Instrumentation was done in a crown-down technique using rotary Protaper universal system (Dentsply Maillefer SA, Bailligues, Switzerland). Rotary instrumentation was performed with the aid of electrical motor X-Smart device at speed of 250 RPM and torque of 3 N. The sequence of files used during instrumentation was that recommended by the manufacturer as SX, S1, S2, F1, F2, F3 and F4 was used as final apical file. Shaping files were used in brushing motion while finishing files was used in straight in and out motion. Glyde file prep was placed on each file before insertion inside the canals. Smear layer removal was done using 2.5% NaOCl and 17% EDTA solution then the canals were finally flushed with 5 ml distilled water.

Teeth were then divided into six equal groups, four experimental and two control groups of ten teeth each. Obturation was performed according to the grouping.

In group I, ten roots were obturated by CPoint and Endosequence bioceramic sealer. The tip of the syringe was inserted into the canal no deeper than the coronal one third. Gently and smoothly a small amount (1-2 calibration markings) of the sealer was dispensed into the root canal by compressing the plunger of the syringe. The CPoint cone (size F4) was lightly coated with sealer then it was slowly inserted with a slight pumping motion to evenly distribute the sealer until it was fully seated. Obturation was done using Single-cone technique.

In group II, ten roots were obturated with Protaper gutta percha with Endosequence bioceramic sealer. The master gutta percha cone (size F4) was coated with the sealer then it was inserted in the canal with a pumping motion until it was fully seated. The obturation was completed using conventional lateral condensation technique with accessory gutta percha cones.

In group III, ten roots were obturated with Protaper gutta percha with Adseal resin sealer. The base and catalyst were mixed on a mixing pad using spatula until reaching creamy homogeneous consistency, sealer carried to the canal using a #15 hand file, rotated in an anti-clockwise direction, the master gutta percha cone (size F4) was coated with the sealer and then inserted with a pumping motion until it was fully seated. The obturation was completed using conventional lateral condensation technique with accessory gutta percha cones.

In group IV, ten roots were obturated by Protaper gutta percha Points and MTA Fillapex sealer. The self-mixing tip was attached to the syringe then the sealer was immediately placed after mixing, it was applied in the root canal directly using an applicator tip adapted to the self-mixing tip, the gutta-percha was coated with a thin layer of the sealer then inserted until it was fully seated. The obturation was completed using conventional lateral condensation technique with accessory gutta percha cones.

In group V, ten roots were prepared but not obturated and kept opened to act as a positive control group.

In group VI, ten roots were filled with Protaper gutta percha and Adseal resin sealer using lateral condensation technique to act as a negative control group.

After obturation, all specimens were stored at 37°C at 100% humidity for two weeks to allow full setting of the sealer. Good quality radiographs had been taken to
verify the quality of the root fillings. The external surfaces for groups (I, II, III, IV), except the coronal access and the apical 2 mm, were covered with two layers of nail polish and sticky wax. For group V, the external surface was not covered with nail polish. For group VI, root surfaces were completely covered with two layers of nail polish including the apex of the root and coronal access.

A split-chamber model (15) was used for bacterial leakage evaluation using natural human saliva. Briefly, the tapered end of a 2-ml plastic eppendorf tube was cut and each root was placed into the tube. The root was positioned inside the tube so that its apical end was protruding from the cut-end section of the eppendorf tube. The junction between the plastic tube and the root was sealed with cyanoacrylate glue and nail polish. The apparatus (teeth and plastic tubes) were sterilized by exposure to Ultraviolet rays. Then the eppendorf tube containing the samples was placed in a glass bottle containing 3 ml sterile Schaedler broth so that at least 2 mm of the root apex was immersed in the broth (as shown in figure 1).

![Fig. (1): Diagrammatic illustration of the apparatus used in this study.](Image)

The junction between the eppendorf and the glass bottle was sealed tightly with sticky wax and parafilm M. Each sample was enumerated for identification. To verify sterilization, the whole apparatus was incubated for 3 days at 37°C to check any turbidity in the broth of the lower chambers.

The coronal chambers were filled with 1 ml of the mix of human saliva and broth (ratio 3:1). The saliva was collected from a single volunteer who did not brush for 12 hours before collection or were on antibiotic therapy. The system was stored in an anaerobic incubator with an anaerobic gas pack at 37°C and any changes in opacity of the broth in the apical chamber was checked daily for identification of bacterial contamination for up to 60 days (as shown in figure 2). If no turbidity was observed in the apical chamber for up to 7 days, the medium in the coronal chamber was changed.

Data was submitted to the non-parametric Kaplan-Meier statistical analysis to a 5% significance level (p < 0.05), estimating the mean time of leakage in days for all groups. The results were statistically analyzed using Kruskal Wallis and Monte Carlo test.

**RESULTS**

The daily contamination of obturated root canals of all groups after exposure to natural human saliva for a period of 60 days is shown in Table 1.

All samples of the positive control group showed leakage on the second day, indicating the ability of saliva to penetrate the prepared root canals. Also, none of the negative control specimens leaked within 60 days, indicating that the seal created between the two-chambers of the systems was efficient.

Regarding the days of contamination, all experimental groups showed leakage throughout the study period. Group I showed the best results with a mean of (37.30 ± 23.61) days while Group II and III showed nearly equal results with a mean of (32.10 ± 22.57) and (33.10 ± 19.90) days, respectively. Group IV showed the worst results with a mean of (16.90 ± 8.21) days and was found to be statistically significant than the other groups (P = 0.048). (As shown in table 2)
Microleakage is one of the most crucial causes of endodontic failure and is defined as the passage of bacteria, fluids, and chemical substances between the root structure and filling material (18). Microleakage in endodontics is multi-factorial, as it may depends on several factors as root filling technique, root filling material, type of sealer used and bonding to root canal walls. So sealing all the communication pathways between the coronal and apical portions of the root canal system is necessary for the long-term success of the root canal therapy.

In this study we examined the hypothesis that: the newly self-expanding hydrophilic CPoint obturation system and BC sealer would be able to prevent or decrease bacterial microleakage when compared to gutta percha with different type of sealers.

Mandibular premolars with single canals were selected for this study as they have oval shaped canals, providing space between the rounded gutta percha and the root canal walls, such space will need greater amount of sealer between the filling material and root canal walls, which requires the use of a sealer with optimum sealing ability. 

Formalin solution was not used for storage of teeth as it has strong bactericidal effect (19) which might affect the bacterial leakage through the obturation material and it also might affect the bonding to dentin since it promotes cross-linking of collagenous proteins present in the dentin matrix leading to an accumulation of insoluble products along the canal wall after instrumentation which might influence sealer penetration resulting in the alteration of the seal (20).

For standardization, the teeth were decoronated and the length of all roots were fixed to 12 mm, as it was found by Metzger et al (21) and Mozini et al (22) that the length of the filling material would affect the speed rate of bacterial leakage. Also the volume of the canals was standardized to the same taper and size by the use of universal ProTaper rotary files in a Crown down technique till size F4.

During instrumentation, a smear layer is formed covering the dentin surface of the prepared root canals. This layer consists of organic and inorganic substances which includes microorganisms and necrotic tissues, occluding the dentinal tubules. This will prevent penetration of sealers and the complete adaptation of the obturating material to the prepared root surfaces which might increase leakage. Grandini et al (23) and Lim et al (24) recommended the removal of both organic and inorganic components of the smear layer using a combined application of Glyde file prep and 2.5% NaOCl, in accordance with the present study. Glyde file prep was used due to the adjunctive effect of EDTA on the smear layer and the effervescence which occurs due to the reaction between carbamide peroxide and sodium hypochlorite allowing dentinal shavings and pulpal remnants to be readily removed (25). It was also found by Baumgartner et al (26) that the removal of the smear

<table>
<thead>
<tr>
<th>Table (2):</th>
<th>Comparison between the different studied groups according to the days of contamination of each obturated root canal after exposure to natural human saliva.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contamination</td>
<td>10.3 0.048</td>
</tr>
<tr>
<td>Min. – Max.</td>
<td>6.0 – 60.0</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>37.30 ± 23.61</td>
</tr>
<tr>
<td>Median</td>
<td>43.0</td>
</tr>
<tr>
<td>Post hoc adjusted analysis</td>
<td>Group IV is significantly different from the others</td>
</tr>
</tbody>
</table>

Regarding the percentage of samples that leaked at the end of the observational period, it was found that 60% of roots in group I showed leakage, while in group II 70% of roots showed leakage, 90% of roots showed leakage in group III and 100% of roots in group IV showed leakage. The observed differences were found to be statistically non-significant (P = 0.208) (As shown in table 3).

<table>
<thead>
<tr>
<th>Table (3):</th>
<th>Comparison between the different studied groups according to the % of contaminated and non-contaminated samples after exposure to natural human saliva for 60 days.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contamination</td>
<td>MC: Monte Carlo test *: Statistically sig. at p ≤ 0.05</td>
</tr>
<tr>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Non - Contaminated</td>
<td>4</td>
</tr>
<tr>
<td>Contaminated</td>
<td>6</td>
</tr>
</tbody>
</table>

**DISCUSSION**

All stages of endodontic therapy have equal importance, although more emphasis is given to obturation as its quality can determine the success or failure of therapy (16).

Ingle and backland stated that 60 % of failures in endodontics can be attributed to incomplete obturation of the root canals (17). This demands three-dimensional obturation to decrease the microleakage that may occur through the root canal filling.

<table>
<thead>
<tr>
<th>Sealer</th>
<th>CPPoint + BC (n = 10)</th>
<th>GP + BC (n = 10)</th>
<th>GP + Resin (n = 10)</th>
<th>GP + MTA (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>kw</td>
<td>2</td>
<td><strong>P</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contamination</td>
<td>6.0 – 60.0</td>
<td>6.0 – 60.0</td>
<td>5.0 – 60.0</td>
<td>5.0 – 32.0</td>
</tr>
<tr>
<td>Min. – Max.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>37.30 ± 23.61</td>
<td>32.10 ± 22.57</td>
<td>33.10 ± 19.90</td>
<td>16.90 ± 8.21</td>
</tr>
<tr>
<td>Median</td>
<td>43.0</td>
<td>30.50</td>
<td>39.50</td>
<td>18.0</td>
</tr>
</tbody>
</table>

| Post hoc adjusted analysis | Group IV is significantly different from the others |

**Table (2):** Comparison between the different studied groups according to the days of contamination of each obturated root canal after exposure to natural human saliva.

**Table (3):** Comparison between the different studied groups according to the % of contaminated and non-contaminated samples after exposure to natural human saliva for 60 days.

**MC:** Monte Carlo test *: Statistically sig. at p ≤ 0.05
layer from the instrumented canal would leave a smoothly planed surface with patent tubular orifices that range from 2 to 4 µm in diameter.

17% EDTA solution was used for 1 min only, as increasing the time of exposure makes dentin susceptible to erosion (27). NaOCl was not used as a last irrigant as it is a strong oxidizing agent leaving behind an oxygen-rich layer on the dentine surface, which results in a reduced bond strengths to sealers. So distilled water was used as a final flush in order to minimize the compromising effect of NaOCl (28).

For standardization of obturation technique, lateral condensation technique was used in all groups except the CPoint group, which was used as a single cone. The main advantage of the CPoint system, according to the manufacturer, is its lateral expansion which occurs until it becomes prestressed by the contact with the canal walls. This exclusive property may be lost if it was used in conjunction with the lateral condensation technique.

Several methods have been described to evaluate the sealing quality of obturated root canals. Although each technique has its proponents, there is no general consensus in the profession as to which technique is the best (29). The model used in this study was the split-chamber bacterial leakage model which was firstly designed by Torabinejad et al (1990) (13). It has been modified and used by several other researchers for coronal leakage studies (12, 30). This model was used as it is more clinically relevant, producing more precise and reproducible data (13), using the etiologic agent of apical periodontitis (14).

The results of the negative control group in this study were 0 % as none of the ten teeth used showed any leakage. Their root remained clear all through the study period. This proved that the seal between the two chambers of the system was efficient.

Group I (CPoint + BC sealer) showed the best coronal seal by reducing the speed of bacterial leakage. These findings may be attributed to the expansion property possessed by the CPoint cones, where the cones were designed to expand laterally without expanding axially by absorbing residual water from the instrumented root canal space and the naturally present moisture in the dentinal tubules. This post-setting expansion shows an approximately 14% expansion after 20 minutes (10) which could provide better sealing and adhesive properties, as it decreases the gaps and voids found between the main cone and the root canal walls. Also the use of bioceramic sealer with CPoint cones provided better sealing ability due to the slow setting time of bioceramic sealer which provides more time for the expansion of the CPoints pushing the sealer towards dentinal walls so better filling of the canals irregularities. Also it has been shown that the release of calcium and hydroxyl ions from the calcium silicate-containing material results in the formation of an apatite layer. Formation of this interfacial layer develops a chemical bond between calcium silicate-based materials and dentinal walls decreasing the marginal leakage and gaps (3). Moreover, BC Sealer’s extremely small particle size (2 µm) (31) and hydrophilic nature allow it to flow into all aspects of the canal anatomy. The present results confirmed the findings reported by Hegde and Arora (15, 32) that CPoint cones with BC sealer showed better sealing ability than laterally condensed gutta percha with resin sealer. Furthermore, El-Sayed et al (33) showed that the novel hydrophilic Smart-Seal system provided better sealing ability when compared to other single-cone obturation systems.

According to group II (GP + BC sealer) and group III (GP + Resin sealer), both groups showed nearly equal results in delaying coronal bacterial leakage. Both types of sealers form chemical bond to dentinal walls which might provide better sealing ability which in turn should reduce leakage in clinical situations. Bonding of resin sealer may be associated with its ability to react with any exposed amino groups in collagen to form covalent bonds between the resin and collagen upon opening of the epoxide ring (34, 35). The results of this study coincide with that of Zhang et al (36) who found that there was no statistically significant difference between BC and Resin sealer in sealing ability when EDTA was used as irrigant. On the other hand, Al-Zaka et al (37) found that resin sealer provided better sealing ability than bioceramic sealer when EDTA used as an irrigant. It may be attributed to the effect of EDTA as it alters the surface energy of dentin which significantly decrease the wetting ability of dentin (37) thereby providing a suitable dentin substrate for adhesion of materials of a hydrophobic nature like Resin sealers. On the contrary, it would affect the adhesion of hydrophilic materials like BC sealer.

Group IV (GP + MTA sealer) showed the worst results, where all samples showed leakage within 32 days. This results are in line with Sonmez et al (5) who found that MTA Fillapex sealer showed inferior sealing ability when compared with resin sealer and MTA, they attributed that to salicylate resin component of the MTA Fillapex, which might adversely affect its sealing properties (38). Also Borges et al (39) used scanning electron microscopy to show that porosities and cracks occurred in the resin matrix of MTA Fillapex after subjecting to solubility test by immersion in water after setting, which might affect its molecular stability. On contrary, Ferreira et al (40) stated that MTA-based sealers showed reduction in leakage values over time, which could be attributed to the volumetric expansion after setting.

It was difficult to directly compare results of this study with those of other investigators as many different factors in the spectrum of the model systems used could account for variability of penetration data reported. These include type of teeth, amount of remaining tooth structure, preparation technique, obturation materials, model design, selection of organisms, sterilization techniques, and sample size.
CONCLUSIONS
It was concluded from this study that:
1) None of the tested materials were able to provide a complete hermetic seal, where all groups showed leakage within the study period.
2) CPoint with endosequence bioceramic sealer provided the best coronal seal by reducing the speed of bacterial penetration through the root canal filling. MTA Fillapex had an inferior coronal seal compared to the other groups.
3) Single-cone obturation with well-fitted CPoint cones and bioceramic sealer is an alternative for traditional lateral compaction technique.

ACKNOWLEDGEMENT
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CONFLICT OF INTEREST
The authors declare that they have no conflicts of interest.

REFERENCES
CLINICAL EVALUATION OF THE USE OF PROCEED® MESH IN RECONSTRUCTION OF ORBITAL FLOOR FRACTURES: CLINICAL TRIAL
Oraby M S1 BDS, Sharara A A2 PhD, Fahmy M H2 PhD

Abstract:
Introduction: Orbital fracture is a common type of midfacial trauma. Its proper treatment is considered a challenging situation. Otherwise orbital complications; as diplopia, limited ocular motility, enophthalmos, hypoglossus and infraorbital dysesthesia; may occur. Proceed® mesh consists of polypropylene soft mesh encapsulated with polydioxanone (PDS) and layered with oxidized regenerated cellulose (ORC). It is a widely used mesh in hernia repair which is proved to be safe and suitable. The ORC component provides the mesh with some properties differ from other materials. It provides a physical barrier to decrease the adhesion between the mesh and orbital tissue. It is a commonly used hemostatic agent. In addition, it is proved to be antibacterial against many resistant species. The PDS component attaches the ORC with polypropylene soft mesh.

Objectives: We aimed in this study to evaluate Proceed® mesh clinically in reconstruction of orbital floor defects in seven patients.

Materials and Methods: Indications for intervention were diplopia, enophthalmos, minimal clinical improvement over time under drug therapy, ocular limited motility, dystopia, defect more than 50% of the orbital floor and progressive infraorbital hypoesthesia. The ORC side of the mesh was placed facing orbital soft tissue, while polypropylene side was placed towards orbital bony wall. The follow-up schedule was 3 days postoperatively then once weekly for two weeks and then monthly for 6 months.

Results: Clinically, most patients recovered from diplopia, dystopia, ocular restriction and enophthalmos. Two patients had transitory postoperative diplopia (for 1 week), that may be due to postoperative muscle weakness. One patient out of four suffered from preoperative ocular restriction, had no or slight improvement postoperatively. Two patients complained from inflammatory reaction within 2-4 weeks postoperatively, resolved by anti-inflammatory and antibiotic administration.

Conclusion: The study concluded that Proceed® mesh is a suitable material for orbital floor reconstruction, especially in cases of small to medium defects (< 3 cm2).

Keywords: orbital floor, Proceed® mesh, implant material, prolene mesh.

INTRODUCTION
Maxillofacial injuries rate is raising due to the high speed travel as well as increasing violence. Road traffic accident, personal assault, direct/indirect trauma, sport activities, falls, and firearms are considered common causes to facial injuries (1).

Orbital fracture occurs as a result of the application of forces that overcome the resistance of orbital bony structures. It is one of the most frequent fractures following midfacial trauma. Orbital fractures account for 40% of craniofacial injuries. They represent 21.4% of midfacial fractures (2-6).

The orbital complications resulted from these fractures may be: diplopia, restriction of ocular motility, enophthalmos, hypoglossus, and infraorbital dysesthesia (7). So proper examination, diagnosis and treatment should be performed in suspected cases. Improper treatment or misdiagnosis of orbital floor fractures may result in permanent diplopia, enophthalmos or nerve dysesthesia (8).

Methods for orbital floor reconstruction have great controversy among the surgeons. The choice of material depends on the size and complexity of the orbital fracture. The ultimate goal for orbital floor repair is to restore function and form in the safest possible way (9).

Although the autogenous bone graft remains the gold standard material for orbital reconstruction; donor site morbidity is considered its major disadvantage. Other disadvantages are altered resorption rate, increased operation time, limited graft size and thickness, and limited ability of contouring (9, 10).

Resorbable alloplastic materials are less likely to have late complications such as infection and extrusion. But they have inferior strength, need of overcorrection, lack of osteoconductivity and have late enophthalmos possibility (9, 10).

Titanium mesh is a widely used implant material, as it showed success and good results in reconstructing and spanning large defects. On the other hand, it is expensive, needs fixation, not easily contoured and positioned, sharp edges may catch periorbital fat, dense fibrosis occurs within mesh’s pores and difficulty to be removed if needed (9, 10).

Prolene mesh is one of the non-resorbable alloplastic materials. Prolene is biocompatible, cheap, strong yet flexible, easy to be contoured and shaped, has unlimited supply and has both tensile strength and stability (11-13).

Proceed® mesh consists of oxidized regenerated...
cellulose (ORC) fabric, and Prolene Soft Mesh which is encapsulated by a polydioxanone polymer (PDS). The polypropylene mesh side of the product allows for tissue ingrowth. The ORC side provides a bioresorbable layer that physically separates the polypropylene mesh from underlying tissue during the wound-healing period, which minimizes periorbital tissue attachment to the mesh. The polydioxanone provides a bond between the ORC layer to polypropylene mesh (14).

Oxidized regenerated cellulose is a widely used hemostatic agent, so it may aid in decreasing of retrobulbar hemorrhage possibility. It also has antimicrobial activity and is effective against antibiotic-resistant microorganisms like Methicillin-resistant Staphylococcus aureus (MRSA) and Methicillin-resistant Staphylococcus epidermidis (MRSE) (14, 15).

Our aim in this study was to get the benefits of the combination between these three materials; including reduced adhesions with surrounding tissues, hemostasis and antibacterial properties, in order to overcome the drawbacks of other widely used orbital floor implant materials. So we evaluated this material clinically in cases of orbital floor fractures indicated for surgical repair.

MATERIALS AND METHODS

It is a prospective observational study during the period from September 2014 to May 2015 including the follow up for 6 months. Seven patients were admitted to the Oral and Maxillofacial Surgery Department, Faculty of Dentistry, Alexandria University; suffering from orbital floor fractures indicated for surgical repair.

Patients suffered from orbital floor fractures; either unilaterally or bilaterally or associated with other maxillofacial injuries were included in the study. Patients were diagnosed by computed tomography (CT) (Figure 1 A & B) and evaluated for trauma of the ocular globe by ophthalmologist.

The indications for surgery included impaired eye movement, diplopia more than 7 days, enophthalmos, hypoglobus, radiographically the defect was more than 50% of orbital floor, progressive infraorbital hypoesthesia and minimal clinical improvement over time under drug therapy.

Our choice was orbital reconstruction with Proceed® Surgical Mesh. It is composed of oxidized regenerated cellulose (ORC) fabric, and Prolene Soft Mesh; a non-resorbable Polypropylene mesh, which is encapsulated by a Polydioxanone polymer. (Figure 2)

Before the surgical intervention of the orbital fracture, forced duction test was done to examine the limitation. The approach to orbital floor was through an infraorbital incision. After reaching the orbital rim, subperiosteal dissection was performed to visualize the entire orbital fracture. Entrapped soft tissues were freed and repositioned intraorbitally. (Figure 3)

The defect was then measured by metallic ruler and the mesh was tailored and contoured according to the size of the

![Fig 1a: Preoperative CT scan showing left orbital floor fracture - Coronal view](image)

![Fig 1b: Sagittal view](image)

![Fig 2: Proceed® mesh (polypropylene soft mesh encapsulated with PDS and layered in one side by ORC)](image)
defect. The mesh was folded 2-3 times. Insertion of the Proceed® mesh was done as follows: prolene side of the mesh (the side with blue strea) was facing the orbital bone, while ORC side was toward the periorbital soft tissue. Placement of the mesh was done without fixation. Forced duction test was done to ensure freeing of all herniated soft tissue. Postoperatively patients were evaluated for diplopia, visual acuity, enophthalmos and ocular movement in the ophtalmologic department, faculty of medicine. Clinically, infraorbital sensation was examined subjectively.

RESULTS
In the present study seven patients (seven males) with age range from 19 to 60 years were included. The causative factors for fractures were road traffic accident in three cases, personal assault in two cases and motorcycle accident in two cases. Early intervention was done (within 2 weeks) in all patients.

In addition to orbital floor fracture, in all seven cases there was associated zygomatico-maxillary complex fracture. There was additional frontal bone fracture in two cases, a case associated with nasal fracture and a case associated with mandibular fracture.

Diplopia was noted in six cases, limitation of ocular motility in four cases, enopthalamos in three cases and ocular dystopia in two cases, ptosis in two cases, epiphora in one case and proptosis in one case.

All seven patients enrolled in the study consented to participate. After orbital floor reconstruction, a clinical evaluation was done to evaluate patients on follow-up visits. All the patients of preoperative diplopia showed significant improvement postoperatively. One case complained from transient diplopia postoperatively resolved within one week. One case out of four with preoperative ocular restriction shows no or minimal improvement postoperatively although patient didn’t complain from postoperative diplopia.

All patients with preoperative enophthalmos showed improvement in postoperative follow up visits. The two cases of preoperative ocular dystopia showed significant improvement postoperatively. One case out of two with preoperative ptosis showed improvement postoperatively. One case complained from epiphora didn’t improve postoperatively and required ophtalmologic intervention. One case of post-traumatic visual impairment showed some improvement over the follow up visits but not fully recovered. The operated cases with preoperative and postoperative findings were listed in Table 1.

Complications including infection, extrusion, implant migration and retrobulbar hemorrhage did not occur in the current study. Only two out of seven cases complained from late inflammatory reaction (within 2-4 weeks postoperative) and resolved within 4 days by anti-inflammatory and antibiotic administration. (Figures 4 and 5)

DISCUSSION
Orbital floor reconstruction has been and still a matter of controversy in literature with regard to the indication, timing, surgical technique, access and reconstruction materials used (11,16). Techniques for reconstruction of orbital floor fractures continue to evolve and innovative alloplastic materials have been introduced (9).

In our therapeutic protocol, the indications for orbital floor repair include diplopia, evidence of muscle or perimuscular soft tissue entrapment in CT, minimal clinical improvement over time under drug therapy, progressive infraorbital hypoesthesia, and orbital defects greater than 50% of the orbital floor with a resultant enophthalamos which is similar to recommendation of Burnstine et al (17).

Prolonged incarceration of orbital content leads to atrophy and scar contracture, which needs compensation of larger degree of soft tissue loss and enlarged orbital volume (10). According to this fact, we reconstructed orbital floor with unresolved clinical findings within 2 weeks.

A variety of implant materials are used in orbital wall reconstruction but no material has yet proven to be successful without any complications. A composite multilayered implant material (Proceed mesh) used for abdominal hernia repair was assessed in this study. Proceed mesh showed no serious complication and application safety in our study as well as in hernia repair according to Rosenberg et al and Eltayeb et al (18, 19).

Its composition offers an advantage over the other implant materials. It consists of biodegradable components (ORC and PDS) and non-biodegradable component (prolene soft mesh). So after the biodegradation process, the remained foreign mass is reduced, and that will decrease long term complications of non-resorbable implants (20, 21).

Comparing Proceed lightweight mesh with heavyweight polypropylene mesh, Proceed mesh has less filament and larger pores. Therefore Proceed mesh is weaker than polypropylene. So incorporating of an absorbable component (PDS) is needed to reinforce it and improve its
handling. Larger pores of Proceed mesh provide optimal handling, increase the fibrovascular incorporation and decrease the thickness of fibrous capsule, resulting in development of flexible scar mesh (16, 18-23).

As well as Proceed mesh, porous hydroxyapatite and porous polyethylene show fibrous ingrowth and complete vascularization in animal model (24). But they show more adhesion and removal difficulty if needed (22). On the other hand, silicone and nylon has smooth surface that does not bond with bone or soft tissue, which explains their high rate of extrusion and migration (4, 25-28).

### Table 1: Summary of pre and postoperative clinical findings.

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Preoperative findings</th>
<th>Postoperative findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>33</td>
<td>Diplopia - Ocular dystopia - ION parasthesia.</td>
<td>Improvement of all preoperative findings.</td>
</tr>
<tr>
<td>2</td>
<td>35</td>
<td>Diplopia - Ocular limitation - Ptosis - ION parasthesia.</td>
<td>- Improvement in diplopia (within 1 week postoperatively) - no or slight improvement in ocular restriction - no improvement in ptosis</td>
</tr>
<tr>
<td>3</td>
<td>22</td>
<td>Diplopia - Enophthalamos - Ocular dystopia - Epiphora - ION parasthesia.</td>
<td>Improvement of all preoperative findings except epiphora and ION parasthesia</td>
</tr>
<tr>
<td>4</td>
<td>24</td>
<td>Diplopia - Enophthalamos</td>
<td>Improvement of all preoperative findings</td>
</tr>
<tr>
<td>5</td>
<td>60</td>
<td>Proptosis - Ptosis - Visual impairment - Ocular limitation - ION parasthesia.</td>
<td>Improvement of all preoperative findings except visual impairment not fully recovered.</td>
</tr>
<tr>
<td>6</td>
<td>30</td>
<td>Diplopia - Ocular limitation - ION parasthesia.</td>
<td>Improvement of all preoperative findings. (diplopia recovered within 1 week postoperatively)</td>
</tr>
<tr>
<td>7</td>
<td>19</td>
<td>Diplopia. - Enophthalmos - ION paresthesia. - Ocular limitation.</td>
<td>Improvement of all preoperative findings</td>
</tr>
</tbody>
</table>

Oxidized regenerated cellulose layer has proven to be bactericidal against a broad range of gram-positive and gram-negative organisms including various antibiotic resistant bacteria (MRSA, VRE, PRSP and MRSE) (14, 29). The alloplastic implants are more liable to infection than autogenous grafts. This is due to the bacterial load required to infect an alloplastic implant is lowered by a factor of

![Fig. 4: Graph showing number of patients with pre- and postoperative findings.](image)

![Preoperative](image)

![Postoperative](image)

![Fig. 5: Photographs showing inferior view for evaluation of enophthalamos. Preoperative enophthalamos in Lt. eye and postoperative improvement in Lt. eye.](image)
10,000 than for an autogenous graft (30). So the presence of ORC with polypropylene decreases rate of infection lower than other alloplastic materials. This is helpful because orbital floor defects are communicated with maxillary sinus which represents a source of infection. Also regarding to infection, in vitro study supported that the infection rate with the porous materials was less than non-porous material (31).

Placement of ORC side facing the orbital soft tissue decreases but not prevent the adhesions between the mesh and the orbital soft tissue. Harrell AG et al (32), have evaluated the adhesion formation of intra-abdominal prosthetics in a rabbit model. They found that polypropylene mesh alone formed more adhesions with greater tenacity than Proceed mesh. Lee and Nunner (33) reported 10 patients of orbital adherence syndrome as a result of using titanium mesh. The usage of ORC as a barrier over titanium mesh could lower adhesion and subsequent fibrosis especially in large defects.

Rubin et al (34) contraindicated orbital floor reconstruction with porous polyethylene implants if the extraocular muscles are visualized during the orbital floor exploration. This is due to the ingrowth of extraocular muscle’s tissues into porous polyethylene implant may result in limiting the duction of the globe because of adhesions. Villarreal et al (35) recommended in this situation the interposition of a smooth nonporous alloplastic implant, or an autogenous nasal septal cartilage between the orbital tissue and the implant to avoid direct contact of the implant with the extraocular muscles. Choi et al (36) supported in their study the efficacy of porous polyethylene implant with barrier in reducing soft tissue adhesions, without compromising the degree of fibrovascular ingrowth into the implant. They recommend it in cases with exposed extraocular muscles. Our mesh offers this combination and could be beneficial in such cases as it contains the resorbable ORC.

Concerning Proceed handling, it can be cut and tailored easily by scissors without unraveling or leaving sharp edges. So it overcomes the problem of cutting titanium mesh and the sharp edges formed if not probably trimmed (37). Once Proceed mesh is trimmed outside the orbit, it maintains its shape and facilitates easily implantation. In comparison to Prolene mesh, Proceed mesh found to be more malleable, with softer borders and has less recoil memory. On the other hand, the ORC component hinders the ability of repetitive trial as it softens once it contacts the blood. So a template of Prolene mesh was used to transfer the size and contour needed to the Proceed mesh.

No further securing (with screws or suture) was necessary. As the intraorbital content is allowed to fall back onto the mesh and the peristeme is closed at the orbital rim. This provides a barrier which prevents the anterior migration (38).

All the patients of preoperative diplopia showed significant improvement postoperatively. Two patients complained from transient diplopia postoperatively which resolved within 1 week. That may be due to surgically induced muscle weakness.

One case out of four, with preoperative ocular restriction, showed no or minimal improvement postoperatively. Although this patient did not complain from postoperative diplopia. We supposed that the patient has strabismus from before.

All patients with preoperative enophthalmos showed improvement in postoperative follow up visits. The two cases of preoperative ocular dystopia showed significant improvement postoperatively. One case out of two with preoperative ptosis showed improvement postoperatively. One case complained from epiphora did not improve postoperatively and required ophthalmologic intervention. One case of post-traumatic visual impairment is showing increasingly improvement over the follow up visits but not fully recovered.

In two patients, inflammation occurs from 2-4 weeks postoperatively. This may be due to the degradation process of ORC and PDS. The inflammation resolved within 4 days after administration of anti-inflammatory and antibiotic therapy. Mauriello et al (39) also found local inflammatory reaction after use of Vicryl (polyglyactin-910) mesh implant for repair of orbital floor fracture.

The main disadvantage of this mesh is that it is not radiodense. Therefore its position cannot be easily visualized on immediate postoperative CT scans. It is not easily available, sterilized only with radioactive cobalt, and considered more expensive than Prolene mesh. Aramayo AL et al (40) showed that Proceed mesh may produce acute inflammatory reaction more than Prolene mesh.

The Proceed double-sided mesh is a good surgical option to replace missing bone in the reconstruction of the internal orbital wall especially with visualized extraocular muscle. It is considered biocompatible, easily contoured, allows fibrovascular ingrowth, decrease adhesion, antibacterial and hemostatic. In addition to the advantages of alloplastic material, which are decreased operative time, no donor site morbidity, and the ability to adjust the volume of filling as needed.

CONCLUSIONS
We concluded from this study that Proceed mesh has a potential to be a useful reconstructive material in orbital floor defects (less than 3 cm²). It offers advantages of adhesion reduction, hemostasis and antibacterial component.

ACKNOWLEDGEMENT
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CONFLICT OF INTEREST
The authors declare that they have no conflicts of interest.
REFERENCES


THE CORRELATION BETWEEN BONE DENSITY AND IMPLANT STABILITY
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Abstract:
Introduction: Long-term studies have documented the successful treatment of edentulous and partially edentulous patients with endosseous titanium implants. Success rates between 81% and 93% have been reported. Successful treatment with endosseous implants is dependent upon a complex relationship of numerous factors. Bone density and implant stability are important factors for implant osseointegration which has been widely demonstrated by several authors.

Objectives: This study was designed to evaluate the correlation between bone density estimated by Cone-Beam Computed Tomography (CBCT) and stability of dental implants estimated by resonance frequency analysis using Osstell ISQ.

Materials and methods: Ten dentis s-clean tapered implants were inserted in posterior mandibular edentulous spaces in ten adult patients. The bone density of implants recipient sites were determined by density value (HU) using CBCT. And the implants’ stability were determined by quantitative unit called implant stability quotient (ISQ) measured by resonance frequency analysis using Osstell ISQ. Both values were determined immediately post-operatively and on intervals of 3&6 months.

Results: The mean implant stability value was 67.3 ± 9.14 immediately post-operatively, then increased on the 3rd month to be 72.3 ± 3.95 & 75.2 ± 5.33 on the 6th month, there was a statistical significant increase. Also the results of the present study showed that the mean bone density value was 827.96 ± 206.85 immediately post-operatively, then increased to 890.67 ± 138 & 1018.0 ± 149.79 on the 3rd and 6th months respectively, there was a statistical significant increase.

Conclusion: There was no correlation between bone density and implant stability.

Keywords: bone density, implant stability, cone beam CT, resonance frequency analysis.

INTRODUCTION
Osseointegrated dental implants have become an important therapeutic modality since the last decade with success rates ranging between 90%-95% in healthy patients. Still failures of up to 10% are still encountered. In general, these failure rates have been associated with poor bone quality and/or quantity and lack of stability of the implant (1-3).

Bone quality can improve around a functional osseointegrated dental implant due to the positive bone stimulation, the more bone that is present at an implant site, the better the possibility for implant success. Bone quality can be described by factors other than bone density such as skeletal size, the architecture and 3-dimensional orientation of the trabeculae, matrix properties, mineralization and structure (3, 4).

Several approaches such as densitometric measurements, dual energy X-ray absorptiometry, computerized tomography (CT) and dental cone-beam CT In the last years, cone beam computed tomography (CBCT) became widely used for oral and maxillofacial imaging providing a good spatial resolution, gray density range, and contrast, as well as a good pixel/noise ratio (5-8).

Implant stability is a combination of mechanical and biological stability; mechanical stability is the result of compression of bone tissue during implantation (primary stability), while biological stability is the result of newly formed bone cells, which are created on the implant surface during the osseointegration process (secondary stability) (9-13).

Various methods were used for implant stability testing such as histomorphologic research, tensional test, push-out/pull-out test and removal torque test, which are classified as destructive methods. Non-destructive methods include percussion test, cutting torque test while placing implants, Periotest® (Siemens AG, Bensheim, Germany), and resonance frequency analysis (RFA) (14).

Resonance frequency analysis has been introduced to provide an objective measurement of implant primary stability and to monitor implant stability over the healing period using Osstell ISQ (10, 15-18).

This study was designed to evaluate the correlation between bone density estimated by Cone-Beam Computed Tomography (CBCT) and stability of dental implants estimated by resonance frequency analysis using Osstell ISQ.

MATERIALS AND METHODS
A clinical trial was conducted on ten adult patients of both sexes (6 males and 4 females) having missing mandibular posterior teeth indicated for implant rehabilitation. The patients were selected from the Out Patient Clinic of the Oral & Maxillofacial Surgery Department, Faculty of Dentistry, Alexandria University.

The inclusion criteria of this study were; patients’ age ranging from 20-50 years, adequate vertical distance between the alveolar crest and the inferior alveolar canal to accommodate a minimum 8 mm length implants and good...
oral hygiene. While the exclusion criteria were; inadequate interocclusal space, parafunctional habits such as bruxism and clenching, uncontrolled systemic diseases such as uncontrolled diabetes and osteoporosis, chemotherapy or radiotherapy and heavy smokers.

The dentis system implants (Dentis s-clean tapered system, Woram-Dong, Dalseo-Gu, Daegu, Korea) with different diameters (3.7, 4.1 and 4.8 mm) and lengths (8, 10, 12 and 14 mm) were used in this study, and Ostell ISQ was used for measurement of implant stability.

Ostell ISQ (Ostell AB, stampgatan, Goteborg, Sweden) consists of Ostell ISQ instrument, probe, charger, USB cable and test peg.

The system includes the use of a SmartPeg™ attached to the dental implant by means of an integrated screw. The SmartPeg is excited by a magnetic pulse from the measurement probe on the handheld instrument. The resonance frequency, which is the measure of implant stability, is calculated from the response signal. Results are displayed on the instrument as the implant Stability Quotient (ISQ), which is scaled from 1 to 100. The higher the value, the more stable the implant.

All patients underwent pre-operative clinical examination: Patients’ data were collected; name, gender and age, medical and dental histories were taken and the oral mucosa of the edentulous area was examined for color, texture, firmness and thickness. Also all patients underwent pre-operative panoramic radiograph examination to determine the size of the implants, their relation to adjacent structures, to measure the amount of vertical height of bone above the mandibular canal, and to evaluate the condition of bone. (Fig 1)

![Preoperative panoramic radiograph showing missing mandibular right.](image)

Preoperative oral antibiotic one hour before surgery was given (Amoxicillin/clavulanic acid 2 gm) and 0.12% chlorohexidine gluconate mouth wash was used to rinse for 30 seconds before operation.

With the patient under local anesthesia a full thickness mucoperiosteal envelope flap was raised, surgical stent was fitted in the patients’ mouth, osteotomy was carried out in the central part of the alveolar bone, the initial marking or preparation of the implant site was done with a pilot drill of 2.2 mm, the osteotomy was then widened using an intermediate drill and the final drill according to the diameter of the implant, the implant was then threaded into the bone using a Ratchet, the SmartPeg™ was attached to the dental implant, the implant stability was measured by Ostell ISQ then the cover screw was placed, and the flap was sutured around the fixtures using 3/0 black silk suture. (Fig 2)

All patients were advised to apply cold packs extra orally intermittently every 10 minutes for 2 hours on the first day, chlorohexidine mouth wash was started on the 2nd post-operative day 3 times daily for 2 weeks, the sutures were removed after one week post surgically. Antibiotic (Amoxicillin/clavulanic acid 1 gm tab), 2 times daily for 5 days, non-steroidal anti-inflammatory drugs (ibuprofen 400 mg , EIPICO, 10th of Ramadan city, Egypt), 3 times daily for 3 days were given.

All patients were evaluated immediately post-operatively and on intervals of 3 & 6 months, for presence of pain, swelling or infection using Visual Analogue Scale (VAS) (19), gingival inflammation using the Löe and Silness Gingival Index (20) on the 2nd and 7th post-operative days and implant mobility was tested according to Mickney and Koth (21), then the implant stability measurement was examined at the time of insertion and on intervals of 3 and 6 months postoperatively using the Resonance Frequency Analysis via the Ostell ISQ system.

All the implants involved in this study were radiographed by CBCT immediately post operatively and on intervals of 3 & 6 months to assess the bone density around the implants, exposure was performed using “veraviewepocs 3D R100” at 8 MA, 90 KV and at a proper field of view. Densitometric analysis was performed around dental implants on CBCT image at these 3 time intervals using the “Ondemand 3D”software supplied with the previously mentioned machine. This analysis gives the actual bone density around the immersed dental implant that proves the process of osseointegration. (Fig 3)

Final prosthesis (porcelain fused to metal crown) was placed after three months.

The statistical analysis was performed to evaluate the correlation between bone density values and ISQ values immediately post-operative and on intervals of 3 and 6 months.

**RESULTS**

Ten implants were placed in a total of ten patients (4 females and 6 males) having missing mandibular posterior teeth were included in this study. Their ages ranged between 20 and 50 years with mean age of 35 years. They were selected from the Outpatient Clinic of the Oral and Maxillofacial Surgery Department, Faculty of Dentistry, Alexandria University. All patients had undergone surgical procedures for delayed implant placement and loading. All patients were followed up both clinically and radiographically for 6 months.

All patients had been operated under local anesthesia using surgical flap technique and implant placement, and no
Youssef et al. The Correlation Between Bone Density and Implant Stability

Fig. (2): A photograph showing the implant placement surgical procedures and implant stability measurement. (a) mucoperiostal envelope flap reflection. (b) intermediate drill. (c) measurement displayed on the portable instrument screen. (d) cover screw.

Fig. (3): CBCT image of the implant taken (a) immediately postoperatively (b) 3rd month postoperatively and (c) 6th month postoperatively

complications had been recorded during the operation.

All patients had been examined periodically during the follow-up period up to 6 months. Healing was uneventful in all cases, with no post-operative complications. Other clinical parameters had been recorded such as: Pain index, gingival index, implant mobility and Implant Stability.

1) Pain, tenderness, infection and/or swelling after surgery; all patients experienced slight to mild pain at the surgical site. Four had slight pain and showed mild oedema which subsided totally by the 2nd post-operative day. Two others had mild pain and mild oedema which also disappeared completely by the 2nd post-operative day. Four patients experienced mild pain and moderate edema, which subsided totally by the 4th post-operative day. All patients continued the follow up period without any signs of infection, gingivitis, or peri-implantitis.

2) Gingival Index; no signs of gingival inflammation were observed in all patients. (i.e. gingival index score was 0)

3) Implant mobility; all over the evaluation period, none of the implants showed any signs of mobility (i.e. mobility score was 0).

4) Implant Stability Evaluation; immediately post-operative, the mean implant stability value was 67.3 ± 9.14 (that value is known as primary stability) with a minimum recorded value of 56.0 and a maximum recorded value of 83.0. On the third month, the mean implant stability value was 72.3 ± 3.95 with a minimum recorded value of 65.0 and...
a maximum recorded value of 78.0. On the sixth month, the mean implant stability value was 75.2 ± 5.33 with a minimum recorded value of 63.0 and a maximum recorded value of 81.0.

The mean implant stability on the 3rd postoperative month showed no significant difference when compared with the immediate postoperative measurements (p = 0.06). While, the mean implant stability was statistically significant on the 6th month postoperatively when compared with the immediate postoperative measurements (p = 0.01). Also, the mean implant stability was statistically significant on the 6th month postoperatively when compared with the 3rd month postoperative measurements (p = 0.01). (Table 1, Fig 4)

On the sixth month, the mean peri-implant bone density value for the study group was 1018.0 ± 149.79 with a minimum recorded value of 805.85 and a maximum recorded value of 1218.14.

The mean bone density on the 3rd postoperative month showed no significant difference when compared with the immediate postoperative measurements (p = 0.151). While, the mean bone density was statistically significant on the 6th month postoperatively when compared to the immediate postoperative measurements (p <0.001). Also, the mean bone density was statistically significant on the 6th month postoperatively when compared with the 3rd month postoperative measurements (p = 0.001). (Table 2, Fig 5)

5) Radiographic evaluation; evaluation of bone density around the implants immediately post-operative, the mean peri-implant bone density value was 827.96 ± 206.85 with a minimum recorded value of 573.42 and a maximum recorded value of 1174.53.

On the third month, the mean peri-implant bone density value was 890.67 ± 138.31 with a minimum recorded value of 715.38 and a maximum recorded value of 1123.0.

The Correlation Between Bone Density and Implant Stability

The analysis of the effect of bone density on the implant stability showed that there was no statistically significant relationship between the implant stability and bone density in any of the follow-up periods (p=0.62, p=0.19, p=0.37).

DISCUSSION

The successful treatment of dental implants depends on the concept of osseointegration introduced by Branemark which implies the structural and functional contact between the implant and the surrounding vital bone (22). Bone density and implant stability are important factors for implant osseointegration which has been widely demonstrated by several authors (23).
Therefore, the present study was designed to evaluate the correlation between the bone density and the stability of dental implants in the posterior region of the mandible.

In this study 10 patients with missing mandibular posterior teeth were selected from the Outpatient Clinic of the Oral and Maxillofacial Surgery Department, Faculty of Dentistry, Alexandria University. The selected patients were free from any uncontrolled systemic diseases or conditions that may complicate the surgical procedure or the healing process of the implant. This was following a study performed by Bornstein et al in 2009 (24) where they reviewed whether systemic diseases with/without systemic medications increased the risk of implant failure and therefore diminish the success and survival rates of dental implants. They stated that the level of evidence indicative of absolute and relative contraindications for implant therapy due to systemic diseases is low.

CBCT was used to measure the bone density because of its accuracy, lower radiation exposure and fewer cost compared with CT. This was following a study performed by Cassetta et al in 2014 (25) where they scanned twenty dry mandibles with CBCT and conventional CT to evaluate if there is a statistically significant difference between the bone density values they produce, defined as gray density values and to determine any correlation between them. They concluded that the lower radiation dose and reduced costs of CBCT make this a useful substitute for CT, however, they stated that in order to more accurately define the bone density with CBCT, a conversion ratio needs to be applied to the voxel value.

In this study, the mean bone density increased significantly towards the 6th month post-operatively. This can be attributed to the healing of the bone around implants.

These results were in agreement with the results of Al-Sudani in 2014 (26). In her study, twenty implants in the premolar and molar regions of upper and lower jaws were evaluated using CT scan to measure the bone density by using HU around dental implants and after six months after dental implant placement. The mean HU of jaw bone immediately following implant placement was 552.28 HU and increased significantly to 761.33 HU after six months statistically.

Implant stability was measured using the Resonance Frequency Analysis (RFA) via the Osstell ISQ system. RFA was chosen as a noninvasive and reliable method to assess variation in implant stability over time. RFA registrations are directly related to the stability of the implant in the surrounding bone: during healing an increase in implant stability quotient (ISQ) values presumably reflect new bone apposition at the implant-bone interface (15, 27-31).

Meredith et al (15, 32) concluded that RFA is a method that can serve as a useful research technique and it is valuable in studying the behavior of implants in surrounding tissue. Also, Jaramillo et al in 2014 (33) reported that Resonance frequency analysis systems in Osstell Mentor and Osstell ISQ show almost perfect reproducibility, repeatability and accuracy.

In this study the mean implant stability value was 67.3 ± 9.14 immediately post-operatively, then increased on the 3rd month to be 72.3 ± 3.95 & 75.2 ± 5.33 on the 6th month there was a statistical significant increase.

These results are in agreement with Huwiler et al in 2007 (34), where they studied the resonance frequency analysis (RFA) during the early phases of healing. They concluded that during the incorporation and healing phase of the implants, the mean values for the various observation periods the ISQ values seemed to increase.

Also, the surface treatment of the implant had a role in the increase of the ISQ value in the present study during the healing period. All the implants placed were resorbable blast media (RBM) treated, in which the surface of the implant is blasted with calcium phosphate powder in order to increase cohesion of bone tissue, followed by thorough cleaning of the surface.

This is accordance with Glauser et al in 2007 (35), where they reported that implant design and surface treatment have a significant influence on soft bone.

Also, this is agreement with Kim et al in 2010 (36) where they evaluated the effects of different implant surface treatments on implant stability in dog mandibles. A total of 30 implants were placed in 5 dog mandibles. Bone quality was assessed at each site. An Osstell resonance frequency analyzer (RFA) was used to determine the stability at different periods after surgery. The results of this study suggested that surface treatments may have significant effects on the biological stability.

In this study, the analysis of the effect of bone density on the implant stability showed that there was no statistically significant relationship between these two factors throughout the study period.

These findings agreed with the results of Huwiler et al in 2007 (34), when analyzing the morphologic characteristics of the parent bone into which the implants were placed, no correlations could be demonstrated between the ISQ values and the bone volume density and/or the bone trabecular connectivity as revealed by micro CT analysis of bone cores obtained at the time of implant installation.

Moreover, Farre- Pages et al in 2010 (37), studied the relation between the bone quality and primary stability and they concluded that there was no relation between the ISQ value and bone quality.

On the other hand, Turkayilmaz et al in 2007 (38) reported that the bone density value from pre-operative CT examination may provide an objective assessment of bone quality and significant correlations between bone density and implant stability parameters may help clinician to predict primary stability before implant insertion.
Also, Sumera et al in 2013 (39) stated that positive correlation between RFA values and bone density was found for all four regions of the jaws.

Moreover, Salimov et al in 2014 (40) reported that bone density assessment using CBCT is an efficient method and significantly correlated with implant stability parameters and Lekholm and Zarb index. Thus, it is possible to predict initial implant stability and possibility of immediate or early loading using CBCT scans prior to implant placement.

The contradiction between the results of the above studies and this study may be attributed to the use of a higher number of implants with minimum 57 implants, but in this study only 10 implants were used. Also, in their studies they used more than one type of bone according to Lekholm and Zarb classification, but in this study only one region of the mandible was selected. In addition, in their studies they correlated the bone density with primary stability only, but in this study we correlated the bone density with primary implant stability and secondary implant stability on intervals of 3 and 6 months.

CONCLUSIONS
There is no correlation between bone density and implant stability, using cone beam computed tomography is a simple method to measure bone density around dental implants and evaluate the condition of bone before implant placement and resonance frequency analysis is a reliable method to predict bone healing around implants and to measure implant stability throughout the follow up period. There is no correlation between bone density and implant stability.

CONFLICT OF INTEREST
The authors declare that they have no conflicts of interest

INFORMED CONSENT
Appropriate institutional ethical clearance and written informed consent were obtained.

REFERENCES
THE EFFECT OF BIOSCAFFOLD ALVELAC™ IN PRESERVATION OF ALVEOLAR BONE AFTER EXTRACTION OF TEETH
(CLINICAL AND RADIOGRAPHIC STUDY)

Sager M¹, Darwish S², Melek L³

ABSTRACT

Introduction: After tooth extraction, the extraction socket heals by forming a blood clot which leads to the formation of new bone within 3-4 months. Although bone deposition in the socket will continue for several months, it will not reach the crestal level of the neighboring teeth.

Objective: Is to clinically and radiographically evaluate the use of Bioscaffold Alvelac™ in preservation of dimensional measure of alveolar bone after extraction of teeth.

Materials and methods: This study was conducted on twelve patients divided into two equal groups (study group and control group). Indicated for extraction of anterior maxillary teeth, in the study group, the bioscaffold Alvelac™ was inserted into the empty socket after extraction and was supported by 3-0 silk with figure of eight sutures. In the control group, extraction of upper anterior teeth was done without introducing any material and the wound was sutured.

Results: There was a statistically significant decrease of alveolar bone width and height in both groups at three months postoperative interval compared with the bone width and height at the immediate postoperative period.

Conclusion: Immediate tooth extraction stabilizes the bioscaffold Alvelac™ material in the socket and allows it to act as a scaffold for bone deposition. From this study, it is clear that, this material allows preservation of the dimensional measure of the alveolar bone.

Key Words: Tooth extraction, Alveolar bone, socket preservation, Scaffold, Alvelac™.

INTRODUCTION

After loss of natural teeth, bony changes in the jaws begin to take place immediately. Since the alveolar bone no longer responds to stresses applied in this area, it begins to resorb (1).

Resorption of the alveolar ridge following extraction of hopeless teeth is recorded even when the missing teeth is immediately restored (2).

The alveolar ridge beneath the artificial teeth decreases in height and width due to lack of the stimulating effect of the teeth roots. A gap is created by time beneath the prosthesis and the ridge leading to food impaction, mucosal inflammation, and bad esthetics especially in the anterior region (3).

The remodeling process results in a ridge morphology reduced in vertical height and more palatal in relation to the original tooth position (4, 5).

Alveolar bone is a specialized part of the mandibular and maxillary bone that forms the primary support for the teeth. Alveolar bone is composed of bundles of bone which are built up into layers in parallel orientation to the coronal -apical direction of the tooth (6, 7). The bone loss is estimated to be 40%-60% during the first 3 years and decrease to 0.25%-0.5% annual loss (8, 9).

Immediately after tooth extraction, the alveolar socket is filled by blood clot that is replaced by granulation tissue within 1 week (10).

The bio-scaffold is made of PLGA (Polylactic-co-glycolic acid) material and acts as a mechanical support to hold the blood clot at the crest level (11). After tooth extraction, the bundle bone appears to be the first bone to be resorbed (12-14).

PLGA also has the advantage of being capable of delivering drugs, proteins and growth factors to enhance bone healing in both oral-maxillofacial and general orthopedic applications (15-18).

Brown et al (2014) (19) found in his study that sixty percent of implant-supported dental prostheses require bone grafting to enhance bone quantity and quality prior to implant placement. They have developed a metallic magnesium particle/PLGA (Mg/PLGA) composite scaffold to overcome the limitations of currently used dental bone grafting materials. These scaffolds could decrease inflammation observed with clinically used PLGA devices. These characteristics not only increase cell proliferation in vitro, but provide a safe and osteoconductive environment for bone regeneration in vivo. These findings show promising results for the use of Mg/PLGA composite materials for a wide range of bone regeneration applications.

The aim of this study was to evaluate clinically and radiographically the use of Bioscaffold Alvelac™ in preservation of dimensional measure of alveolar bone after extraction of teeth.
MATERIALS AND METHODS
Alvelac™ (International Pte Ltd 61 Science Park Road, #02-05/06, The Galen, Singapore Science Park II, Singapore 117525) is a porous, osteoconductive, biocompatible and biodegradable synthetic scaffold that is synthesized from polylactic-co-glycolic acid (PLGA) and polyvinyl alcohol and produced using proprietary and patented technology.

It is a rigid structure specifically designed to prevent collapse of the buccal and lingual walls in achieving width maintenance. It is strategically placed in the extraction socket with the top of the scaffold in line with the crest of the socket in order to raise the forming blood clot to that level thus achieving height maintenance. The size of Alvelac™ does not occupy the whole socket thus allowing maximum space for blood to fill the socket. This allows for the patient’s own bone to form naturally within that space by the action of Alvelac™ as scaffold (20).

Selection of patients:
Twelve patients, indicated for extraction of maxillary teeth were selected from those attending the outpatient clinic of Oral and Maxillofacial Surgery department, Faculty of Dentistry, Alexandria University.

Inclusion criteria of selection:
- Patients’ age ranged between (25 - 45) years old of both sexes.
- All patients selected were free from any relevant diseases.
- Indicated for extraction of maxillary teeth.

Exclusion criteria of selection:
- Heavy smokers.
- Bone disease (as osteoporosis).
- Uncontrolled diabetes.

Patients were divided into two equal groups; the study group, where the maxillary teeth were extracted and the bioscaffold Alvelac™ was inserted into the empty socket after extraction and was supported by 3-0 silk with a figure of eight sutures. (Fig. 1) Whereas in the control group the extraction of teeth was done without introducing any material and the wound was closed and supported by 3-0 silk with a figure of eight sutures.

Fig (1): Showing insertion of the bioscaffold alvelac into empty socket.

A) Surgical phase: Local anesthesia (Each carpule contains 1.8 ml mepivacaine HCL 2% produced by: Alexandria Co. for pharmaceuticals, Alexandria, Egypt). Extraction of teeth was performed using maxillary forceps.

B) Clinical follow up: All patients in the two groups were examined clinically for infection and healing. Healing was assessed by the uninterrupted (adequate & proper) closure of the socket visually, which was done at intervals of one week, and three months after extraction.

C) Radiographic follow up: All patients in the two groups were examined radiographically immediately postoperative to serve as a baseline for measurement and after 3 months of the extraction.

The radiographic examination was done by cone beam CT (CBCT). Bone height, bone width and bone density were measured using cone beam CT (CBCT) software (On Demand 3DAPP-DBM)

RESULTS:
Twelve patients were divided equally into two groups, group I (control group) and group II (study group). Group I included 4 females (66.7%) and 2 males (33.3%). While group II included 5 females (83.3%) and 1 male (16.7%). For group I the age ranged from 25 to 40 years with mean of (32.17 ± 5.71 years). While in group II, the age ranged from 25 to 45 years with mean of (30.83 ± 7.22 years).

Clinical Results:
1. Infection: Infection was observed by inspection, all cases in both group I and group II showed that there were no signs of infection throughout the postoperative follow up period.
2. Healing: Normal colour of the oral mucosa and adequate closure of the extraction socket were achieved in patients of both groups. (Fig. 2)
Fig (2): Showing figure of eight suture (study case).

Radiographic results:
1. Alveolar bone width (Table 1). There was a statistically significant decrease of alveolar bone width in both groups at three months postoperative compared with the bone width at the immediate postoperative period. (Fig. 3-6)

The percentage of change in alveolar bone width in group I was 18.87% while in group II it was 1.04% There was a statistically significant difference between the two groups ($t=8.292$, $p<0.001$).

2. Vertical bone height (Table 2). There was a statistically significant decrease of alveolar bone height in both groups at three months postoperative compared with the bone height at the immediate postoperative period. (Fig. 3-6)

The percentage of change in alveolar bone height in group I was 3.68% while in group II it was 0.44%. There was significant difference between the 2 groups ($t=5.968$, $p<0.001$).

3. Bone density (Table 3). There was no significant difference in bone density between group I and group II at three months postoperative.

The percentage of change in bone density for the control group was 14.87 %, while for study group was 22.94 % with no statistically significant difference. There was a higher percentage of change in bone density in group II (study group) than in group I (control group).

![Table 1: Comparison between the two groups according to horizontal bone (width) using cone beam CT.](image1)

<table>
<thead>
<tr>
<th>Horizontal (width)</th>
<th>Control (n = 6)</th>
<th>Study (n = 6)</th>
<th>$t_1$</th>
<th>$p_1$</th>
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<tr>
<td><strong>Immediately</strong></td>
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<td>after extraction</td>
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<tr>
<td>Min. – Max.</td>
<td>4.10 – 7.83</td>
<td>6.65 – 10.65</td>
<td>3.332</td>
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<td>5.70 ± 1.39</td>
<td>8.34 ± 1.35</td>
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<td>Median</td>
<td>5.74</td>
<td>8.32</td>
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<td><strong>After 3</strong></td>
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<td>months</td>
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<tr>
<td>Min. – Max.</td>
<td>3.13 – 6.68</td>
<td>6.65 – 10.50</td>
<td>4.746</td>
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<td>8.21 ± 1.29</td>
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<td>Median</td>
<td>4.88</td>
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<td><strong>Change</strong></td>
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<td>after 3 months</td>
<td>1.01 ± 0.27</td>
<td>0.13 ± 0.14</td>
<td>7.012</td>
<td>&lt;0.001</td>
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Fig (3): Showing immediate CBCT sagittal view showing alveolar bone width and alveolar bone height (control case).

Fig (4): Showing post-operative (three months) CBCT sagittal view showing alveolar bone width and alveolar bone height (control case).

Fig (5): Showing immediate CBCT sagittal view showing alveolar bone width and alveolar bone height (study case).
Fig (6): Showing post-operative (three months) CBCT sagittal view showing alveolar bone width and alveolar bone height (study case).

<table>
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<th>Vertical (height)</th>
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<th>Study (n = 6)</th>
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<th>p₁</th>
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<td>Min. – Max.</td>
<td>15.40 – 22.38</td>
<td>5.72 – 20.46</td>
<td>1.685</td>
<td>0.123</td>
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<td>15.78 ± 5.49</td>
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<td>Median</td>
<td>20.38</td>
<td>18.15</td>
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<td><strong>After 3 months</strong></td>
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<td>Min. – Max.</td>
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<td>5.55 – 20.39</td>
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<tr>
<td>Median</td>
<td>19.65</td>
<td>18.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Change after 3 months</strong></td>
<td>0.74 ± 0.10</td>
<td>0.08 ± 0.06</td>
<td>13.971</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

Table (2): Comparison between the two groups according to vertical bone (height) using cone beam CT.

- t: Student t-test
- p₁: p value for student t-test for comparing between the two groups

<table>
<thead>
<tr>
<th>Bone density</th>
<th>Control (n = 6)</th>
<th>Study (n = 6)</th>
<th>t₁</th>
<th>p₁</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Immediate after extraction</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Min. – Max.</td>
<td>210.58– 513.26</td>
<td>312.55 – 645.79</td>
<td>1.291</td>
<td>0.226</td>
</tr>
<tr>
<td>Mean ± SD.</td>
<td>308.58 ± 111.32</td>
<td>397.79 ± 127.60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>297.63</td>
<td>356.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>After 3 months</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Min. – Max.</td>
<td>213.75 – 646.22</td>
<td>325.22 – 821.79</td>
<td>1.604</td>
<td>0.140</td>
</tr>
<tr>
<td>Mean ± SD.</td>
<td>367.06 ± 174.91</td>
<td>547.78 ± 213.54</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>306.69</td>
<td>515.35</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Change after 3 months</strong></td>
<td>-58.48 ± 94.29</td>
<td>-149.99 ± 130.10</td>
<td>1.395</td>
<td>0.193</td>
</tr>
</tbody>
</table>

Table (3): Comparison between the two groups according to bone density using cone beam CT.

- t: Student t-test
- p₁: p value for student t-test for comparing between the two groups

**DISCUSSION**

The rationale for alveolar ridge preservation relies on the knowledge that the alveolar ridge resorption is an unavoidable sequela of tooth loss (21). Its goal is to prevent the loss of 40% - 60% of ridge height and width commonly seen after extractions (8).

Preservation of socket is driven by the desire to minimize the need for future more invasive ridge augmentation procedures. Moreover, it also facilitates successful implant and conventional prosthetic treatment. Extraction site grafting often facilitates the best possible functional and aesthetic results. It is axiomatic therefore that socket preservation should be the treatment of choice to prepare the remaining alveolar ridge for conventional or fixture supported restorations (22, 23).

Several studies have evaluated the effect of PLGA with different grafting materials (24). Defects that were treated with Mg/ PLGA scaffolds had an improved bone height preservation compared with empty defects at both 8 and 16 weeks post-surgery. This effect compares well with other bone-grafting materials (including polymers) that have been successfully used for socket preservation (24, 25).

According to Fan (26), a bio-scaffold, placed immediately after tooth extraction, helps and allows for bone to grow into it and prevents the socket from collapsing. The results of the present study are in accordance with Fan’s results regarding an increase in the bone density and protection of the height and width of the alveolar bone.

A similar osteoconductive scaffold, OsteoScaf, was used by Araujo et al (27). The results obtained from CBCT measurements have clearly shown that OsteoScaf protection impaired the expected bone lost during the post extraction remodeling of the alveolar bone ridge at 120 days post extraction. This result is also in agreement with the results of the current study.

In a study by Serino et al (28), the use of a bioabsorbable synthetic sponge of polylactide–polyglycolide acid was evaluated. The results of this study indicate that alveolar bone resorption following tooth extraction may be prevented or reduced by the use of a bioabsorbable synthetic sponge of polylactide–polyglycolide acid. The quality of bone formed seemed to be optimal for dental implant insertion. The material is similar in content to Bioscaffold Alvelac™ used in the present study.

**CONCLUSIONS**

Within the context of this study, the following conclusions can be listed:

- Immediate tooth extraction stabilizes the bioscaffold Alvelac™ material in the socket and allows it to act as a scaffold for bone deposition.
- It is clear that this material allows preservation of the dimensional measure of alveolar bone.
- Healing seems to be more proper in the study cases.

**REFERENCES**

EFFECT OF COLLATAPE® COLLAGEN WOUND DRESSING ALONE AND COMBINED WITH INGENIOS® SYNTHETIC BONE GRAFT ON THE SOCKET HEALING IN RABBITS

Moustafa H 1, Omar S 2, Osman S 3, Kawana K 4

Abstract:
Introduction: Socket preservation enhances healing and reduces bone loss during healing of extraction socket.
Objectives: To assess the effect of bio-absorbable collagen wound dressing (CollaTape®; Zimmer) on the healing of extraction sockets in rabbits when applied alone and combined with IngeniOs Beta tricalcium phosphate (β-TCP) synthetic bone particles.
Materials and methods: Sixteen rabbits were included in this study in which lower left first premolars were extracted and then divided into three groups including: Control group (4 animals) in which the sockets were left for normal healing, Collatape group (the sockets were closed with CollaTape collagen wound dressing alone) and IngeniOs β-TCP group (6 animals) in which the sockets were filled with synthetic bone particles and were closed with CollaTape collagen wound dressing. Euthanasia were done 2 and 6 weeks respectively. Socket healing was examined and evaluated histologically and analyzed histomorphometrically.
Results: At 2 weeks of healing, it was observed in all groups that the sockets were filled with newly formed woven bone. The percentage of newly formed bone was lowest in control group followed by Collatape group while β-TCP group showed highest values. At 6 weeks healing period, the socket entrance was sealed with compact bone and the rest of the socket was filled with trabecular bone. β-TCP group showed the highest values of the percentage of the formed bone.
Conclusions: It was confirmed that IngeniOs β-TCP enhances bone formation during socket healing and could be used for socket preservation.

Key Words: Socket healing, β-TCP, bone graft, Collatape

INTRODUCTION
Millions of teeth are extracted every year. Tooth extraction is one of the most common procedures that are carried out in dental practice (1). Unfortunately the healing of dental extraction socket is a time dependent complicated process and results in pronounced resorption of alveolar process that hinder the replacement of the missing tooth.

Many attempts were attributed to assist healing and to counteract such resorption (2). Various grafting and barrier materials were used for socket preservation. Bone grafting materials are important in regenerative dentistry. They act in three different modalities involving osteogenesis, osteoinduction and osteoconduction (3). Concerning osteogenesis, the grafting material accommodates osteogenic cells that directly deposit bone. Only freshly harvested autogenous bone graft contains these osteogenic cells (4). For osteoinduction, the graft material has differentiating factors that promote differentiation of mesenchymal cells into osteoblasts. Allografts are working with this concept (5). The last is the osteoconduction in which the bone graft materials act only as a scaffold that support new bone formation.

This property is represented in Xenografts and synthetic bone grafts (6). One of the osteoconductive synthetic bone grafts is beta tri calcium phosphate (β-TCP). The main disadvantage of β-TCP is its fast resorption so its degradation is not always associated with bone healing (7).

Recently, Silificated β-TCP graft material was produced. Silica was added to β-TCP in order to enhance its strength and its biologic properties so that the resorption of graft will be in co-incidence with new bone deposition (8). Researches dealing with using silicated β-TCP for socket healing are limited.

So the aim of this study is to evaluate the effect of bio-absorbable collagen wound dressing (CollaTape®; Zimmer) on healing of extraction sockets in rabbits alone and combined with IngeniOs Beta tricalcium phosphate synthetic bone particles.

MATERIALS AND METHODS
The Ethical committee of Alexandria University approved the protocol of this research. Sixteen healthy male rabbits weighting 2.5 kg aged 14-16 weeks were used in this study. The animals were obtained from the Institute of Medical Research Alexandria University. They were housed in specially designed wire mesh bottom cages. The animals were supplied a regular balanced diet during the whole experiment period. The rabbits were divided into three groups.

Control group (4 rabbits): The sockets were left without application of any material for normal healing. CollaTape group (6 rabbits): The sockets were closed with CollaTape collagen wound dressing only. IngeniOs β-TCP group (6 rabbits): The sockets were filled with IngeniOs Beta tricalcium phosphate synthetic bone particles and were closed with CollaTape collagen wound dressing.

The animals in each group were divided equally into two subgroups each consisting of 3 animals of both CollaTape
group and IngeniOs β-TCP group and 2 animals of control group and were scarified after 2 and 6 weeks from the start of the surgical procedure. During the surgical procedure all animals were anesthetized with general anesthesia using mixture of 13 mg/kg of body weight 2% xylazine hydrochloride and 33 mg/kg of body weight ketamine base.

In all rabbits, only the mandibular left first premolar was extracted. A mouth prop was used to obtain proper accessibility to lower left premolar. The tooth was luxated by using surgical elevators. Once luxation was performed, the tooth was pulled carefully out of the socket by lower remaining root extraction forceps. For the control group, the sockets were left without application of any materials to allow for normal healing.

For CollaTape group, a Collatape sheet was trimmed into small pieces to the size of extraction socket using surgical scissor, then it was sutured to the extraction socket by 4-0 black silk suture using the cross mattress suturing technique to achieve proper stability. For IngeniOs β-TCP group, Particles of IngeniOs β-TCP were firstly mixed with saline to be applied to the extraction socket using periosteal elevator, then Collatape absorbable collagen wound dressing was sutured to the socket using the same technique.

Post-operatively, each animal received the same course of antibiotic of Ampicillin 25 mg/kg body weight for five days after surgery every eight hours. Analgesics were given to animals in the form of Cataflam IM every eight hours for the first two days. The animals were observed daily for the first week to assess the occurrence of any signs of inflammation or infection.

Euthanasia was done at 2 and 6 weeks respectively then the mandibles were dissected out, sectioned into two halves and fixed in 10% neutral buffered formalin.

After fixation, the mandibles were decalcified in 5% trichloroacetic acid, washed, dehydrated in ethanol and embedded in paraffin wax. Serial bucco-lingual sections of 5µm thickness were cut and stained with Hematoxylin & Eosin.

Histomorphometric analysis using Image J software was done to obtain the percentage of surface area of the formed bone compared to the total surface area of the extraction socket in the two studied observation periods of socket healing (9).

Five sections of tissue from different standardized depths were used for quantification from each sample. A rectangle with standardized dimensions was drawn on the desired area to be measured using the image J program. The surface area of this selected region was measured by choosing Region of interest (ROI) manager, from tools from analyze and the measurement was recorded. The surface area occupied by the marrow spaces was selected using the wand tracing tool and the measurement was recorded.

The two recorded measurements were subtracted to obtain the surface area occupied only by bone, and its percentage to the total area selected was calculated. The measurements from three photographs were recorded and their mean was calculated for each of the five sections obtained, from each specimen.

Statistical analysis of the obtained data was done using ANOVA test to compare between three groups and t test to compare between one group and the other two groups.

RESULTS
1. Histological results
Results of the first observation period (two week post operatively):
A- Control group: The apical half of the extraction socket was occupied by newly formed bone that consisted of anastomosing bone trabeculae of moderate thickness. The newly formed bony trabeculae were lined by discontinuous layer of active osteoblasts, contained moderate density of osteocytes and enclosed bone marrow spaces of limited blood supply. (Fig.1)

B- CollaTape group: In this group, a central mass of newly formed bone was seen projecting from the lateral wall of the socket and extending towards its apical part. The coronal portion of the socket contained a mass of newly formed bone beneath Collatape collagen wound dressing. The newly formed bone trabeculae enclosed marrow spaces with rich blood supply and surrounded by dense fibrous connective tissue. They contained numerous osteocytes and lined by a continuous layer of voluminous osteoblasts. (Fig.2)
C- IngeniOs β-TCP group: The apical half of the extraction socket was occupied by newly formed bone surrounded by dense fibrous connective tissue and the marginal portion of the socket harbored fibrous connective tissue. β- TCP particles were apparently enclosed within the newly formed woven bone. The newly formed woven bone was found at the lateral walls and at the central part of the socket surrounding β-TCP particles. The bony trabeculae contained high density of osteocytes, lined by continuous layer of active osteoblasts and enclosed bone marrow spaces containing rich blood supply. Osteoclasts were also found surrounding β-TCP particles. (Fig.3)

Fig.3: LM (β-TCP group, 2weeks) showing the healing socket filled with newly formed bone trabeculae surrounding β-TCP particles (arrows). H&E stain original magnification X40.

Results of the second observation period (six week post operatively):
A- Control group: The socket was sealed by marginal compact bone while its rest was occupied by trabecular bone surrounded by vascular spaces and fibrous tissue. An outstanding fashion of trabecular formation could be traced where the fibrous tissue modeled and mapped the shape and organization of these trabeculae in a step advancing their mineralization. (Fig.4)

Fig.4: LM (Control group, 6weeks) showing compact bone (thick arrow) at the marginal part of the socket while the rest of the socket is filled with trabecular bone (thin arrow) enclosing vascular spaces. H&E stain original magnification X40.

B- Collatape group: In this group, the socket was filled with mature compact bone at its coronal portion of the socket while trabecular bone was seen directed towards the apical part of the socket and surrounded by marrow spaces of rich blood supply. The compact bone was of higher density than in control group consisted of haversian systems enclosing central haversian canals. (Fig.5)

Fig.5: LM (Collatape group, 6 weeks) showing mature compact bone (thick arrow) at the coronal part of the socket with continuation of extension of the underlying trabecular bone (thin arrow) towards the apical part of the socket. H&E stain original magnification X40.

C- IngeniOs β-TCP group: In this group, the socket was entirely filled with dense compact bone and small areas of trabecular bone mass. The compact bone consisted of numerous haversian systems enclosing rich blood supply within their haversian canals. The compact bone was of higher density than in the previous two groups. It consisted of numerous haversian systems enclosing haversian canals. (Fig.6)

Fig.6: LM (β-TCP group, 6weeks) showing dense compact bone mass (arrow) filling the healing socket. Note the presence of rich blood supply adjacent to the formed bone mass. H&E stain original magnification X40.

2. Histomorphometric analysis
The mean values of the percentage of the bone surface area formed in the healing socket in the control group, Collatape group and IngeniOs β-TCP group after 2 and 6 weeks from start of the experiment are demonstrated in table (1).

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Collatape group</th>
<th>β-TCP group</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 weeks</td>
<td>27.77±8.88</td>
<td>35.68±4.58</td>
<td>38.43±12.75</td>
<td>2.45</td>
<td>0.11</td>
</tr>
<tr>
<td>(Mean ±SD)</td>
<td>2*1</td>
<td>2*1</td>
<td>2*1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 weeks</td>
<td>62.71±15.8</td>
<td>76.06±12.86</td>
<td>84.19±12.56</td>
<td>4.30</td>
<td>0.029*</td>
</tr>
<tr>
<td>(Mean ±SD)</td>
<td>2*1</td>
<td>2*1</td>
<td>2*1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Percentage of the newly formed bone occupying the extraction socket. F: F test ANOVA
*: Statistical significance (P≤0.05).
*1: Percentage of bone surface area in control group is statistically lower than in Collatape group and β-TCP group (P≤0.05).
At the second observation period, it was noted that the control group exhibited the lowest value (27.77%) followed by Collatape group (35.68%) while β-TCP group showed the highest value (38.43%). There was no statistical significance between three groups in this interval although numerical values were obtained.

For 6 weeks period of healing, the values of the mean percentage of the formed bone exhibited the lowest value (62.71%) for control group followed by Collatape group (76.06%) while β-TCP group showed the highest value (84.19%). The difference in percentage of the newly formed bone in control group and the other two groups was statistically significant (p=0.029, 0.026) respectively and there was no statistically significant difference between β-TCP group and Collatape group (p=0.19).

**DISCUSSION**

This study was performed in order to evaluate the effect of bio-absorbable collagen wound dressing Collatape on healing of extraction sockets in rabbits alone and combined with IngeniOs Beta tricalcium phosphate synthetic bone particles.

The first mandibular premolars were extracted to study socket healing. Mandibular incisor extraction was not preferred in order not to hinder the animal ability to eat and due to their continuous eruption that might disrupt normal healing. This is supported by Kuboki et al (10) who studied the alteration in collagen crosslinking after tooth extraction in rabbits and Mansol et al (11) who studied different biomaterials in the socket healing in rabbits.

The healing of extraction socket was evaluated at two different intervals 2 and 6 respectively. The choice of such intervals could be explained as the remodeling cycle for rabbit compact bone which is about 6 weeks (12).

In the present study, the results of first observation period (2 weeks after post operatively) revealed the filling of socket with newly formed trabeculae with very few amount of granulation tissue in the control group. These findings are supported by Kuboki et al (10).

The transition of fibrous tissue into bone tissue occurs as osteoprogenitor cells migrate through blood vessels. They differentiate into osteoblasts that lay down newly formed bone (13).

The healing socket of Collatape group showed two striking observations which were the presence of high density of cells within collagen wound dressing and the continuity of bone formation through areas of primary osteons together with adjacent areas of new trabecular bone formation. These findings were consistent with Almazzrooa et al (14) who studied the tissue response of six different collagen membranes.

Osteoconductivity of Collatape collagen wound dressing could be explained on the basis that collagen presents a rough surface geometry for osteoblast attachment. It is hydrophilic and thus favors cell adhesion. Also Fibronectin present on the surface of osteoblasts link specifically with definite regions of the collagen molecules (15). This is also supported by Donzelli et al (16) who confirmed that collagen scaffold supports mesenchymal cells distribution and differentiation.

The presence of primary osteons within formed bone mass which was noted in the second observation period indicates the active remodeling, high density and organization of the formed bone mass in this group. The rich blood supply of the healing socket in this group is considered the main promoter for active bone remodelling (17). This is explained by Twardowski (18) et al who found that type I collagen potently stimulates angiogenesis in vitro and in vivo.

For β-TCP group, the main findings in this interval were the presence of β-TCP bone graft particles incorporated in the newly formed bone mass, the newly formed bone trabeculae were lined by voluminous osteoblasts with few osteoclasts and profound blood supply within bone marrow spaces of the formed bone mass as well as around β-TCP bone graft particles. These findings are in accordance with Berberi et al (19) who evaluated a number of physical and chemical properties in a variety of granulated mineral-based biomaterials used in dentistry.

The bone graft material used in this work was silicated β-TCP bone graft. Adding silica to β-TCP has proved to enhance its osteoconductive properties. Fei et al (20) who studied the osteogenic differentiation on different bio-ceramic materials. The results of their study demonstrated that adding silica to β-TCP enhanced osteoblastic differentiation and bone mineralization. Silica induced osteoblastic differentiation through induction of osteoblasts to secrete bone morphogenetic protein 2 (BMP2) that stimulates osteogenic differentiation by autocrine and paracrine action. BMP2 also regulates the expression of osteogenic marker genes such as alkaline phosphatase and osteocalcin which are responsible for bone mineralization (20).

Increasing the level of porosity of β-TCP bone graft allows the blood vessels to reach the healing extraction socket and provides an environment for collagen formation that leads to deposition of apatite crystals within its micropores (21). The bone graft used in this study is IngeniOs β-TCP which has porosity up to 75% and of pore size 250-1000 μm. Such large pore size explains the rich blood supply of the healing socket and the rapid rate of bone deposition which were seen in this study. This is in accordance to Galois et al 2004 (22) who studied Bone ingrowth into two porous ceramics.

Osteoclasts are essential for cell mediated resorption of β-TCP in order to be replaced with new bone, however the rapid resorption of β-TCP disrupts the healing process (21). In this study, few osteoclasts were observed in these intervals. This could be explained on the fact that the bone graft used in this study was silicated β-TCP. Adding Silica to β-TCP was found to enhance strength of β-TCP as well as its biologic response thus silicated β-TCP characterized by its lower rate of resorption to be compatible with new bone formation (8, 23).

The results obtained from the second observation period (6 weeks) showed that the coronal part of the socket entrance of control group was occupied by cortical bone...
while the rest of the socket was filled with trabecular bone surrounded by marrow spaces. This is in agreement with the findings of Kuboki et al and Cardaropoli et al (10, 13). Corticalization is an important part of socket healing in which hard tissue cap is formed in order to seal the marginal portion of the socket. The hard tissue cap is composed mainly of compact bone (24).

The presence of wide marrow spaces surrounding trabecular bone apical to hard tissue cap could be explained as there are no stresses from forces elicited during mastication so there is no demand on mineralized bone in the areas previously occupied by the tooth (24). Thus, the trabecular bone apical to hard tissue cap remodels into marrow spaces. This explains the wide marrow spaces surrounding trabecular bone.

The organization of fibrous tissue in form of bone trabeculae before mineralization was observed. This indicates the continuity of bone formation in this group as the collagen fibers take the form of bone trabeculae before the mineralization step (17).

Concerning Collatape group, the marginal portion was occupied by larger area of compact bone of rich blood supply than in control group. As a consequence Collatape could enhance bone formation and improve the quality of the formed bone. This is in agreement with Kim (25) et al who observed that formed mature bone was found eight weeks after implantation of collagen wound dressing in comparison with untreated animals.

The presence of wide marrow space surrounding trabecular bone apical to hard tissue cap could be due to the rapid degeneration of Collatape collagen wound dressing (4-5 weeks) so there was no longer stimulation of new bone formation. This is supported by Donzelli et al (16) who confirmed that the rapid degeneration of collagen membranes might be a limitation for their use in bone regeneration.

In β-TCP group, the coronal portion of the socket was filled with compact bone of higher density and vascularity than in control group and Collatape group. The trabecular bone formed apical to the marginal cap was of higher thickness than in the previous two groups and were surrounded by small marrow spaces. These observations are in agreement with Brkovic et al and Jensen et al (26, 27). On the other hand Araujo et al (28) showed that use of the β-TCP graft might in fact have retarded bone formation in the animal model used.

Such observations confirmed that Ingenios β-TCP bone graft could effectively enhance bone formation and reduce the amount of bone resorption associated with bone remodeling.

CONCLUSIONS
1- Application of Collatape alone to the healing socket improved the early phases of bone healing, however, it did not provide long term ridge preservation.
2- Combination of Collatape and Ingenios β-TCP could be used for socket preservation. Ingenios β-TCP has osteoconductive properties and its resorption is compatible with new bone formation. Increasing porosity of the β-TCP graft material enhances blood supply of the healing socket and accelerates the rate of bone deposition.

STATEMENT OF CONFLICT OF INTEREST
The authors declare that they have no conflicts of interest.

REFERENCES


EVALUATION OF THE EFFECT OF CHOLECALCIFEROL ON TITANIUM IMPLANT OSEointegration (AN EXPERIMENTAL STUDY)

Gaber M¹, Saleh M², Fahmy M², Elba G³

ABSTRACT

Introduction: Cholecalciferol (Vitamin D3) is essential for bone mineralization and for the subsequent maintenance of bone quality. Supplementation with Cholecalciferol (vitamin D3) is reported to show positive effects on bone mineral density.

Objectives: Evaluation of the effect of Cholecalciferol on dental implant osseointegration.

Materials and Methods: The study was conducted on fourteen adult male mongrel dogs. Dogs were divided equally into two groups, control group and study group, seven dogs in each group. Extraction of mandibular right premolar and insertion of immediate titanium implant had been done for all dogs in both groups, and then only the dogs of the study group received Cholecalciferol for four weeks. Histological and radiographical evaluations were carried out after twelve weeks postoperatively for both groups.

Results: Histological results revealed improved bone healing in the study group, as shown by marked osteoblastic activity and accelerated woven bone formation, and absence of chronic inflammatory cells. The highest rate of bone ingrowth occurred in the study group. Radiographical evaluation revealed that the peri-implant bone density had increased significantly in the study group.

Conclusion: These results indicated that Cholecalciferol vitamin D3 has systemic effects on accelerating bone formation around titanium implant.

Keywords: Cholecalciferol, Vitamin D3, Dental implants, Osseointegration.

INTRODUCTION

Over the last two decades, research has validated the success of osseointegrated implants as a viable replacement for partial and complete edentulism. Although techniques and materials have been developed which are capable of high degree of clinical success, the ultimate long-term success of implants is dependent upon the effort of both the patient and dentist in maintaining the health of peri-implant tissue (1). Recently, the clinical utilization of dental implants has accelerated and it is now a well recognized form of tooth placement, and a safe method for oral rehabilitation with high success rates (2).

Various factors may enhance or inhibit implant osseointegration. Factors enhancing osseointegration include implant-related factors such as implant design and chemical composition, topography of the implant surface, material, shape, length, diameter, implant surface treatment and coatings (3), the status of the host bone bed and its intrinsic healing potential (4), the mechanical stability and loading conditions applied on the implant (5), the use of adjuvant treatments such as bone grafting, osteogenic biological coatings and biophysical stimulation (6-8) and pharmacological agents such as simvastatin and bisphosphonates (9, 10). Supplementation with Cholecalciferol (vitamin D3) is reported to show positive effects on bone mineral density (11-15).

The term “vitamin D” actually refers to a group of fat-soluble vitamins. There are five different forms of vitamin D, but the two major forms are vitamin D2 (Ergocalciferol) and vitamin D3 (Cholecalciferol). Vitamin D2 is produced by plants, while vitamin D3 is produced by the skin of animals in response to sunlight (Ultra Violet light) exposure. UV light reacts with an enzyme called 7-dehydrocholesterol to create pre-vitamin D, which rearranges its structure to form vitamin D3, then converts vitamin D3 into a compound called calcitriol, which is the active form of vitamin D that is responsible for the numerous health benefits. After its conversion from Vitamin D3, calcitriol exerts its effects on the body by binding to and activating vitamin D receptors (VDRs), which are located in the nuclei of target cells (16).

The major physiological role of vitamin D is to facilitate the intestinal absorption of calcium, by stimulating the expression of proteins involved in calcium transport. Vitamin D also plays a crucial role in providing the proper balance of minerals necessary for bone growth and function. However, it turns out that VDRs are present in the cells of most organs in the body, suggesting that there is wide diversity in the types of responses that vitamin D3 can promote (17-19).

Vitamin D3 is recognized as a regulator of both osteoblast mediated bone formation and osteoclast mediated bone resorption (20-22). Besides these, numerous other disease associations have been reported with VD3 deficiency, including cardiovascular disease, common obesity, and diabetes mellitus (23, 24). Therefore, the purpose of this study was to evaluate histologically and radiographically the role of Cholecalciferol on osseointegration on dental implants.

MATERIALS AND METHODS

This study was conducted on fourteen male mongrel dogs, about 18-24 months old, and average weight 10 to 12 kgs.
The animals were kept under the same nutritional and environmental conditions in the Animal house, Physiology Department, Faculty of Medicine, Alexandria University. The total number of dogs was divided equally into two groups each group consisted of seven dogs:

- **Control Group**: consisted of seven dogs which had extraction of mandibular right premolar and insertion of immediate titanium implant.
- **Study Group**: consisted of seven dogs which had extraction of mandibular right premolar, insertion of immediate titanium implant and received a daily dose of Cholecalciferol immediately after the operation.

Devarol-S™ (Memphis Pharmaceutical & Chemical Industry, Cairo, Egypt) is a form of vitamin D, also called vitamin D3. Devarol-S™ was provided in a form of 2 ml glass ampoule. Each ampoule contains Cholecalciferol (Vit.D3) 5mg (equivalent to 200000 I.U.) in a sterile clear solution (Fig. 1).

![Devarol-S](image)

**Fig 1**: A photograph showing Devarol-S.

Dentis™ (Dentis Co., Ltd. 951, Woram-Dong, Dalseo-Gu Daegu, South Korea) titanium implant is the implant of choice for this experimental study. This implant is designed with the following characteristics: Resorbable blast media (RBM) surface treatment whereby micro-particles of Hydroxyapatite-derived Beta-Tricalcium Phosphate are impregnated into the titanium surface through high pressure blasting techniques in order to obtain micro-surface roughness, safe cutting edge for reduction of bone stress, allowing smoother insertion, dome end to decrease perforation possibility, tapered body with optimized thread designs for easy initial fixation at the time of placement surgery and implant sizes range from 3.00-6.00 mm in diameter and from 8-14 mm in length.

Before surgery, all dogs were healthy as documented by a veterinarian report. The dogs were kept under the same nutritional and environmental conditions and were kept on the same balanced diet consisting of milk, broth and meat throughout the whole period of the study. Each animal received a dose of antibiotics in the form of ampicillin (25 mg/kg body weight; Epicocillin, provided by: Eipico Pharmaceutical Co., 10th of Ramadan city, Cairo Egypt) just before the operation by intramuscular injection. All operating procedures were performed under general anesthesia and sterile conditions in an animal theatre. Each animal was generally anaesthetized via intravenous injection of Thiopentone sodium (30 mg/kg body weight; Barbiturate provided by Glazer export Co. Dinshwa Waccha India).

After dogs were generally anesthetized, a blood sample about 10 ml was collected preoperatively from each dog in a sterile tube to measure serum calcium level. Atraumatic extraction of the mandibular right premolar was performed using forceps without any damage to adjacent soft or hard tissues and the extraction sockets were irrigated with normal saline. Drilling was performed as recommended by manufacturer and extended 3 mm beyond the root apex. Implants diameter and length ranged from 3.7 mm to 4.1 mm and from 10 mm to 12 mm respectively.

After the final drill, the implant was carried with its mount to the socket, and then pressed a little bit with threading movement clockwise until resistance was encountered. This was followed by removal of the implant cap and final seating of the implant by ratchet wrench till the implant shoulder is at the level of the alveolar bone crest and then screwing of the cover screw was performed followed by suturing of the wound. Study group received a daily dose of Cholecalciferol 12µg/kg/day by intramuscular injection immediately postoperatively (Fig. 2).

![Implant positioning](image)

**Fig 2**: A Clinical photograph, illustrating the positioning of implant in the premolar area.

After the surgical procedure, each dog received the same course of antibiotics of ampicillin 25 mg/kg body weight for five days every eight hours. Ketolac (1 mg/kg body weight; Ketorolac Tromethamine by ELAMRIA Company) subcutaneous injection every twenty four hours was given as analgesic and anti-inflammatory drug to the animals for three days post-operatively. The animals of each group were isolated in separate cages to be kept under observation to assess the presence or absence of any post-operative complications as infection, wound dehiscence or implant rejection.

Then the animals were maintained on soft diet consisting of bread, milk and broth post-operatively for one...
week, after that shifted to normal balanced diet again. Study group dogs received a day after day dose of Cholecalciferol 12 µg/kg/day by intramuscular injection for a period of 28 days. Blood sample about 10 ml was collected from each dog in a sterile tube to measure serum calcium level before sacrifice on the 12th week. The dogs were sacrificed by an over dose of Thiopentone sodium after twelve weeks.

Immediately after sacrifice, the implant-bearing areas were retrieved, labeled and immediately immersed in 10% neutral formalin to be radiographed with periapical x-ray films to evaluate bone density using the Image J software. (Fig.3 and Fig.4).

Fig 3: A periapical x-ray film of control group.

Fig 4: A periapical x-ray film of study group.

After the preparation of the specimens, tissue sections were cut at four microns thickness microscope then sections were stained by Hematoxylin and eosin stain (H&E) and Trichrome stain. Each section was examined under light microscope.

For statistical analysis, data were fed to the computer and analyzed using IBM SPSS software package version 20.0. Quantitative data were described using range (minimum and maximum), mean, standard deviation and median. The distributions of quantitative variables were tested for normality using Kolmogorov-Smirnov test, Shapiro-Wilk test and D’Agostino test. It reveals normal data distribution, and parametric tests were applied. For normally distributed data, comparison between two independent populations were done using independent t-test, also paired t-test is used to analyze two paired data. Significance of the obtained results was judged at the 5% level.

RESULTS

Clinical Results: All animals survived very well the experimental protocol of the present study and remained active and alert all over the course of experiment. The animals did not exhibit any clinical signs of infection, tissue dehiscence, discoloration or other tissue reactions surrounding the implants and this period went without complications in both groups.

Histological Results: After removal of the implant materials, the shape of the implant screw threads was distinctly visible on the walls of the bone block and pointed out the area of the implant osseointegrated interface. Bone growth and remodeling between the implant threads was already visible on the sample. After 3 months of healing, a complete bone volume was built between the implant threads and was observed as an imprint of the screw pattern of the implant.

In the control group a well marked implant threads in a direct contact with mature compact bone inside the concavities and convexities on both sides were found. Intact bone revealed that the bone in direct contact with the surface of the screw threads was without soft tissue intervening. (Fig.5)

Fig 5: Photomicrograph of control group, showing organization and density of newly formed bone trabeculae with their ostocytes developing from the host bone(HB)and run towards the implant threads (arrows).(H&E X40)
In the study group microscopic examination revealed an intense invasion of numerous groups of well formed woven trabecular bone on both sides of the surface of the implant threads. A network of small marrow spaces containing fibrous tissue were scattered between trabecular woven bone. Profound dilated blood vessels were widely distributed among the newly formed trabeculae. An intense newly formed trabecular bone projects from the host. Projections were rich with blood supply. It is an evident feature of angiogenesis. The newly formed bone in the study group varies in size. It is well noted that the organized woven bone trabeculae were osseointegrated with host and directed towards the surface of the implant threads. The osteoid tissue is well detected with many osteocytes. Functioning osteoblasts bordered the new woven bone trabeculae. The early deposition of new calcified matrix on the implant surface is followed by arrangement of the woven bone trabeculae. (Fig.6)

Laboratory Results: There was no significant difference between the serum calcium levels in the control group and study group (Table 1).

Radiographic Results: In comparison between the two groups, there was significant increasing difference in bone density in the study group (Table 2).

DISCUSSION

In the current work the overall histological observation in control group illustrated masses of newly formed bone with different degrees of maturity. The newly formed compact bone with Haversian system with volkman’s canal and osteocytes with interstitial lamellae exhibited osseointegration in the bone of the socket. Implant osseointegration is the final goal of implant surgery and the prerequisite to achieve long-term success of endosseous dental implants.

Davis (25), Gailit (26) and Franchi M et al (27) reported that Peri-implant osteogenesis can be in distance and in contact from the host bone. Distance osteogenesis refers to the newly formed peri-implant bone trabeculae that develop from the host bone cavity towards the implant surface. In contrast, contact osteogenesis refers to the newly formed peri-implant bone that develops from the implant to the healing bone. The newly formed network of bone trabeculae ensures the biological fixation of the implant and surrounds marrow spaces containing many mesenchymal cells and wide blood vessels. A thin layer of calcified and osteoid tissue is deposited by osteoblasts directly on the implant surface. Blood vessels and mesenchymal cells fill the spaces where no calcified tissue is present.

Murai et al (28) were the first to report a 20-50 mm thin layer of flat osteoblast-like cells, calcified collagen fibrils and a slight mineralized area at a titanium implant-bone interface. The newly formed bone was laid down on the

<table>
<thead>
<tr>
<th>Study</th>
<th>Serum calcium</th>
<th>Preoperative</th>
<th>After 3 months</th>
<th>% of change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 7)</td>
<td>Min. – Max.</td>
<td>10.90 – 11.20</td>
<td>10.90 – 11.20</td>
<td>-1.80 – 1.83</td>
</tr>
<tr>
<td>Mean ± SD.</td>
<td>11.05 ± 0.10</td>
<td>11.06 ± 0.10</td>
<td>0.27 ± 1.36</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>11.0</td>
<td>11.10</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>p1</td>
<td></td>
<td></td>
<td>0.631</td>
<td></td>
</tr>
</tbody>
</table>

Table (1): Comparison between the two studied groups according to serum calcium

<table>
<thead>
<tr>
<th>Bone density</th>
<th>Control (n = 7)</th>
<th>Study (n = 7)</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD.</td>
<td>Min. – Max.</td>
<td>10.90 – 11.20</td>
<td>79.05 – 99.30</td>
<td>4.536*</td>
</tr>
<tr>
<td>Median</td>
<td>11.04 ± 0.10</td>
<td>11.04 ± 0.08</td>
<td>0.01 ± 1.17</td>
<td></td>
</tr>
<tr>
<td>t</td>
<td>1.79</td>
<td>1.80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p1</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table (2): Comparison between the two groups according to difference in bone density

* Statistically significant at p ≤ 0.05
reabsorbed surface of the old bone after osteoclastic activity. This suggested that the implant surface is positively recognizable from the osteogenic cells as a biomimetic scaffold which may favor early peri-implant osteogenesis. Osteoblasts cannot always migrate so rapidly to avoid being completely enveloped by the mineralizing front of calcifying matrix; these osteoblasts became clustered as osteocytes in bone lacunae.

The early deposition of new calcified matrix on the implant surface is followed by the arrangement of the woven bone and bone trabeculae. This is appropriate for the peri-implant bone healing process as it shows a very active wide surface area, contiguous with marrow spaces rich in vascular and mesenchymal cells. Marrow tissue containing a rich vasculature supports mononuclear precursors of osteoclasts so bone trabeculae remodel faster than cortical bone (27).

Initially, rapid woven bone formation occurs on implants to restore continuity, even though its mechanical competence is lower compared to lamellar bone based on the random orientation of its collagen fibers. Woven and trabecular bone fill the initial gap at the implant-bone interface. Arranged in a three-dimensional regular network, it offers a high resistance to early implant loading. Its physical architecture including arches and bridges offers a biological scaffold for cell attachment and bone deposition that is biological fixation. The early peri-implant trabecular bone formation ensures tissue anchorage that corresponds to biological fixation of the implant. This begins at 10 to 14 days after surgery (27, 29).

Vitamin D was originally described as a steroid hormone controlling calcium and phosphorus metabolism. Consequently, vitamin D deficiency is a pathogenic factor for osteoporosis and the occurrence of fractures (30). However, preclinical studies suggest that vitamin D deficiency also negatively affects bone regeneration, including fracture healing (31) and the osseointegration of implants (32). While vitamin D supplementation is mandatory in pharmacologic osteoporosis therapy, few studies are available that would justify this treatment to support bone regeneration, and thus osseointegration (33).

Our histological findings in the study group showed intense and continuous new bone formation within all the threads of the implants. A well formed new woven bone with numerous osteoblasts and osteocytes were in close vicinity to implants threads.

A large number of osteoblast cells bordering the newly bone trabeculae as well as osteocytes within woven bone. These findings are consistent with Franchi et al. (27) and Probst (29). They revealed that osseointegrated implants is confirmed by the presence of medullary or marrow spaces containing osteoclasts, osteoblasts, mesenchymal cells and lymphatic/blood vessels next to the implant surface. During the remodeling of the peri-implant bone, new osteons circle around the implant with their long axis parallel to the implant surface and perpendicular to the long axis of the implants. Osteoid tissue is produced by osteoblasts suggesting that osteogenesis is underway. The remodeled bone can extend up to 1 mm from the implant surface.

In the present study, after three months, all the screws appeared osseointegrated, being almost completely covered by a compact, mature, newly formed bone. The peri-implant tissues showed areas of fibrous tissue alternating with direct bone contact. This was supported by Abrahamsson et al (34) and Vercaigne et al (35).

The histological observation in this study described peri-implant connective tissue corresponds to a marrow tissue in marrow spaces. The presence of a highly vascularised tissue rich in undifferentiated cells is helpful for the biological turnover of the peri-implant bone. Marrow spaces have always been observed around and next to the different implant surfaces; they were wide and filled by dense connective tissue or calcified tissue in 3-month-old samples. Their presence next to the implant surface during early peri-implant healing ensures a biological support for the turnover of mineralized tissues. During implant healing bone matrix mineralizes and envelops the osteoblasts, which produce the osteoid tissue and, if new bone is required, new osteogenic cells must migrate to that surface, and this was advocated by Davies in 2003 (36).

Interestingly microscopic examination in the study group of the current work revealed continuous newly formed bone is in direct contact with implant. It was originated from the compact bone of the socket bone. Complete osseointegration between bed bone and newly formed woven bone was originated from the bed bone with many marrow spaces within bony trabeculae. Vitamin D activates osteoblasts and increases the production of extracellular matrix proteins by osteoblasts. Vascularization is of critical importance for the process of osseointegration. Differentiation of osteogenic cells strictly depends on tissue vascularity.

Our findings were also in agreement with Franchi et al (27) who concluded that peri-implant bone contains regular osteons and host bone chips enveloped in mature bone. The bone-implant interface shows inter-trabecular marrow spaces delimited by titanium surface from one side and by newly formed bone from the other one rich in cells and blood vessels.

CONCLUSIONS
Within this context, it can be concluded that Cholecalciferol vitamin D3 has systemic effects on accelerating bone formation around titanium implant and can favor the biological turnover of the peri-implant bone.

CONFLICT OF INTEREST
The authors declare that they have no conflicts of interest.

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32. Kelly J, Lin A, Wang CJ, Park S, Nishimura I. Vitamin D and bone physiology: demonstration of vitamin D
EVALUATION OF THE EFFECT OF THE HIGH INSERTION TORQUE ON THE IMMEDIATELY LOADED DENTAL IMPLANTS

(CLINICAL & RADIOGRAPHIC STUDY)

Gawish A^1 BDS, Osman S^2 PhD, Shokry M^3 PhD

ABSTRACT

Introduction: The ideal insertion torque values for placing dental implants to be immediately loaded is unknown, therefore it may be useful to evaluate the effect of insertion torque values on immediately loaded dental implants.

Objectives: was to evaluate clinically and radiographically the placement of immediately loaded single implants inserted with high insertion torque (> 70 N.cm).

Materials and Methods: This study was conducted on twelve patients divided in to two groups (study group and control group) with missed mandibular posterior teeth, Zimmer Swissplus Implants were inserted using Aseptico motor implant system, insertion torque was measured digitally. In the control group implants were inserted with medium torque (25-35 N.cm) while in the study group implants were inserted with high torque (>70 N.cm).

Results: There was a statistically significant increase in bone density around the implants in the study group at 6 months postoperative compared with the control group.

Conclusions: the use of high insertion torque (up to 70 N.cm) neither prevented osseointegration nor increased marginal bone resorption around tapered multithreaded dental implants placed in posterior mandible. Also there is statistically significant correlation between insertion torque and bone density around implants placed in posterior mandible.

Key Words: Dental implants, Insertion torque, Immediate loading.

INTRODUCTION

Implant stability depends on the direct mechanical connection between implant surface and the surrounding bone and can be divided into primary and secondary stability (1).

The goal of achieving primary stability at the time of implant placement is to limit excessive micromotion at the bone-implant interface, which could fracture regenerating bone and prevent osseointegration (2-4).

The use of a slightly narrower final drill with a tapered implant design has been often associated with elevated insertion torque (IT) (5-6) and localized bone compression (7). Both of these factors may help to increase primary implant stability.

Factors such as bone density, length, width, type of implant and surgical technique may interfere with primary implant stability (8).

The clinical measurement that assesses primary implant stability is IT. High IT indicates that the implant is well fixed and mechanically stable within the bone tissue (9).

Implant stability can be measured by non-invasive clinical test methods, one of these quantitative methods is the insertion torque described by Johansson and Strid (10).

This method records the torque required to place the implant and provides valuable information about the local bone quality.

Measurement of the cutting resistance of the jaw bone (insertion torque) (measured in Newton-centimeters), where 1 Ncm is the torque generated by a force of 1 N acting on a lever of 1 cm in length is performed intraoperatively (11, 12).

IT corresponds to a combination of the cutting friction of the tip of the implant in the bone, and the friction between the implant surface and the hole in the bone. If the hole is narrow or the bone quality is high the torque will be higher. The torque will also depend on how sharp the cutting tip of the implant is, on the surface properties of the implant, on the lubrication of the preparation (blood) and also on the design of the implant itself. For instance if the preparation is cylindrical and the implant is tapered, the insertion torque will be higher. May be most important is the diameter of the implant, a narrow implant will have lower insertion torque than a wide implant in the same bone (13).

The aim of this study was to evaluate the outcome of immediately loaded single implants inserted with high insertion torques (> 70 N.cm).
MATERIALS AND METHODS
A total of 15 implants were placed in the mandibular posterior region. Each patient was informed about the study’s aims and gave informed consent.

Inclusion criteria
Age ranged between 25 and 40 years, good oral hygiene, mandibular posterior missing teeth, suitable inter-occlusal distance at the edentulous area to allow implant placement and its fixed prosthesis, adequate bone quantity and quality, acceptance of treatment plan and signature of informed consent.

Exclusion criteria
Radiotherapy at the head/neck region within the last 12 months, uncontrolled diabetes, pregnancy, poor oral hygiene and/or motivation, drug or alcohol abuse, active inflammation/infection in the sites of implant insertion, smoking >10 cigarettes/day and severe bruxism or clenching.

Patients were divided to two groups each group containing 6 patients (control group and study group). Zimmer SwissPlus implant system was used in this study (Zimmer implant system company1800 West Center Street Warsaw, USA). A preoperative orthopantomogram and cone beam computerized tomography (CBCT) (Kodak CS 9300 CBCT machine) with software (OnDemand 3d app) (Fig.1) were done for patients of the two groups.

Surgical technique
Inferior alveolar nerve block and long buccal nerve block local anesthesia were given, the surgical field was then cleaned with a tincture iodine swab. The flap design used was three incision-lines flap (Pyramidal flap), to adequately expose the surgical field which was done using Bard-Parker scalpel number 15, then full thickness mucoperiosteal flap was reflected, the osteotomy was prepared by drilling using pilot drill followed by the successive drills till reaching the final drill which corresponds to 0.5 mm less than the diameter of the selected implant. Drilling was made under copious external irrigation by normal saline as cooling system, depth of the drilling was monitored using depth gauge, and parallelism was checked using paralleling pins.

After drilling and debridement, the implant was held by its cover and inserted into the osteotomy site and screwed using Aseptico implant motor system (Aseptico dental equipment 8333 216th Street SE Woodinville, USA) (Fig.2), the insertion torque was adjusted digitally according to resistance of bone which corresponds to bone quality, the inserted implants were then grouped according to insertion torque.

Control group: Implants were inserted with medium insertion torque (25-35 N.cm) using Aseptico implant motor system. Study group: Implants were inserted with high torque (> 70 N.cm) using the same motor.

Immediate loading with ready-made acrylic crowns (Provyl, Dentsply®, USA) which were prepared to be 2 mm free from occlusion and then placed using temporary cement. After 3 months temporary crowns were removed and final porcelain crowns were inserted.

Clinical follow up: All the patients in the two groups group1 (control group) and group 2 (study group) were examined clinically for probing depth, papillary bleeding and implant mobility after implant placement immediately and at 3 and 6 months intervals. Mobility
was tested using back and forth pressure of approximately 500 gm by two instrument handles.

Radiographic follow up: All the patients in the two groups were examined radiographically immediately postoperative by standardized periapical x-ray films using XCP film holder to serve as a baseline for measurement (Fig.3), and after 3 and 6 months using periapical x-ray & CBCT (Fig.4). Bone height was measured using CBCT software, while bone density was measured (in pixels) using Image J software.

RESULTS
This study was done on 15 implants placed in 12 patients, in all the studied patients the age ranged from 25-40 years, with a mean of 27 years, the sizes of the implants used are shown in the table of sample distribution (Table 1).

After implant placement cases were classified into 2 groups (n= implants). Group 1 (control) (n=7): Implants were inserted with medium torque (25-35 N.cm). Group 2 (study) (n=8): Implants were inserted with high torque (>70 Ncm)

As regards gender, there were 8 males and 4 females in all the studied patients with equal distribution in both groups, 4 males and 2 females in the study group and the same in the control group.

Table 1: Showing sample distribution in the current study.

<table>
<thead>
<tr>
<th>Group I (Control group)</th>
<th>Case 1</th>
<th>32</th>
<th>male</th>
<th>6</th>
<th>4.1mm × 12 mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 2</td>
<td>28</td>
<td>male</td>
<td>5</td>
<td>4.1mm × 10mm</td>
<td></td>
</tr>
<tr>
<td>Case 3</td>
<td>37</td>
<td>male</td>
<td>6</td>
<td>4.1mm × 12 mm</td>
<td></td>
</tr>
<tr>
<td>Case 4</td>
<td>26</td>
<td>female</td>
<td>6</td>
<td>4.1mm × 12 mm</td>
<td></td>
</tr>
<tr>
<td>Case 5</td>
<td>35</td>
<td>male</td>
<td>6</td>
<td>4.1mm × 12 mm</td>
<td></td>
</tr>
<tr>
<td>Case 6</td>
<td>27</td>
<td>female</td>
<td>6</td>
<td>3.8mm × 12 mm</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group II (Study group)</th>
<th>Case 1</th>
<th>26</th>
<th>female</th>
<th>6</th>
<th>3.8mm × 10mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 2</td>
<td>33</td>
<td>male</td>
<td>7</td>
<td>4.1mm × 12 mm</td>
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</tr>
<tr>
<td>Case 3</td>
<td>28</td>
<td>male</td>
<td>6</td>
<td>4.1mm × 12 mm</td>
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<tr>
<td>Case 4</td>
<td>35</td>
<td>male</td>
<td>15</td>
<td>4.1mm × 10mm</td>
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<td>Case 5</td>
<td>37</td>
<td>male</td>
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<tr>
<td>Case 6</td>
<td>34</td>
<td>female</td>
<td>7</td>
<td>4.1mm × 10mm</td>
<td></td>
</tr>
</tbody>
</table>

Clinical Follow Up:
Probing pocket depth: Mean probing depth values and standard deviation for both groups were measured immediate post-operative, at 3 months and 6 months. The difference in probing depth values was found to be statistically insignificant (Z= 1.206, p-value= 0.254).

Papillary bleeding index (PBI): The intensity of any bleeding provoked was recorded on a 0-4 scale (Muhlemann 1981) (14). The difference in (PBI) readings between the two groups was found to be statistically insignificant (LR= 2.773, p-value = 0.428).

Mobility: According to the clinical implant mobility scale (0-4 scale) Mckinney and Koth (15) none of the implants of both groups showed any signs of mobility throughout the evaluation period except one implant in group 2 which showed mobility after two weeks and was removed and excluded from statistical analysis. i.e. mobility score was 0.

Radiographic results
Evaluation of marginal Bone level around the implant: Mean marginal bone level values and standard deviation at 3 months and 6 months of both groups are shown in (Table2, Fig.5)

![Fig.3: Immediate postoperative periapical x-ray.](image1)

![Fig.4: Postoperative CBCT after 3 months.](image2)

![Fig.5: Comparison between the study group and the control group according to the marginal bone height.](image3)
Immediate post-operative

The mean bone density value for control group was $82.23 \pm 27.36$, while the mean bone density value for study group was $85.23 \pm 39.03$. This difference in bone density values was found to be statistically insignificant ($Z=0.128$, p-value = 0.548).

At 3 months, the mean bone density value for control group was $87.65 \pm 49.51$, while the mean bone density value for study group was $90.22 \pm 35.20$. This difference in bone density values was found to be statistically insignificant ($Z=0.256$, p-value = 0.612).

At 6 months, the mean bone density value for control group was $98.84 \pm 41.6$, while the mean bone density value for study group II was $112.8 \pm 39.53$. This difference in bone density values was found to be statistically significant ($Z=0.575$, p-value = 0.008).

**DISCUSSION**

The successful outcome of any implant procedure depends on a series of patient-related and procedure-dependent parameters, including general health conditions, biocompatibility of the implant material, the microscopic and macroscopic nature of the implant surface, the surgical procedure and the quality and quantity of the local bone (16).

Several other researchers have also reported that they sometimes had to use a manual wrench to finalize implant placement, and therefore had no mean to register the true peak insertion torque (4, 17).

In the present study implants were placed using Aseptico motor implant system which is rated up to 80 N.cm for implant applications and works with any conventional or mini implant system. The insertion torque can be adjusted and measured digitally.

In the present study, no negative effects of high insertion torque on marginal bone loss could be detected. It is possible that other more heterogeneous implant designs with marked steps and edges along the implant surface may result in bone resorption when using high insertion torque because of stress concentration.

Regarding the implant mobility, no detectable clinical mobility of anyone of the used implants was detected throughout the evaluation period. This was confirmed by radiographic evaluation that revealed the absence of peri-implant radiolucency. This indicates proper osseointegration of all implants. The absence of implant mobility is considered to be the most important criteria for implant success in accordance with Porter and Von Fraunhofer in 2005 (18).

From our clinical evaluation throughout the follow up period which was extended up to six months, only one patient from group II complained from pain, tenderness and swelling after two weeks and the implant failed and excluded from the study, this was attributed to bad oral hygiene and patient negligence.

As regarding bone density around the implant it was measured by Image J software (Image J, U. S. National Institutes of Health, Bethesda, Maryland, USA) on serial

<table>
<thead>
<tr>
<th>Marginal bone height (mm)</th>
<th>Control group (n=7)</th>
<th>Study group (n=7)</th>
<th>Test of significance (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>After 3 months</strong></td>
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<tr>
<td>Min-Max</td>
<td>1.50-2.60</td>
<td>2.00-3.00</td>
<td>Z=1.431 (0.19)</td>
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<tr>
<td><strong>After 6 months</strong></td>
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<td></td>
</tr>
<tr>
<td>Min-Max</td>
<td>1.40-2.30</td>
<td>2.00-2.80</td>
<td>Z=2.014 (0.53)</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>1.98±0.30</td>
<td>2.32±0.28</td>
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</tr>
</tbody>
</table>

**Table 2:** Comparison between the study group and the control group according to the marginal bone height.

**Fig. 6:** Comparison between the study group and the control group according to the Bone density.

**Table 3:** Comparison between the study group and the control group according to the Bone density.

**Table 3:** Comparison between the study group and the control group according to the Bone density.

**Fig. 6:** Comparison between the study group and the control group according to the Bone density.

**Table 2:** Comparison between the study group and the control group according to the marginal bone height.

**Z= Mann Whitney test   * Significant at p ≤ 0.05**
periapical standardized radiographs made by long cone paralleling technique using XCP film holder. These standardized radiographs were taken immediately post-surgery and at 3 and 6 months follow up periods to detect changes in bone density around the implants. Both groups showed an increase in bone density from immediately after implant placement to three months and continued to increase at six months. Group II showed higher bone density values than group I immediately after implant placement and throughout the whole follow-up period, this difference was found statistically significant.

The increase in peri-implant bone density may be attributed to the immediate loading of implants of both groups. The effect of immediate loading on peri-implant bone density was demonstrated in a clinical study by Barone (19) in 2003 which compared immediately loaded implants in partially edentulous patients, the radiological assessments showed that the mean bone density was higher in the immediately loaded group than in the unloaded group, the differences between the two groups of oral implants were statistically significant. Thus it was concluded that immediate loading seems to increase the ossification of the alveolar bone around endosseous implants.

A recent study by Dorjpalam and Hee (20) in 2013 showed that the IT and initial stability increased according to the increase in the bone density, resulting in a strong positive correlation. In other words, the initial stability was shown to be highly dependent on the bone density. The IT also increased according to the thickness of the cortical bone, and a slight increase was observed for initial stability. This shows that the volume of high dense cortical bone affects the initial stability.

CONCLUSIONS
Within the limitations of this study, the following conclusions can be listed:

- The use of high insertion torque (up to 70 N cm) neither prevented osseointegration nor increased marginal bone resorption around tapered multithreaded dental implants placed in posterior mandible.
- Based on the results of this study, the values of insertion torque do not affect bone healing, and there are no radiologic signs of bone necrosis for values of insertion torque above 70 N cm.
- Immediate loading of dental implants have no adverse effects on osseointegration and may increase bone density around the implant.

CONFLICT OF INTEREST
The authors declare that they have no conflicts of interest.

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APICAL SEALING EFFICIENCY OF DIFFERENT OBTURATION TECHNIQUES OVER APICALLY BROKEN ROTARY NICKEL-TITANIUM FILES (AN IN VITRO STUDY)

Abd El-Wahed MI\textsuperscript{1} BDS, Zaaou AM\textsuperscript{2} PhD, El Mallakh BF\textsuperscript{3} PhD

Abstract:

\textbf{Introduction:} A broken instrument within the root canal is a panic problem which one should never wish to occur during routine endodontic therapy. However, the decision regarding how to manage this problem is complex. Therefore, the clinician must evaluate carefully the options of treatment; retrieving the instrument; bypassing the instrument; or leaving the fractured instrument in the canal as part of the final root canal filling.

\textbf{Objective:} The objective of this study is to compare the sealing ability of three obturation techniques over apically broken nickel-titanium (Ni-Ti) ProTaper file.

\textbf{Materials and methods:} Forty eight extracted human single-rooted mandibular premolars were prepared using ProTaper files (PT) and subjected to breakage of 3mm from F5 tip at the apical one third of the canal. The canals were randomly divided into three groups of 12 teeth each according to type of obturation technique; E&Q plus obturation, using a Thermafil obturator, or lateral compaction technique. The remaining 12 teeth served as positive controls which contained canals with apically broken files only without any type of obturation. Apical leakage was assessed by the dye penetration method in which all teeth were immersed in 2\% methylene blue dye for 48 hours. Then the specimens were longitudinally split into two halves. Linear dye penetration was measured in millimeters under a stereomicroscope for each specimen. Statistical analysis of the results was performed using Kruskal-Wallis and post hoc tests.

\textbf{Results:} The results showed no statistically significant difference in apical leakage between the three obturation techniques used over apically broken PT files.

\textbf{Conclusions:} All obturation techniques used over apically broken PT files played a significant role in the sealing ability.

\textbf{Key words:} apically broken file, ProTaper, obturation technique, apical leakage

INTRODUCTION

In practice, there are many procedural accidents that affect the prognosis of root canal therapy. One of the most common procedural accidents is instrument separation that usually occurs due to incorrect use or overuse of an endodontic instrument (1). Although rotary nickel-titanium (Ni-Ti) instruments have high flexibility, they are more frequently fractured than stainless steel hand instruments without warning (2-5). The fracture of rotary NiTi files results from torsional failure or cyclic fatigue (6). The majority of separated Ni-Ti instruments occur in the apical third of the canal where they are difficult to retrieve (7).

Despite technological advancements of instruments and techniques for removing a separated instrument from the root canal, the retrieval of the separated instrument still has been a complicated procedure in endodontic clinical work. Therefore, when the fractured instruments are impossible to retrieve, endodontists prefer to incorporate them as part of the final root canal filling (8). In such cases, a good quality obturation is required so that the sealer or the obturation materials may seal the spaces between the flutes of the broken file resulting in an adequate apical seal (9). Recently, a variety of thermo-plasticized gutta-percha techniques have been introduced to the market which provide better sealing ability for the root canal.

Consequently, the goal of this study is to evaluate the quality of apical sealing ability of three different obturation techniques namely E&Q plus obturation, Thermafil, and cold lateral compaction over apically broken Ni-Ti ProTaper. The null hypothesis tested was that there would be no significant difference in the apical sealing ability of the three aforementioned obturation techniques when used over apically broken Ni-Ti ProTaper file.

MATERIALS AND METHODS

Forty eight freshly extracted human single rooted permanent mandibular premolar teeth were selected to be mature with fully formed apices, no root caries, resorption or fracture; radiographed to confirm the presence of a single canal then were stored in 10\% formalin solution. A standard endodontic access cavity was prepared using a #2 high speed round carbide bur then followed by an endo-Z bur for lateral extensions and flaring of the cavity walls, then explored by an endodontic explorer to confirm straight line access.

Canal patency was established by passing a #10 K-file into the canal until it was just visible at the apical...
foramen. The working length was determined with a #10 K-file, by subtracting 1 mm from the canal length. An initial file was selected according to canal anatomy that bound within the canal walls then this file was used to perform a smooth glide path. The canals were subsequently prepared using ProTaper Universal nickel titanium rotary files (Dentsply Maillefer, Ballaigues, Switzerland) in the following sequence; (S1, S2, F1, F2, F3, F4, F5) using an endodontic electric motor with reduction 20:1 contra angle hand piece (EndoMate DT, NSK Japan) at a speed of 250 rpm and torque setting 3.5. Irrigation between each successive file was done by using 2.5% sodium hypochlorite (NaOCl) together with Glyde (Dentsply Maillefer Ballaigues, Switzerland) applied to the tip of each file prior to use.

Root canal preparation was carried out until F5 file was used 2mm short of the working length. Another F5 file was previously nicked with a diamond stone 3mm from the tip then was introduced under steady pressure till separation occurred. All canals were subsequently radiographed to ensure that separation occurred in the apical one third of the canal. Then they received irrigation of 5 ml of 17% ethylene-diamine-tetra acetic acid (EDTA) for one minute then washed with 5 ml of 2.5% NaOCL solution, followed by 5 ml of saline solution as a final rinse to eliminate the residual effect of the irrigant. Then they were dried by paper points to be ready for obturation. The canals were randomly divided into four groups of 12 teeth each according to the technique used for obturation.

**Group I (E&Q plus obturation):** The diameter of the tip of the broken file was measured using a gutta percha gauge, and a single ProTaper gutta percha point (Dentsply Maillefer Ballaigues, Switzerland) corresponding to a size F5 file was trimmed to the same tip diameter with a scalpel. The suitable size of E&Q pen tip and hand plugger that goes 3 mm short of the broken file were selected as a binding point. Topseal sealer was applied in the canal on the trimmed gutta-percha tip of the master cone. The E&Q pen (Meta biomed Korea) was set at a temperature of 200 °C and the selected pen tip was activated to seal off the master cone at canal orifice and inserted into the canal to condense the gutta percha till a binding point. The backfill of the remaining part of the canal was done by using E&Q gun of obturation unit that was set on a temperature of 180 °C. Then the gutta percha was injected into the canal using a gun needle gauge 25 and condensed by using a hand plugger to avoid voids in the obturation.

**Group II (Thermafil obturator):** A Thermafil obturator F5 was cut at 3 mm by a scalpel corresponding to ProTaper points, then Topseal was applied into the canal as with group I. It was heated in a Thermaprep oven for (30-45) seconds then slowly inserted into the canal to the working length with continuous firm apical pressure over the apical broken file to limit shrinkage of the gutta percha while cooling. The shaft of the obturator was cut off at the canal orifice using a Thermacut bur. The coronal gutta percha around the plastic carrier was compacted using hand pluggers.

**Group III (lateral compaction technique):** The size of the ProTaper F5 gutta-percha was cut at 3mm by scalpel then Topseal sealer coated the trimmed gutta-percha tip and was applied to the canals. Each canal was obturated by the trimmed ProTaper cone and accessory gutta-percha cones over the apical broken file using lateral condensation technique by using finger spreaders.

**Group IV (Positive control):** Canals contained apically broken files only and the remainder of the canal was left unobturated. This group acted as a positive control.

After obturation, the coronal access was sealed with Cavit (Espe, Germany) and all specimens were radiographed bucco-lingualy and mesio-distally as presented in (Fig.1 A,B, C) then were stored for two weeks at 37°C and 100% humidity to allow for complete setting of the sealer.

**Evaluation of the sealing ability:** In all groups, the teeth were dried with compressed air then coated with a double coating of nail polish and a layer of sticky wax except 1 mm of the apical foramina of roots. All specimens were immersed in 2% methylene blue solution for 48 hours. Then they were washed under tap water and dried with compressed air. Sticky wax and coating layers were removed from external teeth surfaces. Then all specimens were grooved longitudinally from buccal and lingual surfaces using a diamond disc in a low speed hand piece, in a direction parallel to the long axis of the tooth through the apex then they were split by a chisel into two halves (10). All specimens were finished and polished using wetted smooth sand paper. Linear dye penetration was measured by two observers in millimeters under 30X magnification power using an optical stereomicroscope. Statistical analysis of the results was performed using non parametric Kruskal-Wallis test which was used to compare median leakage and followed by post hoc test to identify the different groups responsible for statistical significance. The P value was set to ≤ 0.05.
RESULTS
Collected data were described using range (minimum and maximum) mean, standard deviation and average (median), as presented in (Table 1) and (Fig. 2). The results of this study showed no statistically significant differences in apical leakage when comparing E & Q plus, Thermafil, and Lateral condensation as obturation techniques over an apical broken F5 ProTaper file. Group IV (positive control) showed the highest apical leakage, and was significantly different when compared with the other three groups at P <0.001.

![Fig 2: Box Plot showing the range and average of apical dye penetration (mm) of the four experimental groups.](image)

**Table 1:** The average of apical leakage, mean, standard deviation, minimum and maximum values for each group.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>SD</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>E&amp;Q plus obturation</td>
<td>12</td>
<td>0.00</td>
<td>0.98</td>
<td>0.44</td>
<td>0.38</td>
<td>0.43</td>
</tr>
<tr>
<td>Thermafil</td>
<td>12</td>
<td>0.00</td>
<td>1.32</td>
<td>0.39</td>
<td>0.45</td>
<td>0.29</td>
</tr>
<tr>
<td>Lateral condensation</td>
<td>12</td>
<td>0.12</td>
<td>1.99</td>
<td>0.52</td>
<td>0.53</td>
<td>0.37</td>
</tr>
<tr>
<td>Teeth without obturation</td>
<td>12</td>
<td>0.68</td>
<td>2.98</td>
<td>1.90</td>
<td>0.83</td>
<td>2.07d</td>
</tr>
</tbody>
</table>

N= number of specimens. SD= standard deviation. H: Kruskal-Wallis test. * P < 0.05 (significant), d: significantly different group identified by Post Hoc test.

DISCUSSION
Fracture of endodontic instruments within the root canal is a frustrating problem that creates a major obstacle during routine endodontic therapy (1, 11). Nickel-titanium rotary instruments have been developed to simplify and improve the efficacy of endodontic shaping procedures. However, instrument separation occurs without warning during clinical use (11-14). Numerous factors have been contributed to the fracture of NiTi instruments including cyclic fatigue (1, 4, 15-17), operator skill / experience, instrumentation technique, dynamics of instrument use, number of uses, instrument design, anatomical configuration of the canals, and effect of sterilization (18).

Removal of fractured files has been technically difficult, time consuming and high risk of procedure errors (18). On the other hand, some studies concluded that the presence of a fragment of the fractured instrument in the root canal had little influence on the rate of endodontic failure when the root canal treatment was performed under ideal conditions (19, 20).

A ProTaper universal Ni Ti rotary file was selected as it is one of the most commonly used NiTi rotary systems. According to debates concerning the extent of apical enlargement, it has been recommended to enlarge apical preparation to more than #25 to allow more efficient irrigation (21). In addition, the apical preparation larger than #40 has been advocated for removing a higher amount of infected dentin and for promoting proper cleaning of the apical region of root canals (22).

Therefore, in the present study, F5 ProTaper file with 5% taper and 0.50 tip diameter was used as an apically broken file when comparing the apical sealing ability of three obturation techniques as found by Madarati et al in 2010 (23). While most of the previous studies (10, 24, 25) evaluated the apical sealing ability of different obturation techniques over apically broken F3 ProTaper files. Regardless of the instrument size, the master file was used 2 mm shorter than the working length to create a tighter apical diameter to facilitate file separation at the apical one third of the tooth. Another file which was previously nicked with a diamond stone 3 mm from the tip to ease the separation process was then introduced under steady pressure till separation occurred. This method of file separation allowed the broken file firmly bind to the canal wall as well as simulating the clinical condition.

Three dimensional obturation of radicular space is essential for long-term success of endodontic treatment. There are various techniques used to obturate the root canal system, as cold lateral compaction which is widely used by practitioners and is proven as a clinically effective filling technique. Nevertheless, some studies reported the creation of voids, spreader tracts, excessive volume of sealer, and lack of surface adaptation to canal walls (9). The thermo-plasticized obturation techniques were recently introduced to improve the homogeneity and surface adaptation of gutta percha (26). The Thermafil system is one of the most common carrier based obturation systems at which thermo-plasticized gutta-percha could be more easily introduced into the root canal using a carrier to provide a void free obturation along with minimal sealer thickness and a higher degree of homogeneity (27, 28). Also E&Q plus obturation is one of the combined obturation systems, based on continuous warm vertical compaction as System B and thermo-plasticized injectable obturation technique as Obtura II. This combination provides significantly superior sealing ability over a separated F3 ProTaper tip when compared to both lateral condensation...
and ultrasonic thermo-plasticized gutta percha obturation techniques, as found by Hussein, and Zaazou in 2008 (10).

Therefore, in this study, the apical sealing ability of the three aforementioned obturation techniques in conjunction with Topseal sealer were compared over an apical broken F5 file. Topseal was used as epoxy amine resin based sealer which is characterized with its excellent biocompatibility, low viscosity, self-adhesive properties, and dimensional stability (29).

Dye penetration has been long used as an apical microfiltration assessment method. Although new methods have been introduced to evaluate microfiltration, but the dye penetration still showed no significant difference when compared to these methods (30, 31). According to Ahlberg et al in 1995, methylene blue has been proved to be a useful aid in endodontics (32). Methylene blue dye was used in the present study as it has been inexpensive, easy to manipulate, has a high degree of staining and a molecular weight even lower than that of bacterial toxins (33). All specimens were grooved longitudinally from buccal and lingual surfaces using a diamond disc then were split by a chisel into two halves (10). This enabled examination of the exposed filling material and any dye penetration into the material or at the dentin-material interface on one side as supported by Ahlberg et al (32).

The results of this study showed no statistically significant difference in apical leakage when compared E & Q plus, Thermafil, and Lateral condensation as obturation techniques over an apical broken F5 ProTaper file. The results were in agreement with the results of Saunders et al (2004) and Mohammadi, and Khademi (2006) who reported that the presence of 3 mm of fractured instrument within the apical third of a root canal had no influence on the time required for bacterial penetration, and it did not compromise the obturation of the root canal space (8, 34). As well, they found that the fractured instrument has flutes, so it is questionable to completely obturate the root canal space by itself. So when the sealer extruded between the flutes of the fractured file, it will become equivalent to any other obturation material (8). On the contrary, the results of the present study were in disagreement with previous studies (9, 10, 24, 25, 35). This disagreement might be due to the difference in leakage test used, obturation techniques, filling materials and sealers, as well as the use of different sizes of ProTaper or different types of rotary. Also other factors might have been contributed to that conflict such as variations in root canal anatomy of the selected teeth and the difference of operator’s experience.

Regarding fractured ProTaper instruments, Altundasar et al in 2008 (24) reported that teeth with fractured ProTaper instruments showed significantly less leakage than those filled without fractured ProTaper instrument, regardless of the obturation technique used as consistent with Taneja et al in 2012 (9). The method of file separation in this study occurred under stress that was created through increased contact and binding of the file within the canal. So this screwing force that led to the separation of ProTaper files could have forced the instrument firmly into the dentin, allowing a more intimate contact between the file and the root canal walls at the apical one third (9). Moreover, ProTaper files are triangular cross sectional design with sharp cutting edges and no radial land which results in better debris removal and less irregularities in prepared canal. That is why; the broken F5 ProTaper file provided a good apical seal by itself.

CONCLUSIONS
The thermo-plastic obturation techniques demonstrated better sealing ability over apically broken PT file when compared to lateral condensation technique. As well, the fractured file would be incorporated as part of the final obturation to minimize the hazards of apical leakage.

ACKNOWLEDGEMENT
The authors would like to extend their gratitude to everyone who participated in accomplishment of this research.

STATEMENT OF CONFLICT OF INTEREST
The authors declare that they have no conflicts of interest.

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THE EFFICACY OF ARTHROCENTESIS ON RESTORING MANDIBULAR FUNCTION IN UNILATERAL SUBCONDYLAR FRACTURE FOLLOWING MAXILLOMANDIBULAR FIXATION (A CLINICO-RADIOGRAPHIC STUDY)

Sinbel A¹BDS, Shaaban A²PhD, Shokry M³PhD.

Abstract:
Introduction: Arthrocentesis can be used as a treatment modality in cases of subcondylar fracture treated with maxillomandibular fixation to restore the normal mandibular movements.
Objective: Was to evaluate the efficacy of arthrocentesis on restoring mandibular function in unilateral subcondylar fracture following maxillomandibular fixation.
Materials and Methods: This study was conducted on twenty patients suffering from unilateral subcondylar fracture with/without contra-lateral parasymphyseal or body fracture where open reduction with plate fixation for the contralateral fracture and closed reduction with 2 weeks MMF for the subcondylar fracture was done. The patients were divided into two groups: Group I (study group) in which ten patients were treated with active mobilization and arthrocentesis following MMF removal, and Group II (control group) in which ten patients were treated with only active mobilization following MMF removal. Groups I & II were also subdivided into A & B each involved five patients with isolated subcondylar fracture and five patients with contralateral parasymphyseal or body fracture in addition.
Results: There was a statistically significant decrease in pain and edema and a statistically significant increase in the mouth opening, lateral excursive and protrusive movements in addition to the bite force through-out all follow up periods in the study group especially after the application of arthrocentesis in comparison to the control group.
Conclusions: Arthrocentesis (TMJ lavage) was an effective procedure for washing out inflammatory mediators present within the TMJ following the exposure to trauma and showed a significant improvement in mandibular movements immediately after performing it. This helped in improving the quality of the patients’ life enabling them to practice their usual activities and return back to normal life starting from the second week postoperatively i.e patients rehabilitation occurred in a short period.
Key Words: Arthrocentesis, subcondylar fracture, maxillomandibular fixation.

INTRODUCTION
The mandible is the second most commonly fractured part of the maxillofacial skeleton because of its position and prominence (1, 2). Among which mandibular condylar fractures account for 17.5–50% of all mandibular fractures (3).

Injury to the maxillofacial skeleton may be caused by a variety of mechanisms and causes which includes motor vehicle accident, interpersonal violence, work related incidents, sporting accidents and falls (4). The type of fracture produced following an injury depends on the age of the patient and is affected by the direction and magnitude of the force. For example; a blow directed horizontally to the mandibular body, such as that provided by a fist, results in a fracture of the ipsilateral mandibular body and the contralateral condyle (4).

The proper management of the fractured mandibular condyle is one of the most controversial topics in maxillofacial trauma (4). Restoration of mandibular function, in particular, as part of the stomatognathic system must include the ability to masticate properly, to speak normally, and to allow for articular movements as ample as before the trauma (5).

Treatment is generally divided into conservative or non-surgical and surgical approaches (4). Open reduction with internal fixation (ORIF) is indicated in case of bilateral condylar fractures, limitation of function, inability to maintain occlusion or where closed reduction will result in a high degree of failure but the risk of avascular necrosis and osseous or fibrous ankylosis is high in patients when treated with ORIF which make conservative treatment more preferred (5, 6). Conservative treatment requires varying periods of maxillomandibular fixation (MMF) followed by aggressive physiotherapy (7).

This period currently ranges from 7 to 21 days which may be increased or decreased based on the age of the patient, level of fracture, degree of displacement and the presence of additional fractures (4).

The period of immobilization is controversial and must be long enough to allow initial union of the fracture segments but short enough to prevent complications such as
Muscle atrophy and joint ankylosis. It is better that maxillomandibular fixation be discontinued in all patients at approximately 10 to 14 days. In case of the presence of contralateral body, or parasymphysal fractures open reduction and fixation of them will allow early mobilization of an associated condylar fracture which allow for the early release of MMF without compromising the healing of these other fractures (5).

The effect of MMF on the masticatory system must be taken in consideration together with the negative musculoskeletal changes that prolonged MMF may cause despite the advantages of conservative treatment (8). These changes include atrophy of the muscles of mastication, thinning of the condylar cartilage of the temporomandibular joint (TMJ), and decreased range of mandibular opening (9-11). Some of these changes are reversible to varying degrees, depending on when function is restored via intensive physiotherapy. It has also been shown through several studies that arthrocentesis of the upper compartment of the TMJ (a method of flushing out TMJ) may be highly effective method to restore manual mouth opening and functioning (12).

Since the presence of the pro-inflammatory cytokines interleukin (IL)-6 is detected in patients with subcondylar fractures (13) because these inflammatory mediators initiate pain and limit the normal mandibular movements arthrocentesis can be used as a treatment modality for subcondylar fractures where it wash away the inflammatory mediators, leading to quick recovery of jaw function (6, 14).

This study was performed to hypothesize whether arthrocentesis is efficient in restoring mandibular function in unilateral subcondylar fracture or not in comparison to active mobilization only following the removal of maxillomandibular fixation.

MATERIALS AND METHODS
This study was performed as a controlled randomized clinical trial and was performed on twenty patients suffering from unilateral subcondylar fracture with/without contralateral parasymphysal or body fracture where open reduction with plate fixation for the contralateral fracture and closed reduction with 2 weeks MMF for the subcondylar fracture was done. The ethical clearance was obtained by the ethical committee before starting the study, the selected patients were informed about the nature of the study and informed consents were obtained. The patients were divided into two groups:

Group I (study group): Ten patients were treated with active mobilization and arthrocentesis following MMF removal. (Fig. 1a-c)

Group II (control group): Ten patients were treated with only active mobilization following MMF removal.

I- Preoperative assessment and examination:
Every patient was assessed and evaluated by proper history taking, thorough clinical and radiographical examination as follows:

A- History of the patient: The onset of the mandibular fracture should not exceed more than one week from the day of the trauma. The pre-operative data was collected and recorded in full details in an examination sheet including name, age, gender, occupation, address, onset, etiology of the fracture and present complain in addition to the past medical history.

B- Clinical examination: Both extraoral and intraoral examination were done through inspection and palpation to detect site of tenderness, step defects and bony in addition to examination of TMJ which was done by palpation over the condyle area through placing the little finger in the external auditory canal while the palm directed forward and the patient was instructed to attempt to move the mandible in all directions in order to detect any condylar movement impairment during opening and closing, dislocation of condylar head from the glenoid fossa and/or tenderness over the preauricular area.

C- Radiographic examination: Standard orthopantomogram (OPG) and reverse Towne’s view were taken for all patients at the time of presentation. Other necessary radiographic examination was requested according to each case.

II- Pre-operative preparation:
For all patients, maximum interincisal mouth opening, lateral excursive and protrusive movement were measured preoperatively as a baseline by vernier-calibrated sliding calipers using the incisal edge of the maxillary central incisor and that of the mandibular central incisor as reference points (15).

The bite force was also measured preoperatively as a baseline using Pressure Indicating Film which is an easy tool that reveals the distribution and magnitude of the force between any two contacting, mating or impacting surfaces.

Clinical follow up was carried out to assess the previous measures at intervals of two, four, six and twelve weeks post operatively, removal of MMF was done within 2 weeks after which the group I (study group) received arthrocentesis while in the group II (control group) was instructed for active mobilization only.

The Procedure of superior joint space arthrocentesis was as follows (16-18): (Fig. 1)

• The joint was palpated during mandibular movements to locate the condyle and the mandibular fossa.

• The pre-auricular region was cleaned with Betadine swab (Povidone-Iodine U.S.P. 10% W/V. Manufactured by: the Nile Co. for pharmaceuticals and Chemical Industries Cairo - A.R.E. Licensed by Mundipharma AG – Basel – Switzerland) and the area was isolated with sterile towels.

• A mark was made 1 cm in front of the tragus along with the lateral canthal-tragus line (canthotragal line) and 2 mm below the tragus.

• Block analgesia was done to the auriculotemporal nerve with Mepecaine-L. (Each carpuile is 1.8 ml. Each ml of Mepecaine-L contains: Mepevacaine HCl 3%. Produced by: Alexandria Co. for pharmaceuticals, Alexandria, Egypt. www.alexopharma.net).

• Shepherd arthrocentesis instrument 18g x 2.5” (Shepherd Arthrocentesis Instrument, 18gx2.5-ACE Surgical Supply) was inserted into the superior chamber
The Efficacy of Arthrocentesis in Unilateral Subcondylar Fracture

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According to the mark previously done
- About 50 cc sterile normal saline solution was used for lavage of the superior joint space through the in part of the Shepherd arthrocentesis instrument.
- A free flow of the solution was collected in a kidney dish through the out part of the Shepherd arthrocentesis instrument.
- After removing the Shepherd arthrocentesis instrument, the jaw was gently manipulated in vertical, protrusive and lateral excursions.
- Hair or beard over the joint was shaved.

Fig. 1: Showing arthrocentesis procedure a) Landmarks for arthrocentesis, b) Insertion of shephered arthrocentesis instrument, c) Arthrocentesis using normal saline.

III- Radiographic follow up:
The patients were radiographically assessed immediately after the operation, six and twelve weeks post-operatively. It included orthopantomogram to assess the adequacy of reduction of the fractured segments and the fracture healing progression. OPG and Towne’s view were required preoperatively to assess the results. (Fig. 2)

Fig. 2: Showing preoperative OPG and Towne's view showing isolated right subcondylar fracture.

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0 (19, 20). Quantitative data were described using range (minimum and maximum), mean, standard deviation and median. Comparison between two independent samples was done using independent t-test, also paired t-test was used to analyze two paired data, comparison between different periods using ANOVA with repeated measures. Significance of the obtained results was judged at the 5% level.

RESULTS
The study was performed on twenty patients; seven males and thirteen females; with a ratio of 1:2. They were treated in the Oral and Maxillofacial Surgery Department, Faculty of Dentistry, Alexandria University. Patients’ age ranged from 20 to 50 years old with a mean age of 30.45 ± 9.638.

The etiological factors of the fractures were (45%) road traffic accidents (RTA), (30%) interpersonal violence (IPV), and (25%) fall.

Clinical results:
Every case was monitored at the intervals of two, four, six and twelve weeks postoperatively to evaluate bite force, occlusion, and range of mandibular movements (mouth opening, lateral excursive and protrusive movements). (Fig. 3a-c & Fig.4a-d).

Fig. 3: Showing occlusion a) Preoperative occlusion, b) Two weeks postoperative occlusion, c) Six & twelve weeks postoperative occlusion.

Fig. 4: Showing mouth opening a) Preoperative mouth opening, b) Two weeks postoperative mouth opening following removal of MMF, c) Two weeks postoperative mouth opening following application of arthrocentesis, d) Six and twelve weeks postoperative mouth opening.

Regarding the maximum mouth opening, a limited mouth opening was obvious preoperatively but the mouth opening improved gradually postoperatively. The maximum mouth opening scores at 2 weeks, 4 weeks, 6 weeks and 12 weeks postoperatively is shown in table 1. The maximum mouth opening (MO) was increased in all cases in both groups through-out the follow up periods where the increase in the mean of the MO scores after two, four, six and twelve weeks was found to be statistically significant in comparison to that measured preoperatively in both groups (p1 value <0.05). There was a significant increase in MO score in group I by the end of the second week after arthrocentesis in comparison to that just before arthrocentesis in the same week as p2 = 0.001 (P2 < 0.05).

There was no statistical significance difference in MO scores between group I and II in the first two weeks (before arthrocentesis was done in cases of group I) as p value > 0.05. After arthrocentesis was done in group I, there was a significance difference in MO scores between group I and II by the end of the second, fourth, sixth and twelfth week (p value < 0.05).

The lateral excursive movements either towards the affected side or non-affected side in were measured in group
I & II. They were increased in all cases in
both groups through-out the follow up periods, this increase in the mean of these scores after two, four, six and
twelve weeks was found to be statistically significant in comparison to that measured preoperatively in both groups
(p1 value <0.05). There was a significant increase in these score in group I by the end of the second week after
arthrocentesis in comparison to that just before arthrocentesis in the same week as p2 = 0.001 (p2 < 0.05)

Table 1: Showing the maximum mouth opening scores at 2 weeks,
4 weeks, 6 weeks and 12 weeks postoperatively.

<table>
<thead>
<tr>
<th>Week</th>
<th>Preoperative</th>
<th>2nd Before</th>
<th>2nd After</th>
<th>4th</th>
<th>6th</th>
<th>12th</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Min.</td>
<td>15.40 – 15.00</td>
<td>15.20 – 15.00</td>
<td>15.20 – 15.00</td>
<td>15.20 – 15.00</td>
<td>15.20 – 15.00</td>
<td>15.20 – 15.00</td>
</tr>
<tr>
<td>Max.</td>
<td>15.70 – 15.00</td>
<td>15.70 – 15.00</td>
<td>15.70 – 15.00</td>
<td>15.70 – 15.00</td>
<td>15.70 – 15.00</td>
<td>15.70 – 15.00</td>
</tr>
<tr>
<td>Mean</td>
<td>15.50 ± 0.95</td>
<td>15.50 ± 0.95</td>
<td>15.50 ± 0.95</td>
<td>15.50 ± 0.95</td>
<td>15.50 ± 0.95</td>
<td>15.50 ± 0.95</td>
</tr>
<tr>
<td>Median</td>
<td>15.50</td>
<td>15.50</td>
<td>15.50</td>
<td>15.50</td>
<td>15.50</td>
<td>15.50</td>
</tr>
<tr>
<td>p1</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>p2</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

Table 2: Showing comparison between the two studied groups
according to lateral excursive movement.

<table>
<thead>
<tr>
<th>Lateral Excursive movement</th>
<th>Preoperatively</th>
<th>2nd Before</th>
<th>2nd After</th>
<th>4th</th>
<th>6th</th>
<th>12th</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Min. – Max.</td>
<td>14.0 – 17.00</td>
<td>12.0 – 15.00</td>
<td>16.0 – 19.00</td>
<td>19.0 – 23.00</td>
<td>21.0 – 25.00</td>
<td>25.0 – 27.00</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>15.70 ± 1.06</td>
<td>13.30 ± 0.95</td>
<td>17.60 ± 0.97</td>
<td>20.80 ± 1.48</td>
<td>23.0 ± 1.15</td>
<td>26.10 ± 0.74</td>
</tr>
<tr>
<td>Median</td>
<td>15.50</td>
<td>13.0</td>
<td>17.50</td>
<td>20.50</td>
<td>23.0</td>
<td>26.0</td>
</tr>
<tr>
<td>t</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>p1</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>p2</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

p1: Stands for adjusted Bonferroni p-value for ANOVA with
repeated measures for comparison between 1st week with each
other period
p2: p value for Paired t-test for comparing between 2nd week
before and after

There was no statistical significance difference in scores
between group I and II in the first two weeks (before
arthrocentesis was done in cases of group I) as p value >
0.05. After arthrocentesis was done in group I, there was a
significance difference in these scores between group I and
II by the end of the second and fourth week (p value < 0.05),
but became nearly the same by the end of sixth and twelfth
week in both group I and II. (Table 3)

The protrusive movements were measured in group I &
II. They were increased in all cases in both groups through-
out the follow up periods, this increase in the mean of these
scores after two, four, six and twelve weeks was found to be
statistically significant in comparison to that measured
preoperatively in both groups (p1 value <0.05). There was a
significant increase in these score in group I by the end of
the second week after arthrocentesis in comparison to that
just before arthrocentesis in the same week as p2 = 0.001
(p2 < 0.05)

There was no statistical significance difference in scores
between group I and II in the first two weeks (before
arthrocentesis was done in cases of group I) as p value >
0.05. After arthrocentesis was done in group I, there was a
significance difference in these scores between group I and
II by the end of the second and fourth week (p value < 0.05),
but became nearly the same by the end of sixth and twelfth
week in both group I and II. (Table 3)

The percentage of change in the bite force was
measured in group I & II. It was increased in all cases in
both groups through-out the follow up periods, this increase in
the mean of these scores after two, four, six and twelve
weeks was found to be statistically significant in comparison to
that measured preoperatively in both groups (p1 value
<0.05). There was a significant increase in these score in
group I by the end of the second week after arthrocentesis in
comparison to that just before arthrocentesis in the same
week as p2 = 0.001 (p2 < 0.05)

There was no statistical significance difference in scores
between group I and II in the first two weeks (before arthrocentesis was done in cases of group I) as p value > 0.05. After arthrocentesis was done in group I, there was a significance difference in these scores between group I and II by the end of the second and fourth week (p value < 0.05), but became nearly the same by the end of sixth and twelveth week in both group I and II. (Fig. 5)

Concerning wound healing and sensory function in cases of ORIF, no infection or wound dehiscence were detected nor impairment in the normal sensation.

**Table 3:** Showing comparison between the two studied groups according to protrusive movement.

<table>
<thead>
<tr>
<th>Protrusive movement</th>
<th>Week</th>
<th>Preoperative</th>
<th>2nd Before</th>
<th>2nd After</th>
<th>4th</th>
<th>6th</th>
<th>12th</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group I</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Min. – Max.</td>
<td>2.0 – 5.0</td>
<td>1.0 – 4.0</td>
<td>3.0 – 6.0</td>
<td>5.0 – 7.0</td>
<td>4.0 – 6.0</td>
<td>4.0 – 6.0</td>
<td></td>
</tr>
<tr>
<td>Mean ± SD.</td>
<td>3.40 ± 0.97</td>
<td>2.40 ± 0.97</td>
<td>4.40 ± 0.84</td>
<td>6.20 ± 0.79</td>
<td>4.90 ± 0.74</td>
<td>5.30 ± 0.82</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>3.0</td>
<td>2.0</td>
<td>4.0</td>
<td>6.0</td>
<td>5.0</td>
<td>5.5</td>
<td></td>
</tr>
<tr>
<td>p₁</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p₂</td>
<td>0.003</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Group II</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Min. – Max.</td>
<td>2.0 – 5.0</td>
<td>1.0 – 3.0</td>
<td>4.0 – 6.0</td>
<td>4.0 – 6.0</td>
<td>4.0 – 6.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD.</td>
<td>3.30 ± 1.06</td>
<td>2.10 ± 0.57</td>
<td>4.70 ± 0.82</td>
<td>5.0 ± 0.82</td>
<td>5.30 ± 0.82</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>3.0</td>
<td>2.0</td>
<td>4.50</td>
<td>5.0</td>
<td>5.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p₁</td>
<td>&lt;0.001</td>
<td>0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p₂</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>T</strong></td>
<td>0.221</td>
<td>0.848</td>
<td>7.155</td>
<td>4.160</td>
<td>0.287</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>0.828</td>
<td>0.408</td>
<td>0.001</td>
<td>0.001</td>
<td>0.777</td>
<td>1.000</td>
<td></td>
</tr>
</tbody>
</table>

p₁: Stands for adjusted Bonferroni, p-value for ANOVA with repeated measures for comparison between 1st week with each other period
p₂: p value for Paired t-test for comparing between 2nd week before and after
p: Student t-test
*: Statistically significant at p ≤ 0.05

**DISCUSSION**

This study was performed to detect whether arthrocentesis can overcome the disadvantages of MMF and restore the normal mouth opening or not. In the current study, the period of maxillomandibular fixation did not exceed 14 days in all cases of both the study and the control group. This is advised to avoid any negative musculoskeletal changes or decrease in the range of mandibular opening that may occur if maxillomandibular fixation (MMF) was left long (9-11).

The age distribution involved in this study ranged from 20 – 50 with a mean age of 27 years which is consistent with previously published reviews (14, 21-26). This could be related to the fact that young adults represent a wide section of the population and are involved in violence and take part in dangerous exercises or sports (11).

In this study, the females showed a higher prevalence than male with a ratio 2:1 unlike a retrospective study made on the medical records and radiographs of 509 patients treated for mandibular fracture at the University of Alexandria Hospital between 1991 and 2000 (11), where the male to female ratio was 3.6:1.

Regarding the etiology of fracture, this study showed that traffic accidents, falls and assaults are the main causes of fracture representing 45%, 30% and 25% respectively with the primary cause being the traffic accidents in males and assaults or falls in females. This is consistent with a retrospective study done to show that among male patients

**Fig. 5:** Line graph showing comparison between the two studied groups according to the percentage of change in the bite force.

**Fig. 6:** a) Immediate postoperative OPG view, b) Six weeks postoperative OPG, c) Twelve weeks postoperative OPG.

**Radiographic results:**

Immediate postoperative panoramic X-ray, showed satisfactory reduction in all cases having contralateral parasymphyseal or body fracture with proper bony alignment at the lower border of the mandible and proper occlusion. Radiographic follow up was continued at the intervals of four, sixth and twelve weeks postoperative which showed progressive improvement in bone healing and stability of the fracture segments. (Fig. 6a-c)
road traffic crashes accounted for the highest percentage (42%) followed by falls (32%), assaults (18%), and sports injuries (6%) while among female patients the highest percentage (51%) were caused by falls followed by crashes (36%), assaults (9%) and sports (4%) (27). This may be due to the availability of car and motor vehicles to young people, insufficient stress on the use of seat belts, high-speed driving, and less enforcement of traffic rules and regulations in the Middle East (22, 25).

Regarding the site of fracture, this study involved 10 cases showing isolated subcondylar fractures and 10 cases with contralateral parasymphyseal or mandibular body fracture in addition. The site of fracture is affected by the type, magnitude and direction of traumatic force (25, 26). This is also proven through a 5 year study made in 2003 and showed that 32% of fractures are seen in the condylar region; 29.3% in the symphyseal-parasymphyseal region, 20% in the angle region, 12.5% in the body, 3.1% in the ramus, 1.9% in the dentoalveolar, and 1.2% in the coronoid region (9).

In the current study, there was a significant increase in the percentage of bite forces in both study and control group across the follow up period with a significant difference in the study group in relation to the control one especially in the second and fourth week due to the application of arthrocentesis. This is in agreement with the study made in 2004 which proved that arthrocentesis (TMJ lavage) was found to be effective for washing out bradykinin, interleukin-6, and protein from TMJ (14) this helped to bite normally because the pain mediators are removed.

Concerning the range of mouth opening, lateral excursive and protrusive movements, there was a significant improvement within each group through-out the follow up periods. Regarding the lateral excursive and protrusive movements the study showed obvious significant difference between the two groups especially at the second week following the application of arthrocentesis and this improvement continued through-out the fourth week but with no significant difference in sixth and twelfth week. However, the range of mouth opening continued to show remarkable significant difference between the two groups up to the twelfth week postoperatively. This is consistent in part with a study made to clinically compare between arthrocentesis and conventional conservative treatment in 2014 and proved that there were no significant differences in protrusive, lateral excursive movement or incidence of malocclusion but the mandibular range of motion and joint pain showed good improvement from the early stages of treatment and showed better outcomes (28). The significant improvement in the mandibular movements in study group immediately after the application of arthrocentesis showed that arthrocentesis has a great impact on restoring the normal mouth opening and improving the quality of life in patients (12).

In cases with contralateral parasymphyseal or mandibular body fracture and who were treated by ORIF, small incision and gentle soft tissue dissection for the placement of the conventional miniplates which decreased the risk of infection and soft tissue dehiscence This was in agreement with the study made in 2013 showing that one of the advantages of conventional miniplate is low rate of infection (29).

CONCLUSIONS
From the results of this study it was concluded that Arthrocentesis (TMJ lavage) is an effective procedure for washing out inflammatory mediators present within the TMJ following the exposure to trauma. Such lavage had a significant improvement of quality of life of patients and showed immediate significant difference on the spot of performing the arthrocentesis in increasing the mouth opening, lateral excursive and protrusive movements in addition to the bite force and caused significant decrease in the intensity of pain. These changes are valuable to the patients exposed to trauma where they became able to practice their usual activities and return back to normal life starting from the second week postoperatively i.e patients rehabilitation occurred in a short period.

CONFLICT OF INTEREST
The authors declare that they have no conflicts of interest.

REFERENCES
EVALUATION OF TWO DIFFERENT MATERIALS FOR
PRE-ENDODONTIC RESTORATION OF BADLY
DESTRUCTED TEETH
Hamed S\textsuperscript{1} BDS, Zaazou A\textsuperscript{2} PhD, and Leheta N\textsuperscript{3} PhD

Abstract:

\textbf{Introduction:} Pulpal and periradicular diseases develop when microorganisms and/or their by-products contaminate these tissues. Therefore, the major goal of endodontic restoration is to prevent the penetration of microorganisms into the coronal pulpal space and root canal system.

\textbf{Objectives:} To evaluate and compare the sealing ability of two intermediate temporary filling materials in badly destructed teeth reinforced with stainless steel bands and to evaluate the adaptation of these materials upon application of clamps and rubber dam material.

\textbf{Materials and methods:} 65 mature maxillary premolar teeth were used in this study. This study was divided in two parts. Part I: evaluation of coronal leakage by glucose penetration model and spectrophotometer. Teeth were randomly divided into two experimental groups (n=10) and a control group (n=15). Access cavities and stimulation of loss of tooth structure were done. Instrumentation till apical size # 35 and reinforcement with stainless steel bands were done. Group I: teeth were filled with Cavit-G (3M ESPE, Seefeld, Germany); Group II: teeth were filled with CLIP (Voco, Cuxhaven, Germany); Group III: used as positive and negative control group. Measurement of coronal leakage was performed at the 3rd, 7th and 15th day. Part II: evaluation of adaptation of coronal restoration by using 2.5x magnifying loupes and USPHS criteria, grouping was done as before but ten teeth were used as positive controls.

\textbf{Results:} Part I; CLIP (group II) showed the least glucose leakage with median change of leakage (1052.45) mg/dl followed by Cavit-G (Group I) with median change of (2828.55) mg/dl. The difference was statistically significant (P= 0.001). In part II; results were statistically significant (p=0.001), where the CLIP group showed better adaptation than Cavit-G group.

\textbf{Conclusions:} CLIP seals against marginal leakage better than Cavit-G. CLIP provides better adaptation than Cavit-G on application of clamps and rubber dam.

\textbf{Keywords:} Endodontics, temporary filling materials, coronal leakage, Cavit-G, CLIP.

\textbf{INTRODUCTION}

Loss of integrity of coronal tooth structure and invasion of microorganisms into dentin and pulp space play a very important role in pulpal and periradicular diseases. Coronal microleakage appears to be of equal or greater clinical relevance as a factor in endodontic success or failure than apical leakage due to risk of recontamination (1).

Teeth with root canal fillings should immediately receive definitive restorations, as coronal microleakage could occur in a few days (2).

The coronal filling material is considered to be effective when it is able to fulfill certain properties, including good sealing of tooth margins, lack of porosity and dimensional changes to hot and cold temperatures, good abrasion and compression resistance, easy insertion and removal, compatibility with intracanal medicaments, and good esthetic appearance (3).

The pretreatment of broken-down teeth with a stainless steel band helps in improvement of isolation during endodontic treatment. Its use transforms a complex endodontic access cavity preparation into a simple class I cavity, thus assisting the in-between visits sealing quality of some temporary filling materials (4).

Cavit (3M ESPE, Seefeld, Germany) is one of the most commonly used temporary restorations among endodontists for both anterior and posterior teeth. However, Cavit is not esthetic and it is not durable against the force of mastication especially in complex cavities as it leads to extensive cracks and extrusion from the tooth preparations (5).

In an effort to improve the sealing and mechanical properties of temporary restorations, different materials have become available in the market. Recently, a light cure composite resin (Clip; Voco, Cuxhaven, Germany) was introduced as a temporary restorative material in endodontics (6). It contains hydroxyethylmethacrylate, butylhydroxytoluene, acrylic-ester, and polymers. The application of clamps and rubber dam could affect the adaptation of the core materials which can be easily cracked and lose their adaptation under the force applied from the clamps throughout the endodontic treatment.

There are controversies regarding the test methods and tracer substances used to detect microleakage and compare the relative sealing performance achieved with different materials (7, 8).

The glucose leakage test was first introduced in 2005 to evaluate endodontic leakage. Glucose has been proposed as a tracer substance for evaluating endodontic leakage because of its small sensitive molecular size (9). The amount of glucose leakage is quantified with spectrophotometry.

The aim of the present in vitro study was to evaluate and compare the sealing ability of two intermediate temporary
filling materials in badly destructed teeth reinforced with stainless steel bands using a glucose model test and spectrophotometry. Furthermore, the adaptation of the temporary filling materials on application of clamps and rubber dam was done by macroscopic examination using magnifying loupes. The null hypothesis was that, the CLIP temporary filling material was as efficient as Cavit-G temporary filling material in sealing ability.

MATERIALS AND METHODS
Sixty-five mature maxillary premolar teeth with mature apices were used in this study. Teeth were cleaned from soft tissue or debris using a sharp scalpel, then teeth were stored in isotonic saline solution at 100% humidity and 37°C till use.

Part I of the study:
Sample preparation: Thirty-five teeth were randomly divided into two experimental groups of 10 teeth each according to the temporary material used for restoration, and a control group of 10 teeth for positive control group and 5 teeth for negative control group. Access cavities were done by using a round diamond bur mounted on a high speed hand piece followed by a non-cutting end bur as the Endo-Z bur (Dentsply Maillefer, Ballaigues, Switzerland) to smooth and flare the walls of the access cavity. Stimulation of loss of tooth structure was done by preparing mesio-occluso-distal (MOD) intracoronal cavities. The palatal cusps were removed coronal to the cemento-enamel junction (CEJ) by using a fissure diamond bur mounted on a high speed handpiece with water cooling.

A periodontal probe was used to measure the depth of the access cavities; occlusal adjustment was performed so that the depth of the access cavities was 5 mm corresponding to the final thickness of the temporary filling material. Periapical patency was done then a size 15 K-file (Dentsply Maillefer, Ballaigues, Switzerland) was inserted into the root canal until the tip became visible at the apical foramen; then 1 mm was subtracted and taken as the working length. After accurate determination of working length, all root canals were enlarged in a step-back technique reaching an apical preparation size 35. During instrumentation, the canals were flushed with 3% NaOCl as an irrigating solution using disposable syringes and 30-gauge needles (Ultradent Product, Inc., South Jordan, Utah, USA).

After completion of instrumentation the root canals were flushed for 1 minute with 2.0 ml of 17% EDTA solution, then washed with 2.0 ml of 3% NaOCl solution followed by copious rinsing with 5.0 ml saline. Finally the canals were dried with paper points. A dry cotton pellet was placed in the pulp chamber.

The best fitting premolar stainless steel band was selected for each tooth and tested for size and fit. Ketac Cem easy mix (3M ESPE, Seefeld, Germany) was used as the luting agent to cement the band. Glass ionomer cement (GIC) powder and liquid of equal ratio was mixed according to manufacturer's instructions then applied to the stainless steel bands and seated on the teeth. Excess material was removed and GIC was left to set.

The temporary filling materials were packed according to manufacturer’s instruction by the same operator at the site of access opening according to the grouping:

In group I, a readymade paste of Cavit-G was applied with a suitable instrument to fill the required quantity into the wet cavity of the experimental teeth. Excess materials were removed and left to set. The hardening process started after a few minutes.

In group II, teeth were rinsed and dried, CLIP was applied to the cavities with a suitable instrument (spatula or plastic filling instrument), contoured, excess material was removed and the material light cured for 40 seconds using LED light curing device (Woodpecker, LED, China).

After that, teeth were thermo-cycled between 5°C and 55°C for 300 cycles, for 10 seconds at each temperature (10). The external surfaces of each tooth from the experimental groups were covered with two layers of nail varnish except for the apical 2 mm.

Preparation of the glucose penetration model: The coronal part of the tooth was attached to the end of an Eppendorf vial. A hole was created in the cap of the eppendorf vial, through which a glass tube of at least 15 cm long was connected. The assembly was placed in a sterile 5 ml glass bottle with a rubber cap. Leaksages at all connections were eliminated by use of cyanoacrylate glue and sticky wax. The tracer in the present study was (1 mol/L, pH 7.0) glucose solution, with density of 1.09×103 g/L and viscosity of 1.18×10-3 (pas.) at 37°C. About 5 ml glucose solution containing 0.2% sodium azide (NaN3), was injected into the Eppendorf vial from the glass tube until the top of the solution was 14 cm higher than the top of the root, creating a hydrostatic pressure of 1.5 KPa. Glucose that leaks through the tooth crown and root canal was collected in the glass bottle containing 1 mL of 0.2% sodium azide. (fig. 1)

The NaN3 was used here to inhibit the proliferation of microorganisms that might decompose glucose. The seal at
all junctions were checked by connecting the end of the glass tube to compressed air. Any bubbles would indicate leakage of the assembly. So in this case sticky wax was used to eliminate this leak. The model was then transferred to an incubator that provided 100% humidity and 37°C temperature for the duration of observation periods (11).

Spectrophotometric measurement of microleakage: Measurement was performed at the 3rd, 7th and after 15 days. A spectrophotometer was used to analyze the amount of glucose leaked in the glass bottle (12). A total of 10 µL of solution was drawn from the glass bottle using a micropipette and replaced with same amount of sodium azide solution to maintain a constant volume of 1 ml. Each sample from each glass bottle was placed in an eppendorf vial. Fifty microns of glucose marker was added to each sample using a micropipette. This mixture was left for a few seconds and any change in color was examined in which glucose oxidase catalysis the oxidation of glucose to gluconic acid. The formed hydrogen peroxide was detected by a chromogenic oxygen acceptor in the presence of peroxidase. The intensity of the color formed was proportional to the glucose concentration in the samples so if the mixture turned pink this indicates the presence of glucose. The samples were then analyzed using a UV spectrophotometer at 500 nm wave length. The exact amount of leaked glucose appeared on the screen in mg/dl (13).

Part II of the study:
Thirty teeth were randomly divided into two experimental groups of 10 teeth each, and a control group of 10 teeth used as positive controls. After temporary filling materials were applied in the experimental teeth, teeth were clamped using hygienic® bicuspid winged clamp #2A then rubber dam were applied. Standard access cavities were done through the temporary filling materials in each group. The applied clamp was left for 30 minutes then the cavities were redressed with the same materials followed by thermocycling between 5°C and 55°C for 300 cycles, for 10 seconds at each temperature. The adaptation between the temporary filling materials and stainless steel band was examined by macroscopic examination using 2.5X magnifying loupes (figures 2 and 3).

Evaluation of the adaptation: All teeth were evaluated for adaptation using modified United States public health service (USPHS) criteria (14). Two evaluators who were not involved in the placement of the fillings and unaware of the materials used performed the evaluation in this double-blind study. When disagreement arose during evaluation, the evaluators had to reach a consensus. All evaluations were carried out under a dental operating light, using dental explorers and magnifying loupes with a power of 2.5X. A new explorer was used for the evaluation of each tooth. Based on USPHS criteria, adaptations were rated as alpha, bravo and charlie according to the following characteristics; Alpha: Closely adapted, no visible crevice (explorer does not catch), Bravo: Visible crevice, explorer will penetrate and Charlie: The temporary filling is fractured.

All statistical analysis was done using two tailed tests and alpha error of 0.05. The results were statistically analyzed using Kruskal Wallis and Monte Carlo tests.

RESULTS
Part I results: The mean and median leakages of experimental groups at different time intervals are shown in table I.

Table 1: Descriptive comparison of leakage between the experimental groups at different time intervals.

<table>
<thead>
<tr>
<th>Group</th>
<th>Leakage (mg/dl) Day</th>
<th>3rd day</th>
<th>7th day</th>
<th>15th day</th>
<th>X² (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Minimum</td>
<td>Mean</td>
<td>SD</td>
<td></td>
</tr>
<tr>
<td>CAVIT-G (group I)</td>
<td>Minimum</td>
<td>15.6</td>
<td>53.6</td>
<td>12.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Maximum</td>
<td>283.1</td>
<td>147.3</td>
<td>161.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>96.3</td>
<td>1007.3</td>
<td>600.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>82.8</td>
<td>565.1</td>
<td>235.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>80.5</td>
<td>1079.2</td>
<td>192.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>9190.5</td>
<td>15.6 (0.001)*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CLIP (group II)</td>
<td>Minimum</td>
<td>12.9</td>
<td>88.3</td>
<td>4128.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Maximum</td>
<td>161.7</td>
<td>600.2</td>
<td>1800.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>53.6</td>
<td>235.0</td>
<td>1138.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>52.6</td>
<td>192.7</td>
<td>285.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4128.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H (P)</td>
<td></td>
<td>5 (0.001)*</td>
<td>1.3 (0.001)*</td>
<td>9 (0.001)*</td>
<td></td>
</tr>
</tbody>
</table>

X²: Friedman test for repeated measures
H: Kruskal-Wallis test for independent groups
* P < 0.05 (significant) d: significantly different of others

Excessive amount of glucose leakage was observed in the positive control group (n=10), and no leakage was detected in the negative control group (n=5), which
confirmed the functioning and reliability of the experimental model. It was noted that there was a tendency for increased coronal leakage from the 3rd day to the 15th day for all studied groups.

When comparing the leakage throughout the study period, results showed a statistically significant difference between all studied groups (p<0.001). CLIP (group II) showed the least glucose leakage among all studied group with median leakage change of 1052.45 mg/dl followed by Cavit-G (group I) with median change of 2828.55 mg/dl, and positive control group with the highest leakage with median change of 3942 mg/dl (table 2).

**Table 2:** Median change of leakage of different studied groups throughout the study period.

<table>
<thead>
<tr>
<th>Group</th>
<th>Change</th>
<th>H (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Minimum</td>
<td>Maximum</td>
</tr>
<tr>
<td>CLIP</td>
<td>638.7</td>
<td>1638.3</td>
</tr>
<tr>
<td>CAVIT-G</td>
<td>457.9</td>
<td>4027.8</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>5635</td>
</tr>
</tbody>
</table>

H: Kruskal-Wallis test for independent groups  
* P < 0.05 (significant)

Part II results: When comparing the adaptation, it was found that CLIP (group II) showed a statistically significant difference from Cavit-G (group I) (p=0.001). In the CLIP group, nine out of ten teeth gave an Alpha score with 90%, 10% for Bravo score and 0% for Charlie score. While in Cavit-G group, eight out of ten teeth give Bravo score with 80%, 20% for Charlie score and 0% for Alpha score (table 3).

**Table 3:** Adaptation evaluation of CLIP and Cavit-G.

<table>
<thead>
<tr>
<th>Adaptation of temporary filling materials</th>
<th>Group</th>
<th>MCP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CLIP</td>
<td>CAVIT-G</td>
</tr>
<tr>
<td></td>
<td>No %</td>
<td>No %</td>
</tr>
<tr>
<td>Alpha</td>
<td>9 90.0</td>
<td>0 0.0</td>
</tr>
<tr>
<td>Bravo</td>
<td>1 10.0</td>
<td>8 80.0</td>
</tr>
<tr>
<td>Charlie</td>
<td>0 0.0</td>
<td>2 20.0</td>
</tr>
</tbody>
</table>

MCP: Mont Carlo exact probability  
* P < 0.05 (significant)

According to modified USPHS criteria, the CLIP group showed better adaptation than the Cavit-G group.

**DISCUSSION**

Temporary filling materials which prevent the entry of saliva and microorganisms should be used (15). Studies demonstrated that in the absence of an adequate coronal temporary seal, contamination of the root canal system could occur in less than three days (16). Several studies have shown that these materials are incapable of preventing coronal marginal leakage, leading to root canal contamination and inducing the appearance of periapical lesions (17). Temporary sealing materials must not be kept in the root canals for a long period of time due to the risk of contamination (18).

In the present study, a light curing temporary filling material (CLIP) was compared with a commonly used temporary filling material (Cavit-G) to evaluate the coronal microleakage and adaptation of each material.

Cavit-G is an autopolymerized, moisture-initiated, premixed temporary restorative material that contains zinc oxide, calcium sulfate, glycol acetate, polyvinylacetae resins, polyvinyl chloride acetate, tri-ethanolamine and pigments. Cavit-G and Cavit-W are varieties of Cavit that differ in the content of resin and their resulting hardiness and setting. The hardness and dimensional stability of Cavit is higher than that of Cavit-W which in turn is higher than that of Cavit-G. It is a hygroscopic material which possesses a high coefficient of linear expansion, resulting from water sorption which permits the material to adapt to dentin walls. It is widely used between appointments during routine endodontic therapy, probably because of its practical "ready to use" condition (19).

On the other hand, CLIP is a resin based light curing temporary filling material similar to ferritin (Vivadent, France), TERM (DENTSPLY-Caulk) and spacer (Vericom) which has shown good sealing ability in previous studies(20,21). CLIP is composed of BIS-GMA, silicon dioxide, groups of dimethacrylate and organic filler particles. CLIP is a durable material with tight margins, it is ready to use, easy to place, and easy to remove in one piece with no damage to preparation boundaries (21). It does not contain eugenol does not have a negative effect on composite bond strength (22). According to the manufacturer, polymerization shrinkage is minimal and it does not influence sealing.

In the present study, extracted intact maxillary premolars teeth were used, as most fractures occur in the palatal cusps of maxillary premolars which are considered the functional cusps (23). Teeth selected for the current study were of average length and width. Any discrepancy in length was adjusted by occlusal adjustment and the width was adjusted by using a digital caliber. The depth of access cavities of all teeth were measured by using a periodontal probe. A total thickness of five mm in depth was used in this study in order to comply with the recommendations of Webber et al. (24), who found that a 3.5 mm thickness of restorative material was the minimum thickness necessary to prevent microleakage. Periapical patency was done to insure that no blockage occurred at the periapical foramen during instrumentation.

For reinforcement, orthodontic stainless steel bands were used, as it was found by Jensen et al (4) and Heffer (25) that the most important function of stainless steel bands used in endodontic treatment is to help in retaining interim...
restorations during phases of endodontic treatment. Furthermore, stainless steel bands reduced the cuspal flexure by one-half compared to teeth without bands and doubled the fracture strength (26).

Thermocycling procedures attempt to simulate temperature changes that take place in-vivo. Temperature fluctuations can adversely affect the marginal seal of a dental material. To test this factor, thermocycling was incorporated into this study design. The temperature range used in thermocycling (5˚C and 55˚C), corresponds to the extremes of temperatures experienced in the oral environment (27).

A variety of experimental models may be used to measure the coronal leakage; they include dye penetration, fluid transport, bacterial penetration and radioisotope. In the present study, glucose penetration was used (9). Benefits of the glucose penetration model are attributed to the tracer, the possibility of quantitative measurements, reproducibility, and sensitivity (9). Glucose is used as a tracer because it is hydrophilic, has a molecular weight lower than bacteria (MW=180 Da) and serves as a nutrient for bacteria (9). In the present study the level of glucose penetration was measured at 3rd, 7th, and 15th days, representing time intervals for endodontic treatment or when permanent restoration is carried out after root canal treatment. Different observations such as one week, two weeks, or longer, have been used for the evaluation because leakage increases by time regardless of the technique (28).

Under the condition of the present study, the positive control group showed the highest glucose leakage, while none of the five teeth used in the negative control group showed any leakage which confirmed the functioning and reliability of the experimental model.

In the experimental groups, group I (Cavit-G) showed the highest leakage. These findings may be attributed to the fact that expansion of hygroscopic restorative materials leads to poor adaptation at the interface of restorative material and cavity walls (24), also Cavit showed body leakage even when allowed to set in water before immersion in dye this may be due to sorption property of this material (29). The present results confirmed the findings reported by Anderson et al (20) that Cavit restorations were deemed clinically unacceptable in complex endodontic access preparations. This may be attributed to extensive cracks, expansion and extrusion from the tooth preparations. Furthermore, Beach et al (30) showed that Cavit hardness, wear resistance, slow-setting reaction and deterioration with time are key disadvantages. For these reasons, Cavit can be recommended for short-term temporization in small cavities without contact with the antagonizing tooth. In addition, Ludlow (31) found that Cavit-G demonstrated significantly higher leakage after thermocycling. On the contrary, Gilles et al (32) and (Oppenheimer & Rosenberg) (33) found that thermocycling did not adversely affect Cavit products, indicating good dimensional stability, which could be attributed to linear expansion.

According to group II results, CLIP showed the least glucose leakage. The good sealing properties of CLIP could be attributed to minimal shrinkage and the mode of insertion of this material which eliminates the possible inclusion of gaps within the body of the material or at the margins. In addition to the setting reaction which was initiated by exposure to a visible light source. This property enables CLIP to be placed and set, offering no postoperative delays to achieve maximum function. Moreover, Uranga et al (34) found that composite resin based temporary filling materials provided a better seal against leakage after thermocycling when compared to Cavit. Our results are in line with Odabas et al (35) who found that CLIP exhibited the best sealing ability amongst the five tested materials (IRM, Adhesor, Cavit-G, Coltosol and CLIP). Also Tulunoglu et al (36) found that CLIP provided a better seal against microleakage at amalgam and especially composite interfaces, this material also provided a better seal against microleakage at the tooth tissue interface. On the other hand Ciftci et al (21) found that CLIP seals against marginal leakage as effectively as Cavit-G when used as a temporary filling. The disparity between this result and our findings may be attributed to the difference in experimental methods as they used dye penetration test for the evaluation while the present study used glucose penetration model which is more sensitive.

Numerous investigations on microleakage have obtained varying results using Cavit. According to Lim (37), the contradictory reports on the microleakage of Cavit may be due to the differences related to the duration and methods of evaluating microleakage in the different studies.

The lack of saliva and masticatory forces may create inaccuracies in in-vitro leakage studies. Qvist (38) found that occlusal loading had significant effect on the marginal leakage of resin restoration.

In part II of the study, application of clamps after access opening throughout the temporary filling materials was performed. The adaptation of CLIP and Cavit-G during adequate isolation was evaluated for the first time in the present study. CLIP was found to provide better adaptation than Cavit-G with statistically significant difference between them. This is may be attributed to Cavit having weak compressive strength, so there is a need for sufficient bulk to overcome poor strength qualities and provide an adequate seal (24, 39). Furthermore, Rutledge & Montgomery (40) found that light cured temporary filling materials had higher hardness, tensile and compressive strengths than Cavit.

CONCLUSIONS

It was concluded that:

1. None of the studied materials were able to prevent microleakage, where all groups showed leakage within the study period.
2. CLIP sealed against coronal leakage better than Cavit-G when used as temporary filling materials.
3. There was a tendency for increased coronal leakage from the 3rd day to the 15th day for all groups.
4. CLIP provided better adaptation than Cavit-G on application of clamps and rubber dam. The authors declare that they have no conflicts of interest.
ACKNOWLEDGEMENT
The authors would like to express their extreme sincere gratitude and appreciation to all staff members of the Endodontic department. The first author would also like to thank his dear wife Alaa for her support throughout the study period.

CONFLICT OF INTEREST
The authors declare that they have no conflicts of interest.

REFERENCES
33. Oppenheimer S, Rosenberg PA. Effect of temperature change on the sealing properties of Cavit and Cavit G.
WEAR BEHAVIOUR AND SURFACE ROUGHNESS OF POLYMER INFILTRATED CERAMIC MATERIAL COMPARED TO PRESSABLE GLASS CERAMIC

Elhomiamy E\textsuperscript{1} BDS, Aboushady Y\textsuperscript{2} PhD, El Malakh B\textsuperscript{3} PhD.

Abstract:

Introduction: Multiple restorative materials with various chemical, mechanical and physical characteristics are used as permanent dental restorations. The appropriate selection of a restorative material is crucial for preserving the occlusal harmony and normal masticatory function. Several aspects during selection of restorative materials should be considered, among which are the wear behaviour and abrasive nature of the restorative materials and natural enamel.

Objectives: The study determines the two-body wear and surface roughness measurement, of polymer infiltrated ceramic (Vita Enamic) and pressable lithium disilicate based ceramic (IPS e.max press).

Materials and methods: Two-body wear was investigated in custom made tooth wear brushing machine (60,000 cycles, 20 N and 60 cycle/min). The test specimens were divided into two groups, each group consisted of 8 ceramic specimens and 8 enamel cusp antagonists. Quantitative analysis of wear was carried out with subtractive weight loss of all specimens before and after wear test. Surface roughness measurements were evaluated before and after the wear test using a white light interference microscope. The data was collected and statistically analyzed using Mann-Whitney (U) test.

Results: Statically significant differences were found for two-body wear, with Vita Enamic samples show lower wear loss than IPS e.max press samples and cause less enamel wear loss. The Vita Enamic showed lower surface roughness and caused less surface roughness to enamel cusp antagonist than IPS e.max press samples.

Conclusions: Vita Enamic revealed lower wear loss contributed by terms of weight loss and surface roughness change than IPS e.max press. IPS e.max press contributed to more surface roughness and wear loss of opposing enamel than Vita Enamic.

Keywords: CAD/CAM - Two-body wear - Tooth brushing wear - Surface Roughness

INTRODUCTION

With the increase demand of esthetic materials, all ceramic restoration appeared and replaced old ceramo-metal restorations. The all ceramic restorations appear to mimic the natural dentition appearance as they lack metal core, they presented with good properties as biocompatibility, color stability and durability (1).

Wide ranges of all ceramic materials with various chemical, mechanical and physical characteristics were developed. Dental restorative materials should have good mechanical properties and wear resistance to withstand masticatory process and with low abrasive nature to opposing dentition (1). Several aspects during selection of restorative materials should be considered, among which are the wear behaviour and abrasive nature to natural enamel (2). Ideally, restoration materials should have wear resistance similar to that of enamel. The normal vertical loss of enamel from physiological wear was estimated to be approximately 20–38 \( \mu \text{m} \) per annum (3).

Wear is a complex cumulative process of multi factorial etiology, that characterized by progressive loss of material from its surface. Wear alters the anatomy of occlusal surface and affect the occlusal harmony and masticatory function (4, 5).

Pressable glass ceramic systems have gained their popularity due to their ease of fabrication, good mechanical properties, and relative kindness to natural dentition. The dimensional stability of pressed porcelain has made these ceramic materials excellent restorations. With the development of CAD/CAM technology new ceramic and composite materials were introduced that can be incorporated in all ceramic restoration fabrications (6).

Ceramic materials introduced tend to be more rigid and brittle and with potential hazard of excessive wear to opposing dentition. On the other hand the composite materials exhibit low mechanical properties and poor wear resistance but with low abrasive nature. Composite crowns showed preservation of occlusal anatomic form of 26.5% only versus 96% for ceramic crowns (7). A recent analysis mentions excess wear and loosening as the major clinical weaknesses of composite crowns (8), notwithstanding recent structural improvements, of resin-based materials may also be an issue if used for large restoration and multiple restorations in a quadrant (9).

Subsequently the developments of esthetic dental restorative materials have switched to more polymer based resin materials. New generation of restorative material was developed in the benefit of gaining both strength and color stability of dental ceramic and low abrasive nature of composite. These materials are known as hybrid dental ceramics, polymer infiltrated ceramic material is one of these hybrid materials (10).

One of the most recently introduced fully sintered CAD/CAM block, the Vita Enamic material, is a polymer infiltrated ceramic form of feldspathic ceramic 86 wt% and polymer 14 wt%. Vita Enamic polymer infiltrated ceramic material is manufactured by first infiltrating a porous...
feldspathic ceramic base structure with a monomer mixture of new cross linked polymethacrylate polymer material and then cured under high pressure and temperature. Vita Enamic is single visit monolithic restoration and surface shading and glazing is performed using special Polymerizable (light curing) stain and glaze kit. It is used as a single tooth restoration in the anterior or posterior zone (11).

The study was conducted to evaluate two-body wear and surface roughness measurement between the contact area on sliced block of polymer infiltrated CAD/CAM ceramic material and pressable lithium disilicate based glass ceramic materials on enamel cusp antagonist.

MATERIALS AND METHODS

Two types of ceramic materials were included in this study, esthetic CAD /CAM block Polymer infiltrated ceramic material (Vita Enamic) and pressable glass ceramic (IPS e.max press). Natural enamel (antagonist enamel cusp) was used as antagonist.

Specimen preparation: Sixteen freshly extracted caries free permanent upper first molars were collected from various general public hospitals intended for diabetic patient treatment. The extracted teeth were ultrasonically cleaned to remove any calculus and soft tissue remnant and then polished with non-fluoridated polishing paste and stored in saline solution. The antagonist enamel cusp specimens (n=16) were prepared from the mesio-palatal cusp of upper maxillary first molar using high speed handpiece and long carbide fissure bur under water coolant (12). The antagonist enamel cusps were made into block using custom made copper mold and chemical cured acrylic resin (Self-cure acrylic resin, Vertex Dental Co., B.V., Netherland) showing approximately 3 mm of the enamel cusp (Fig. 1).

Fig. 1: Tooth antagonist cusp specimen illustration diagram.

The enamel antagonist cusp block was then stored in saline solution which was changed every 2 days to prevent dehydration of the enamel specimens.

Eight specimens of IPS e.max press (IPS e.max press, Ivoclar Vivadent, Schaan, Liechtenstein, Germany) ingot shade A2 was heat pressed in press furnace (Programmat EP 600) following the manufacturer recommendations. The IPS e.max press was made into slice of (14 x 12 x 2 mm thickness) using Special copper mold. The specimen’s surfaces were cleaned with blast of Al2O3 at 1 bar pressure followed by steam of air jet. Finishing was accomplished with glass ceramic finishing and polishing kit (Dialite LD finishing & polishing kit, Brasseler, USA). The surface intended for wear test was finished to obtain smooth surfaces according to manufacture instruction and then glazed according to manufacturer instructions.

Eight test specimens of Vita Enamic (Vita Enamic, Vita Zhanfabrick, Bad Säckingen, Germany) were prepared from size 14 block (18 mm x 14 mm x 12 mm) using saw microtome (Micracut 150, precision cutter, Metkon instrument Inc., Bursa, Turkey). Precision cutting instrument diamond coated cutting disc (Diamond Coated Wavering Blade No 11-4276, Buehler) was used to cut off the block into a slice with size of (14 mm x 12 mm x 2 mm thickness). The location of cuts was controlled using travelling stage and a horizontally displaced digital micrometer (13). The surface intended for wear test was finished with Vita Enamic polishing and finishing kit technical, then it was etched with 5% hydrofluoric acid gel for 60 seconds. The surface was carefully cleaned under running water to remove all acid remnants and air dried. Vita Enamic glaze was applied with fine brush evenly all over the surface and light cured for 60 sec with Elipar LED curing unit (Elipar TM S10 led curing unite, 3M ESPE, St. Paul, MN, USA).

Wear testing procedures: Two-body wear for ceramic samples and their antagonist enamel cusp was conducted using special custom made tooth brushing wear machine (Dental Biomaterial department, Alexandria University). The custom made tooth brushing machine formed of two articulating parts; movable upper parts and fixed lower part. The antagonist enamel cusp blocks were fixed in the upper movable articulating bars while the ceramic samples were fixed in the lower fixed part using special plastic holder. Contact point geometry was established between the movable antagonist cusp specimens and fixed flat ceramic specimens as shown in (Fig. 2). Artificial saliva was used as a lubricating medium (14).

Fig. 2: Antagonist enamel cusp occluding flat ceramic specimen.

Wear test parameters: Two-body wear was conducted to total of sixteen ceramic specimens (n=8 per material) antagonized by sixteen antagonist enamel cusp specimens. The wear test cycle strokes were to be a total of (60,000 cycles) with frequency of 60 cycle/ min and reciprocating displacement distance of 4-5 mm. A static load of 2 kg (20 N) was used.
Quantitative and qualitative analysis of two-body wear:

Quantitative analysis of two-body surface wear of ceramic specimens and their antagonist enamel cusp specimens was subjected to weight loss assessment and surface roughness (Ra) change (15).

The samples were weighed before and after the wear test using sensitive electronic balance (Analytical Balance, Sacletec SPB 31, Sacletec instruments GmbH, Robert-Bosch-Breite, 1037079 Göttingen, Germany). Wear in a test sample was defined as the weight loss of specimens to have occurred by subtracting initial weight from the final weight measurements. Surface roughness (Ra) change was calculated using white light interference microscope (Interference Microscope, ZYGO Maxim-GP 200, ZYGO Lot GmbH, Boston, Middlefield, CT, USA). The occluding surface for each specimen was scanned and the surface roughness was measured before and after the wear test.

Qualitative analysis of wear patterns, the selected specimens were examined under backscattering scanning electron microscope (SEM) (Jeol JSM 5300, Stoneridge, 122 Pleasanton, CA, USA). Each sample was scanned under two different level magnification, overall view of the wear scar with (X500) magnification and magnified view with (X2000) magnification to give detail analysis of the wear scar.

Statistical analysis

The data sets were analyzed with statistical software (IBM SPSS Version 20, IBM Germany). Descriptive statistics with mean, standard deviation and 95% confidence intervals for all tests and groups were computed. For the two-body wear results, statistical differences between the tested materials as well as the corresponding antagonists regarding (mean weight loss weight loss percent, mean surface roughness change and surface roughness percent change) were assessed with Mann-Whitney (U) test.

Mean value weight change: (weight before wear test) – (weight after wear test).

Table 1: Mean & standard deviation (SD) values and results for comparison in the weight loss between the two ceramic groups and natural teeth antagonist.

<table>
<thead>
<tr>
<th>Weight change</th>
<th>Ceramic material</th>
<th>Teeth antagonist</th>
<th>Teeth antagonist</th>
<th>U (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vita Enamic</td>
<td>IPS e.max press</td>
<td>U (P)</td>
<td></td>
</tr>
<tr>
<td>Mean weight loss ± SD</td>
<td>1.5 mg ± 0.7</td>
<td>6.2 mg ±1.60</td>
<td>3.3 (0.001)*</td>
<td></td>
</tr>
<tr>
<td>Mean weight loss % ± SD</td>
<td>0.24% ± 0.11</td>
<td>0.71% ±0.25</td>
<td>3.2 (0.001)*</td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Mean & standard deviation (SD) values and results for comparison in the weight loss between the two ceramic groups and natural teeth antagonist.

<table>
<thead>
<tr>
<th>Weight change</th>
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<th>Teeth antagonist</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Vita Enamic</td>
<td>IPS e.max press</td>
<td>U (P)</td>
<td></td>
</tr>
<tr>
<td>Mean weight loss ± SD</td>
<td>1.7 mg ± 0.6</td>
<td>10.2 mg ±2.8</td>
<td>3.3(0.001)*</td>
<td></td>
</tr>
<tr>
<td>Mean weight loss % ± SD</td>
<td>0.16% ± 0.05</td>
<td>0.88% ±0.27</td>
<td>3.00(0.001)*</td>
<td></td>
</tr>
</tbody>
</table>

U: Mann-Whitney test
* P < 0.05 (significant level)

RESULTS

Quantitative analysis

Regarding weight loss: There was statistically significant difference between the two ceramic groups. The higher weight loss occurred in the IPS e.max press samples with mean value of (6.2 ± 1.6 mg) and total percent change of (0.71% ± 0.25), while the Vita Enamic samples revealed lower mean weight loss with value of (1.5 ± 0.07 mg) and total percent change of (0.24 % ± 0.11) as shown in (Table 1).

There was statistically significant difference in the enamel cusp antagonist samples. The higher weight loss occurred in the enamel cusp antagonist opposed by IPS e.max press group with a mean value of (10.2 ± 2.8 mg) and total percent (0.88% ± 0.27). While it was lower in the enamel cusp antagonist samples opposed by the Vita Enamic group with mean weight loss value of (1.7 ± 0.6 mg) and weight percent loss of (0.16% ± 0.05) as shown in (Table 1) & (fig. 3).

Regarding surface roughness: Surface texture analysis of test specimens revealed that there was statistically significant difference between the two ceramic groups, the higher surface roughness change occurred in the IPS e.max press samples with mean value of (0.15 ± 0.10 µm) and total percent change of (82.6% ± 37.1). While Vita Enamic samples revealed lower mean surface roughness change value of (0.06± 0.03µm) and total percent change of (48.7% ± 23.9) as shown in (Table 2).

There was statistically significant difference in the enamel cusp antagonist samples with the higher surface roughness change occurred in the enamel cusp antagonist opposed by IPS e.max press group with mean change value of (0.18 ± 0.11 µg) and total percent change value of (189% ± 147). While it was lower in the enamel cusp antagonist
samples opposed by Vita Enamic group with mean surface roughness change value of \((0.06 \pm 0.08 \mu g)\) and surface roughness percent change of \((78\% \pm 108)\) as shown in (Table 2) & (fig. 4).

Table 2: Mean ± standard deviation (SD) values and results for Mann Whitney test, for comparison in surface roughness change between the two ceramic groups and natural teeth antagonist.

<table>
<thead>
<tr>
<th>Surface roughness change</th>
<th>Ceramic material</th>
<th>Teeth antagonist</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vita Enamic</td>
<td>IPS (e_{\text{max}}) press</td>
</tr>
<tr>
<td>Mean surface roughness change ± SD</td>
<td>0.06 ± 0.03µg</td>
<td>0.15µg ±0.10</td>
</tr>
<tr>
<td>Mean surface roughness % change ± SD</td>
<td>48.7% ± 23.9</td>
<td>32.6% ± 37.1</td>
</tr>
</tbody>
</table>

U: Mann-Whitney test  
* \(P < 0.05\) (significant)

On high magnification the surface presented with needle shape crystals and fragment of antagonist teeth. Antagonist enamel show large wear scar area with multiple surface cracks, rough furrows and enamel flakes. High magnification imaging of enamel surface revealed the formation of multiple small cracks and peeling-off the enamel surface.

Qualitative analysis

The results of the qualitative SEM analysis are presented in (Fig. 5, 6) showing image pairs of contact areas on ceramic specimen and corresponding enamel cusp. SEM analysis of Vita Enamic samples revealed narrow, shallow and smooth wear scar as shown in (Fig. 5), with high magnification the wear surface revealed multiple small surface cracks and chipping off the ceramic surface. Antagonist enamel revealed smooth and small area of wear scar with small surface cracks accompanying with the formation of pit-like structure defects.

SEM observation of IPS \(e_{\text{max}}\) press samples revealed wider and deeper wear scar. The wear scar revealed multiple surface irregularities corresponding to area of abrasion (Fig. 6).
DISCUSSION
Tooth wear is a complex process that involves many variables, such as age, para-fuctional habits, neuromuscular force, thickness and hardness of enamel, properties of saliva, masticatory pattern and nature of restorative materials (16). The study of dental wear become common through the biomaterials literatures due to wide variety of dental restorative materials introduced (17).

Laboratory wear testing procedure (in-vitro) may have limitations in reflecting the intraoral condition. It can’t perfectly simulate the intraoral masticatory movement; they can only simulate simple movement such as grinding and clenching (18). The obtained results are helpful in comparing materials under controlled condition, as well as useful in prediction of their clinical performance (19). Wide variety of in-vitro wear test parameters revealed in the publications concerning the applied force, numbers of cycles, design and frequency suggested that there is no agreement between studies. Difficulty in standardization and the impact of methodological or operator own modifications could make direct comparisons between studies almost impossible (20).

In this study we followed the regime for wear testing at a rate of 60 cycle/ min for total of (60,000 cycles) under a load of (20 N) in reciprocating distance of (4-6 mm) which represents (120 days) as described by Imai et al (21). The load of 20 N used in this study applied on a small sample, that simulates a tooth cuspid determines high tension values over the restoration according to Coppédé et al. 2013 (22) and Faria et al (23).

The tooth sample of mesio-palatal cusp of upper first maxillary molar was used in this study as recommended by (Kerjici et al) (24). Many authors recommend flat planes of enamel prepared from labial surface or mesial or distal surface of the tooth (25). The cuspal enamel was to be found much stronger than the enamel found on the side of a tooth and is stronger under compression. Consequently using cusp specimens was more clinically relevant as agreed by (Al-Hyasat et al) (26).

In this study under the two-body wear condition, it was revealed that there was statistically significant difference (P < 0.05) between the two ceramic groups. The IPS e.max Press showed higher wear loss and surface roughness change in comparison to Vita Enamic. There was statistically significant difference between the enamel cusp antagonists groups with the higher wear loss and surface roughness change in the enamel antagonist cusp opposed by IPS e.max press; in contrast Vita Enamic resulted in lower wear loss of enamel antagonist cusp. The test results were in agreement with Mormann et al, Hientze et al, Kim et al and Peng et al. (13, 27-29).

Vita Enamic, wear behaviour and surface roughness results obtained in this study are in agreement with Mormann et al (13). They studied the two-body wear and tooth and roughness measurement of different kind of dental ceramic and composite materials including Vita Enamic. Their results revealed that Vita Enamic showed lower wear loss than most tested materials except for zirconia based ceramic, also they reported that Vita Enamic resulted in lower wear loss to the antagonist enamel cusp.

Mormann et al (13), also reported that the wear behavior of vita Enamic was similar to natural enamel, under SEM imaging of the contact area representing as a sharp line with minimal cracks and pitting defects giving criteria of fatigue wear which was confirmed by the present study. This may be attributed due to that Vita Enamic is interpenetrating phase composite with combination of ceramic (feldspathic) and acrylated base resin polymer. Vita Enamic is damage tolerant, the reaction of Vita Enamic to repetitive impact of the antagonist may be influenced by its modulus of elasticity and flexural strength which showed some degree of elastic deformation under load as reported by Coldea et al (30).

The IPS e.max press, wear behaviour and surface roughness results obtained in this study were in accordance with Hientze et al (27). They reported that IPS e.max press showed lower wear loss and in comparison to Empress and Design, however they also reported that IPS e.max press showed higher friction coefficient. Kim et al (28) found that the IPS e.max press showed lower wear loss in comparison to other glass ceramic except for zirconia based ceramic. Hientze, Kim and Peng et al., reported that SEM images of IPS e.max press showed surface roughness with lateral surface cracks and delamination of ceramic surface representing abrasive wear as observed in this study.

The test results were in disagreement with Peris et al (31) and Albashaireh et al (32), they reported that the wear loss of IPS e.max press was lower to zirconia based ceramic. They also added that IPS e.max press caused lower wear loss to antagonist enamel cusp. The wear behaviour observed by Albashaireh et al (32) of the IPS e.max press showed fragment loss with superficial and deep surface cracks giving sign of fatigue wear which could be contributed due to they used zirconia ball as antagonist.

The publication is controversial about glazing and polishing on the wear behaviour of ceramic materials. In this study all ceramic samples surfaces were finished with glaze layer according to manufacturer instruction. Ling Wang et al (14) and Albashaireh et al (32), reported that the wear behavior of polished IPS e.max press has been lower to that of glazed one. The glaze material will often be removed early during wear cycle leaving underlying rough surface. This could be attributed to that the micro structure of IPS e.max press glass ceramic is not completely free of porosities and/or pores. Surface porosities may cause primarily by volume changes associated with thermal differences during processing and from human errors during the preparation procedure and fabrication stages (33).

The inner properties of ceramic affect the wear rate once the effect of surface roughness disappeared with wear progression (34), Vita Enamic presented with low friction coefficient due to the presence of polymer interpenetrating phase which binding the ceramic phase surface causing surface crack deflections as reported by Alvaro Della et al (35).

Ling Wang et al (14) reported that the frictional
The hardness of IPS e.max press was reported to be higher than Vita Enamic (35-37). However in this study the wear loss of IPS e.max press was higher than Vita Enamic. This could be contributed to that Surface hardness IPS e.max press glass ceramic has been reported to be affected by repeating loading especially in wet condition as reported by Wang et al., Belli et al and Won Suck et al (34, 38, 39). Corrosion mechanism of glass matrix occurred by the diffusion of positive water ions into glass matrix, which caused ploughing of surface molecule from the ceramic surface and reducing surface hardness.

On the other hand Vita Enamic is polymer based ceramic fear of influence of water adsorption through the polymer layer and interfacial salinized polymer feldspar interface which could alter the mechanical properties. Ruse and Sadoun 2014 (40), reported that the mechanical properties of Vita Enamic after aging process was merely affected.

CONCLUSIONS
Within the limitations of this in-vitro study, the following conclusions can be drawn:
1. Vita Enamic showed a lower wear loss and causes less wear damage on opposing enamel than IPS e.max Press.
2. The surface roughness change of Vita Enamic was much lower than IPS e.max Press and all ceramic material show lower surface roughness change than opposing enamel.

STATEMENT OF CONFLICT:
The authors declare that they have no conflicts of interest.

REFERENCES
EVALUATION OF PLATELET RICH FIBRIN IN SINUS LIFTING WITH SIMULTANEOUS IMPLANT PLACEMENT
Elbaradie R¹ BDS; Ossman S² PhD; Eldibany R² PhD

Abstract:
Introduction: Rehabilitation of the edentulous posterior maxilla with dental implants is challenging. The deficient alveolar ridge interferes with implant insertion of adequate length placed in the correct position and with the accurate inclination. The maxillary sinus elevation procedure has become an important preprosthetic surgical procedure for bone creation in the posterior maxilla prior to implant placement. The use of PRF during sinus-lift procedures has been advocated for many years.

Objectives: This study was designed to evaluate the use of platelet rich fibrin (PRF) following sinus lifting with piezosurgery and simultaneous implant placement.

Materials and methods: 7 patients were selected to perform sinus lifting with simultaneous implant placement using PRF as a sole agent and piezosurgery device.

Results: Cone beam computed tomography 6 months postoperatively showed statistically significant increase in bone height and density. The mean of the newly formed bone height was (6.55 ± 1.14 mm). The mean postoperative bone height measured from the floor of the maxillary sinus and alveolar crest was (11.35 ± 0.56 mm). The mean of the newly formed bone density was 507 HU. The mean of the postoperative bone density around the implants after 6 months was (547.71 ± 188.42 HU). The mean marginal bone loss was (0.82 ± 0.25 mm).

Conclusion: PRF could be successfully used as a sole agent for bone regeneration in lateral sinus lifting with simultaneous implant placement.

Key words: lateral sinus lifting, platelet rich fibrin, Piezosurgery, implants, bone density.

INTRODUCTION
Replacement of lost natural teeth by osseointegrated implants has been represented as one of the most significant advances in prosthetic dentistry. Endosseous dental implants are widely inserted to restore oral function, including mastication and speech, as well as for aesthetic improvement in partly or completely edentulous patients (1, 2).

However, insufficient height and/or width of the alveolar ridge when placing implants for oral rehabilitation in the atrophied maxilla is a challenge. Ridge resorption and sinus pneumatization in the posterior maxilla, compounded with poor quality of bone, can compromise implant rehabilitation of the patient (3).

The maxillary sinus elevation procedure has become an important preprosthetic surgical procedure for the creation of bone volume in the edentulous posterior maxilla for the placement of dental implants (4).

The most widely used techniques for maxillary sinus floor elevation is the classical lateral antrostomy introduced by Tatum in 1976. Lateral window may be performed using a 1- or 2-step approach. Implants are installed simultaneously with the bone graft (1-stage lateral antrostomy) or after a delay to allow for bone healing (2-stage lateral antrostomy) (5).

The most common intraoperative complication with these surgical approaches is the perforation of the Schneiderian Membrane. Wallace et al (6) stated that the membrane perforation rate has been reduced from the average reported rate of 30% with rotary instrumentation to 7% using the piezoelectric technique. The piezosurgery device provides a clear surgical site, as it maintains a blood-free surgical field during bone cutting. This allows improved visualization of the surgical area. A very small amount of pressure is applied which allows a very precise cut. The typical cavitation effect induces a hydropneumatic pressure in the physiological saline solution that contributes to atraumatic sinus membrane elevation (5).

Although sinus elevation using autogenous bone graft is considered to be the gold standard, many researchers have attempted to modify this procedure, because of the morbidity associated with bone harvesting. Various non-autogenous substitutes, such as xenogenic, allogenic and some artificial materials have been developed to reduce the risks associated with autogenous bone grafts. However, the use of xenogenic or allogenic materials also induces the risk of disease transmission and have been found to be insufficient for bone regeneration, and artificial bone grafts have been found to be insufficient for osteogenic regeneration (7-9).

The use of blood preparations such as plasma concentrates or fibrin glue might be an interesting option to improve this approach. Platelet rich fibrin has many characteristics that make it suitable for application as a filling material for sinus floor augmentation. It is an autologous fibrin matrix that is rich in platelets, leukocytes and growth factors. It has moderate strength, is easy to handle and promotes healing of the sinus membrane and bone (7).

Therefore, the present study was designed to evaluate the effect of PRF on bone regeneration in sinus lifting using piezosurgery and simultaneous implant placement.
MATERIALS AND METHODS
Patients Selection and Evaluation:
This study was conducted on seven patients selected from the Out-patient Clinic of the Oral and Maxillofacial Surgery Department, Faculty of Dentistry, Alexandria University. Patients were seeking prosthetic rehabilitation of missing maxillary posterior teeth and suffering from locally resorbed maxillary ridge, presenting a problem for dental implant insertion. All patients were informed about the aim of the designed study and a written consent was obtained.

The inclusion criteria of this study were: patients having missing maxillary posterior teeth, the vertical height between the alveolar crest and the floor of the maxillary sinus ranged between 4 mm and 7 mm, adequate inter-arch relation and inter-occlusal space that could accommodate the implant abutment and the future restoration, free from acute or chronic maxillary sinusitis as confirmed by Ear Nose And Throat (ENT) examination, recipient site free from any pathology, adequate oral hygiene, patients psychologically accepting dental implants and the involved procedure. While the exclusion criteria were; systemic diseases that contra-indicate implant placement such as uncontrolled diabetes mellitus, bleeding disorders, serious osseous disorders, cardiac arrhythmia, patients on pacemakers and mental disorders, heavy smoking and alcoholism, parafunctional habits such as bruxism and clenching.

Preoperative clinical examination was performed for all patients: Patients data were collected; name, gender and age, medical and dental history were taken and the oral mucosa of the edentulous area was examined for color, texture, firmness and thickness. All patients were clinically examined by ear nose and throat (ENT) specialist. Also, preoperative evaluation for all patients included panoramic x-ray and cone beam computerized tomography (CBCT), to evaluate the residual ridge height and width, the anatomy of the maxillary sinus, and to evaluate the condition of bone, as shown in (figure 1).

Fig.1: Preoperative CBCT (sagittal cut) showing maxillary posterior alveolar ridge deficiency.

Informed Consent:
Appropriate institutional ethical clearance and written informed consent were obtained.

Surgical procedure:
Preoperative oral sedation diazepam 10 mg (Valium 10 mg tab, Roche Pharmaceutical Co., Basel, Switzerland) tablets given 1 hour prior to surgery. Also, preoperative oral antibiotic prophylaxis one hour before surgery was given Amoxicillin (Amoxil 1gm capsule, Smithline Beecham Pharmaceutical Co., Bentford, England ) 2 mg capsule and 0.12% chlorohexidine HCL (Hexitol Mouth wash, The arab drug Co., Cairo, Egypt) mouth wash was used to rinse for 30 seconds before the surgical operation. Ephedrine spray/nasal drops 1.0 % (Otrivin spray/nasal Drops 10ml, Novartis Pharma AG, Basle, Switzerland) was used as a vasoconstrictor in order to decongest the nasal passages to minimize intraoperative bleeding.

The oral mucosa was painted and swabbed thoroughly with antiseptic povidone iodine solution 10 % (Betadine, Nile Company for Pharmaceuticals, Cairo, Egypt) to render the surgical field free from microorganisms. Under local anesthesia, a pyramidal full thickness mucoperiosteal flap was designed distal to the canine area with a crestal incision placed palatally in the edentulous area and vertical extension to the buccal vestibule using Bard Parker blade number 15. The flap was reflected to expose the alveolar bone.

A lateral window was performed using the piezosurgery device (Silfadent, Italy): Initially a bony window was cut using insert SB PO200 on a power 30/ 100 vibration microns, then the window was freed using SB PO321 on a power 20/100 vibration microns, as shown in (figure 2).

Fig. 2: A figure showing bone cutting using piezosurgery.

Finally the sinus membrane was dissected and elevated along with the trap door using SB PO321 on a power 20/100 vibration microns.

Drilling at the implant site was performed using an implant micromotor and Neobiotech implant drilling kit, implants (IS1 bone level Neobiotech Implant System,
Korea.) were used in this study. A platform switching is present between the implant and the abutment, which minimizes the microgap and maximizes the biologic width in order to minimize bone loss. It has a tapered body with powerful threads at the apex to facilitate implant insertion. They were inserted using a ratchet wrench until the implant body was flushed with bone surface.

**PRF preparation:**
Platelet rich fibrin (PRF) was prepared according to the protocol developed by Choukroun et al (10) (3000 RPM for 10 min) using a table centrifuge.

During surgery 20 ml of whole blood was obtained from the brachial vein, the blood was transferred and divided into two 10 ml sterile glass tubes without anti-coagulant. Immediate centrifugation was performed using a table centrifuge. The coagulation cascade starts during centrifugation and the blood is divided into 3 parts in the tube: serum at the upper part, red blood cells (RBCs) at the bottom and PRF in between which is separated and used as the augmentation material.

PRF was prepared and placed over the implants tented under the maxillary sinus membrane, as shown in (figure 3). Then the flap was repositioned and sutured using 3-0 black silk suture material.

All patients were advised to: apply cold packs extra orally intermittently and avoid hot food on the first day, apply hot packs on the second day, avoid eating hard food at the surgical site, chlorhexidine mouth wash was started on the 2nd post-operative day 3 times daily for 10 weeks. Broad spectrum antibiotic Amoxicillin 875 mg + Clavulanic acid 125 mg tablets (Augmentin 1 gm Smithline Beecham Pharmaceutical Co., Bentford, England) in combination with metronidazole 500 mg capsule (Amrizzol 500 mg tablets, Amriya Pharmaceutical Industries, Egypt) twice daily for 5 days to avoid post-operative infection. Non-steroidal anti-inflammatory analgesic in the form of diclofenac potassium 50 mg tablets (Cataflam 50 mg tablets, Novartis Pharma AG,Basle, Switzerland) 3 times daily for 7-10 days to avoid the possibility of inflammation, oedema and pain. Ephedrine nasal drops (Otrivin spray/nasal Drops 10 ml, Novartis Pharma AG, Basle, Switzerland) 3-5 times daily for 5 days. Sutures were removed after 10 days.

**Postoperative evaluation**
All patients were examined the day after surgery then weekly for the first month postoperatively, then on intervals of 1, 4 and 6 months postoperatively. The clinical parameters of importance for determination of implant success included: Absence of pain, tenderness, discomfort, wound dehiscence, implant mobility or any other complications related to the sinus lifting or implant placement. Pain and discomfort were examined using visual analogue scale (VAS). Patients were asked to assess the level of their average pain by placing a mark on a horizontal line that was 10 cm long (11). Tenderness by palpation and swelling was measured by inspection.

Immediate CBCT were obtained to evaluate the surgical procedure and implant placement, as shown in (figure 4).

**RESULTS**
In this study, seven sinus floor augmentations were performed on seven patients. The selected patients were 2 males and 5 females, and their age ranged from 28-60 years with a mean age (39.86 ± 13.02 years). The mean height of the alveolar ridge from the marginal crest to floor of the maxillary sinus was 5.84 mm ± 0.79 mm (Range: 4.49 - 6.8 mm), as shown in (table 1).
Figure 5: CBCT 6 months post-operatively (sagittal cut) showing new bone formation.

Table 1: Description regarding age, gender, implant location, preoperative bone width and height and implant length and diameter.

<table>
<thead>
<tr>
<th>Case #</th>
<th>Gender</th>
<th>Age</th>
<th>Implant location</th>
<th>Preoperative bone height (mm)</th>
<th>Preoperative bone width (mm)</th>
<th>Implant length (mm)</th>
<th>Implant diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>28</td>
<td>Premolar</td>
<td>6.8</td>
<td>5.5</td>
<td>10</td>
<td>3.5</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>28</td>
<td>Molar</td>
<td>6.5</td>
<td>4.7</td>
<td>10</td>
<td>3.5</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>30</td>
<td>Molar</td>
<td>5.2</td>
<td>6.35</td>
<td>13</td>
<td>3.5</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>38</td>
<td>Premolar</td>
<td>4.49</td>
<td>6.2</td>
<td>10</td>
<td>3.5</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>60</td>
<td>Molar</td>
<td>5.1</td>
<td>4.5</td>
<td>10</td>
<td>3.5</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>55</td>
<td>Molar</td>
<td>6.6</td>
<td>4.7</td>
<td>10</td>
<td>3.5</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>40</td>
<td>Molar</td>
<td>5.8</td>
<td>4.4</td>
<td>13</td>
<td>3.5</td>
</tr>
</tbody>
</table>

Seven implants were placed in the maxillary posterior teeth, 5 at the molar region and 2 in premolar region. The patients received implants with length ranging from 10.0 – 13.0 mm. All the placed implants were 3.5 mm in diameter.

1- Pain index:
Pain index recorded its highest ranging from 1-3 with a mean of (2.0 ± 0.82) during the 1st post-operative day, 0-2 with a mean of (1.00 ± 0.82) during the 1st post-operative week, 0-1 with a mean of (0.29 ± 0.49) during the 2nd post-operative week. While no pain was recorded since the 3rd week postoperatively till the rest of the follow up period. It was statistically significant starting from the 1st week postoperatively compared to the 1st post-operative day (p ≤ 0.05).

2- Tenderness
Tenderness was present only in 2 patients during the 1st post-operative day and absent in all patients during the rest of the follow up period. Swelling was observed in 3 patients during the 1st post operative day, and resolved completely in all cases during the 1st post-operative week with the absence of wound dehiscence in all cases.

3- Nasal obstruction and nasal bleeding
Nasal obstruction was a complaint for 3 patients during the 1st postoperative day. Only 2 patients had complained during the 1st and 2nd postoperative weeks. Nasal bleeding was absent in all cases. All implants were clinically stable and remained stable during abutment insertion.

4- Radiographic evaluation
CBCT 6 months postoperatively showed statistically significant increase in bone height and density. The newly formed bone height ranged from 5.12 – 8.10mm with a mean of (6.55 ± 1.14mm). The mean postoperative bone height measured from the floor of the maxillary sinus and alveolar crest was (11.35 ± 0.56mm) with a range from 10.40 – 11.90 mm. A significant change was indicated compared to the preoperative bone height, with a mean percentage of change 97.05%, as shown in (table 1, figure 6). The mean newly formed bone density was 507 HU and ranged from 250- 798 HU. The mean postoperative bone density around the implants after 6 month was (547.71 ± 188.42 HU) and ranged from (245.0 - 803.0 HU), which changed significantly compared to the preoperative bone density, with a mean percentage of change 88.79%, as shown in (table 2, figure 6).

The mean marginal bone loss was 0.82 ± 0.25 mm with a range of 0.48 – 1.20 at the 6th month postoperatively.

Figure 6: Charts comparing between preoperative and postoperative bone height and bone density.

Table 2: Comparison between preoperative and postoperative bone height.

<table>
<thead>
<tr>
<th>Bone height</th>
<th>Preoperative (n = 7)</th>
<th>Postoperative (n = 7)</th>
<th>% of change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Min. – Max.</td>
<td>4.49 – 6.80</td>
<td>10.40 – 11.90</td>
<td>60.88 – 128.85</td>
</tr>
<tr>
<td>Mean ± SD.</td>
<td>5.84 ± 0.79</td>
<td>11.35 ± 0.56</td>
<td>97.05 ± 25.70</td>
</tr>
<tr>
<td>Median</td>
<td>5.80</td>
<td>11.30</td>
<td>103.45</td>
</tr>
<tr>
<td>t (p)</td>
<td>16.729*</td>
<td>0.001</td>
<td></td>
</tr>
</tbody>
</table>

*: Statistically significant at p ≤ 0.05
Table 3: Comparing between preoperative and 6 months bone density around implants

<table>
<thead>
<tr>
<th>Bone density</th>
<th>Preoperative (n = 7)</th>
<th>Postoperative (n = 7)</th>
<th>% of change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Min. – Max.</td>
<td>142.0 – 591.0</td>
<td>245.0 – 803.0</td>
<td>12.39 – 171.83</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>324.29 ± 171.43</td>
<td>547.71 ± 188.42</td>
<td>88.79 ± 59.05</td>
</tr>
<tr>
<td>Median</td>
<td>280.0</td>
<td>540.0</td>
<td>65.87</td>
</tr>
<tr>
<td>t (p)</td>
<td>5.301 (0.002)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*: Statistically significant at p ≤ 0.05

DISCUSSION

Bone resorption and pneumatization of the maxillary sinus, following tooth extraction, are common in the posterior maxilla. They may cause both a quantitative reduction and a qualitative deterioration of bone that leads to inadequate bone dimension for proper size/length implant placement (12).

The present study was designed to evaluate the use of PRF as a sole agent in the augmentation of resorbed edentulous posterior maxilla following sinus lift using piezosurgery and simultaneous implant placement.

The selected patients were systemically free from any disease or systemic condition to avoid any systemic influence on bone formation or bone resorption. This was in accordance with an 11 years retrospective study performed by Moy et al (13) in 2005 that included systemic disease as a high risk factor for implant failure.

Also, heavy smokers were excluded from this study. This was in agreement with a study by Holahan et al (14) in 2008, where they concluded that implants in patients who were smokers during the time of implant placement were 2.6 times more likely to fail compared to implants placed in patients who did not smoke.

In this study, patients with parafunctional habits such as bruxism and clenching were also excluded and a strict oral hygiene was followed by all patients during the preoperative and postoperative follow up. This was in agreement with a study by Porter and von Fraunhofer (15) in 2005.

Also, patients in this study were selected free from any sinus pathosis after ear nose and throat (ENT) consultation. This is in agreement with a study conducted by Torretta et al (16) in 2013, which recommended that a careful multidisciplinary preoperative management, including an ENT assessment is useful in patients undergoing sinus membrane elevation.

In the current study, antibiotics were administrated preoperatively and postoperatively, as maxillary sinus augmentation and implant placement procedures run a risk of introducing new bacteria into the sinus. The antibiotic administration has been demonstrated not only to minimize the incidence of postoperative infection but also to significantly reduce the rate of implant failure as stated by Trieger (17) in 1999, Laskin et al (18) in 2000 and supported by Sharaf et al (19) in 2011.

The initial bone height from the alveolar crest till the floor of the maxillary sinus that received the implants ranged from 4.90 – 6.80 mm (Mean ± SD, 5.84 ± 0.79). This is supported by a recent study conducted by Mardinger et al (21) in 2011 to compare the radiographic dimensional changes of sinus graft height above and between placed implants, and evaluate the factors affecting these changes with 2 different grafting materials and both combination. They reported 92% success rate in patients with 1 to 3 mm of residual vertical bone height compared with 98.7% with residual bone height of more than 4 mm.

The lateral window approach was used in this study for sinus membrane elevation with a 100% success rate with no implant failure until the 6th month postoperatively. This is in agreement with Wallace and Froum (22) in 2003 that stated in their study a survival rate of 91.8%.

Piezoelectric surgery was used to access the maxillary sinus through the lateral window osteotomy and sinus membrane elevation to protect the soft tissues and minimize patient discomfort. This is in agreement with Al-Dajani (23) in 2014 and others (24) who concluded that the use of piezoelectric surgery allows adequate sinus lift while protecting soft tissues and minimizing patient discomfort.

Thus, the use of piezoelectric devices seems to simplify sinus lift surgical procedures and to allow greater predictability with less postoperative complications.

This is in agreement with Carini et al (25) in 2014 where they reported that bone tissue healing showed a reduced rate of bone loss with piezoelectric instruments than with conventional devices, as well as a better healing quality by reducing patient’s postoperative morbidity.

PRF was used in this study because it is a fibrin-matrix in which platelet, cytokines and cells are entrapped and released after a certain time and can serve as resorbable membrane, it is considered to be a healthy biomaterial, and was initially used in oral implantology by its promoters, and presently, its application has been advocated in various disciplines of dentistry (26).

PRF concentrates most platelets and more than half of live and functional leukocytes from the blood harvest (27) which releases high amounts of growth factors (such as transforming growth factor-β1 [TGFβ-1], platelet-derived growth factor-AB [PDGF-AB]. Thus, it is an autologous and inexpensive material, which is considered as an optimized blood clot, as stated by Tajima et al (7) in 2013.

In the present study no wound dehiscence was present postoperatively in any of the cases and radiographic evaluation by CBCT 6 month postoperatively revealed the absence of any fluid level or inflammatory process.

Nasal bleeding was absent in all cases postoperatively during the follow up period. While pain and discomfort scale significantly changed after the first day. Swelling was present in 3 cases on the first day only and nasal obstruction was a postoperative complain in 3 cases on the first day, with its absence in all cases for the rest of the follow up period.

This coincides with Carini et al (24) in 2014, in their study where they observed better healing quality by reducing patient’s postoperative morbidity when using piezosurgery.

Also, in agreement with, Dohan et al (28) in 2006 who stated that PRF might decrease many harmful effects due to...
inflammatory processes that are inherent to the surgical act itself, mainly by correcting certain destructive and noxious excesses during the healing process of wounded tissues.

In addition, He et al (29) in 2009 in an in vitro study reported the superiority of PRF in the expression of alkaline phosphatase and induction of mineralization. They remarked that PRF released autologous growth factors gradually and expressed stronger and more durable effect on proliferation and differentiation of osteoblasts than PRP.

In the present study, CBCT obtained 6 months after surgery revealed sufficient newly formed bone in all treated cases, elevating the sinus membrane showing repneumatization around the implants apices. The new bone showed adaptation in shape and volume with the repneumatized maxillary sinus with a decreased noted visibility of the original sinus floor. The apex of the implants remained surrounded with bone radiographically. This indicated the stability of the newly formed bone and bone maturation. The sinus membrane is maintained elevated and the bone gained is preserved.

This is in agreement with a study conducted by Simonpieri et al (30) in 2011, where they observed that the final level of the new sinus floor was always in continuation with the implant apical end.

In this study, there was a significant change in bone height. The new bone height formed measured from the floor of the maxillary sinus was 5.12 ± 8.10 mm (Mean = 6.55 ± 1.14). The total over all bone height at the end of the procedure at a 6 month follow up period was 10.40 – 11.90 mm (Mean = 11.35 ± 0.56), with a mean percentage of change 97.05%, which relatively corresponds to the actual length of the inserted implants. Because the implants served as a tent to maintain the height of the bone healing space the final vertical bone height was dependent on the implant length.

This is in agreement with a study conducted by Mazor et al (27) in 2009, where the radiographic analysis showed that the final bone gain was always significant with the implant length.

Norton & Gamble (31) in 2001 suggested that Bone density can be evaluated using Hounsfield units, which are directly related to tissue attenuation coefficients taken from CT. And recorded the mean bone density was 682 HU for 139 sites. While, the mean bone densities in the anterior maxilla and the posterior maxilla were 696, and 417 HU respectively.

In the present study, the density of the new bone formed around implants after 6 month ranged from 250.0 – 798 HU (Mean = 507.0 ± 191.24), which is comparable to that of bone normally present in the maxilla, with a significant change in bone density around the implants comparing the preoperative bone density around the implants that ranged from 124.0 – 591.0 HU (Mean = 324.29 ± 171.43) and bone density around the implants 6 month postoperatively that ranged from 245.0 – 803 HU (Mean = 547.71 ± 188.42), with a mean percentage of change 88.79%.

This is in agreement with the results of Sogo et al (32) in 2012, where they studied the bone density of the posterior maxilla in 30 patients and concluded that the bone in the posterior maxilla was classified as D3 (350–850 HU) or D4 (150–350 HU) according to Misch’s classification, comprising 50% and 32% of the entire regions, respectively.

The radiographic evaluation 6 month postoperatively using CBCT measures showed that the marginal ridge resorption ranged from 0.48 – 1.20 mm (Mean = 0.82 ± 0.25). This coincides with a study that mentioned the mean marginal bone loss within the first year was 1.2+/0.7 mm conducted by Neder et al (33).

CONCLUSIONS
The use of piezosurgery is safe and reduces both intraoperative and postoperative complications. Platelet rich fibrin is an autologous and inexpensive material. Sinus floor augmentation with PRF as a sole material is a secure and reliable method for promoting natural bone formation prior to implant placement. It does not require placement of additional bone grafting material.

STATEMENT OF CONFLICT OF INTEREST
The authors declare that they have no conflicts of interest.

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CLINICAL AND RADIOGRAPHICAL EVALUATION OF IMMEDIATE IMPLANT VERSUS DELAYED IMPLANT AFTER SOCKET PRESERVATION OF UPPER ANTERIOR TEETH

El Sayed E¹ BDS, Khalil A² PhD, Saleh M² PhD.

Abstract:
Introduction: After tooth extraction, the alveolar bone remodeled and resorbed. The preservation of ridge resorption following tooth extraction through socket grafting helped to optimize bony fill, thereby maintaining vertical bone height and helped to stabilize the marginal soft tissue at the site. Adequate bone allowed the implant to be placed in the most ideal restorable position in a three dimensional aspect which yielded higher long-term success.

Objectives: Clinical and radiographical evaluation of immediate post extraction implants versus delayed implants after socket preservation.

Materials and Methods: 14 patients were selected for this study and divided into two groups, study group consist of 7 patients with extracted upper anterior teeth and the sockets have been preserved with Easy-graft material, the implants were placed after 6 months of healing. In control group implants were placed immediately after extraction of upper anterior teeth with placement of Easy-graft material around them.

Results: Clinical evaluation of all patients showed no signs of gingival inflammation and none of the implants showed any signs of mobility all over the study period. Regarding peri-implant probing depth and marginal bone height, there was no statistically significant difference between the two groups.

Conclusions: There was no difference between immediate implant and delayed implant after socket preservation with regards to the marginal bone height and bone density.

Key words: Socket preservation, delayed, immediate, implants.

INTRODUCTION

After tooth extraction, the alveolar bone remodels and resorbs. Two-thirds of this occurs within the first 3 months and within 1 year the clinical width of the alveolar ridge is reduced by approximately 50%. The mean vertical loss of tissues at single extracted sites ranges between 1 and 4 mm depending on site location. This localized alveolar bone resorption may affect the possibility of placing dental implants and their aesthetic outcome. Prevention of ridge resorption following tooth extraction seems important, particularly if implant placement needs to be delayed for 6 months or longer. Site preservation through socket grafting will help to optimize bony fill within the extraction socket, thereby maintaining vertical bone height and helping to stabilize the marginal soft tissues at the site. This generally results in a healed site, which lends itself well to implant placement with a high degree of predictability as well as improved soft tissue contour or 'pink' aesthetic (1-3).

A number of different materials have been suggested for use in extraction sockets, however not all are suitable. The decision on what is used should be based on biological principles. A graft material should be biocompatible, support vital bone formation into the socket in order to allow for successful osseointegration of the dental implant that will subsequently be placed into the site. Thus, it is also preferable that the material used is resorbable and ultimately replaced with long term vital bone (4-9).

Easy-graft materials are bioresorbable, completely synthetic bone graft substitutes for bone defects. In contact with body fluids such as blood or saliva the material hardens within minutes and forms a stable, porous bone substitute material. Due to the porosity of the material, the absorption of blood is possible and thus positively influences the healing process (10).

This study aims to compare clinically and radiographically between implants placed immediately after tooth extraction in fresh extraction sockets with implants placed in a preserved socket after 6 months of healing.

MATERIALS AND METHODS

Patients

Fourteen patients were selected for this study from the Outpatient Clinic of Oral and Maxillofacial Surgery Department, Faculty of Dentistry Alexandria University. The Patients were divided into two groups:

Group I (study group): This group consisted of seven patients with extracted upper anterior teeth followed by socket preservation with easy graft material. The implant was placed after 6 months of healing.

Group II (control group): This group consisted of seven patients with upper anterior teeth indicated for extraction. The implant placed immediately after extraction and easy graft was grafted at the coronal third of the socket.

Inclusion criteria

- Extracted upper anterior tooth with the presence of adjacent dentition.
- Presence of adequate and healthy gingiva of surrounding dentition.
- Good oral hygiene.
- Patient age ranging from 20-50 years old.
Exclusion criteria
- Presence of active infection around the tooth.
- Medically compromised patient.
- Dental history of bruxism and parafunctional habits.
- Untreated periodontitis.
- Bone disease that affects bone formation.
- Patient with insufficient inter-occlusal and mesiodistal spaces for implant placement.
- Smokers.

Informed consent
An informed consent was obtained from the patients. All the procedures were described in detail including the benefits and side effects. It was also mentioned that the patient has the right of withdrawal from the study anytime according to their wish.

Materials
Implant System: Dentium implant system (Superine, 3105 Trade Tower 159, Samsung-dong, Gangnam-gu, Seoul, Korea).

The implant is available in different diameters ranging from (3.6 mm to 7.0 mm) and lengths ranging from (7 mm to 14 mm) according to the requirement.

Graft material: Easy-graft TM" classic" (Degradable solution AG, Wagistrasse 23, 8952 Sthlieren, Switzerland). Easy graft classic materials are bioreabsorbable and are completely synthetic bone graft substitutes for bone defect. The material is supplied in a syringe form, which is filled with roundish, porous granules of pure phase beta tricalcium phosphate each granule is coated with 10 micron of fast resorbing polyactic-co-glicolic acid, and an ampoule of liquid activator, biolinker (organic solvent consisting mostly of n-methyl-2-pyrrrolidone) (11). When combining these two components, the easy – graft material becomes a putty-like biomaterial and can be applied directly from the syringe into the bone defect. In contact with body fluids such as blood or saliva the material hardens within minutes and form a stable porous bone substitute material.

Pre-surgical phase
- Primary alginate impression was taken for both arches and casting diagnostic study models.
- Evaluation of interarch relationship, interocclusal space that could accommodate the implant abutment and the future crown restoration both clinically and on the study model.
- Fabrication of surgical guide stent using the primary model.
- Orthopantomogram (OPG) was done for all patients to detect bone quality and approximation to important anatomical structures.
- Cone beam to detect length and diameter of implant.

Surgical phase
Group I (study group)
- All patients were operated under local anesthesia secured with Mepevacaine Hydrochloride 2% with Levonordefrin 1:20000 (Mepecaine L, Alexandria Co. for pharmaceutical & chemical industries, Alexandria, Egypt) and strict aseptic conditions.
- Crestal incision was made and flap reflected.

Group II (control group)
- Pilot osteotomy was drilled with surgical stent in place.
- Final osteotomy was drilled with drills of subsequent sizes up to the proper size of the implant. (Figure 1)
- Implant was inserted using hand wrench and then finally seated down to the full depth using ratchet wrench.
- Implant mount was removed and cover screw derived in place. (Figure 2)
- The flap was sutured with interrupted sutures.

• All patients were operated under local anesthesia.
• Atraumatic extraction of upper anterior tooth in order to protect and preserve the alveolar bone. (Figure 3)
• Curettage and proper debridement of the socket to remove any inflamed tissues.
• The socket was prepared with drills of subsequent sizes up to the proper size of the implant. Osteotomy was extended 3-4 mm beyond the root apex.

Fig. 1: Showing osteotomy for implant placement (study group)

Fig. 2: Showing implant and cover screw in place (study group)

Fig. 3: Showing tooth extraction (control group)
El Sayed et al.

Immediate Versus Delayed Implant After Socket Preservation

- Immediate implant was placed in position.
- Implant mount was removed and cover screw derived in place. (Figure 4)
- The easy graft material was prepared then packed in the socket around the implant at the coronal third.
- Suturing of the surgical site.

**Post surgical phase**
- Postoperative instructions were given to the patients including cold packs on the first day, then warm mouth wash for the following days beside oral hygiene instructions.
- Antibiotic Amoxicillin 500 mg (Emox 500 cap, Medical union pharmaceuticals, Abu-Sultan, Ismailia, Egypt) was prescribed for 5 days, 1 capsule every 8 hours.
- Non-steroidal anti-inflammatory drug Ibuprofen 400 mg (Brufen 400 tab, Kahira pharma and Chem.ind.com, Cairo, Egypt) was prescribed for 3 days, 1 tablet every 8 hours.
- Chlorhexidine mouth wash (Hexitol MW, Arab drug company, Cairo, Egypt) for 7 days.
- Recall visit – the first week for suture removal.

**Prosthetic phase**
- Final restoration (porcelain fused to metal crown) was placed at 3 months following surgery.

**Follow up phase**

A- Clinical evaluation
- Each patient was clinically examined on intervals of 3 and 6 months. Patients were evaluated clinically for:
  - Mobility of the implant according to Mickney and Koth(12)
  - Mobility was tested using back and forth pressure by two instrument handles. The clinical implant mobility scale is:
    - Absence of clinical mobility in any direction (Scale 0), slight detectable horizontal movement (Scale 1), moderate visible horizontal mobility up to 0.5 mm (Scale 2), severe horizontal movement greater than 0.5 mm (Scale 3) or visible moderate to severe horizontal movement and any visible vertical movement (Scale 4).
  - Modified gingival index was used to assess the severity and quantity of gingival inflammation (13). Absence of inflammation (scale 0), mild inflammation or with slight changes in color and texture but not in all portions of marginal or papillary gingival unit (scale 1), mild inflammation, such as the preceding criteria, in all portions of gingival marginal or papillary gingival unit (scale 2), moderate, bright surface inflammation, erythema, edema and/or hypertrophy of marginal or papillary gingival unit (scale 3) or severe inflammation including erythema, edema and/or marginal gingival hypertrophy of the unit or spontaneous bleeding, papillary, congestion or ulceration (scale 4).

  Peri-implant probing depth was done according to Clavind and Loe (1967) (14). After the final prosthesis was placed, peri-implant probing was done at the 3rd and 6th months as follow-up. Probing pocket depth refers to the distance from the gingival margin to the bottom of the pocket. Mesial and distal pockets were measured from the buccal aspect as close as possible to contact points while facial and lingual pockets were measured at the midline of the implant.

B- Radiographic evaluation
- It was done immediately after implant placement, then at 3 and 6 months intervals post operatively. (Figures 5, 6)
  - Standardized periapical x-ray films were taken using paralleling long cone technique by XCP film holder for standardization of serial radiographs, then indirect digital radiography by Image J software (15). These radiographic films were used to verify: bone density and marginal bone level around the implant.
  - Assessment of marginal bone height around the implants: mesial and distal bone height changes of the implants were evaluated using the linear measurement system supplied by the specially designed Image J software.

![Fig. 4: Showing implant and cover screw in place (control group)](image)

![Fig. 5: Periapical x-ray for implants placed in study group; a) Immediate postoperative, b) After 3 months, c) After 6 months.](image)
RESULTS

Clinical evaluation
Implant Mobility was recorded all over the evaluation period, none of the implants showed any signs of mobility (i.e. mobility score was 0).

No signs of gingival inflammation were observed in all patients all over the evaluation period (i.e. modified gingival index score was 0).

Probing depth was measured for all the axial surfaces of all implants and statistical analysis of probing depth scores was done for all patients. Data collected were tabulated (table 1).

Radiographic evaluation
Data were collected regarding the marginal bone height at the mesial and distal aspects of all implants. The mean marginal bone level values and standard deviation at 3 and 6 months of both groups were tabulated. (Table 2)

Table 1: Comparison between the two studied groups according to probing depth.

<table>
<thead>
<tr>
<th></th>
<th>Study</th>
<th>3rd Month</th>
<th>6th Month</th>
</tr>
</thead>
<tbody>
<tr>
<td>Min. – Max.</td>
<td>1.25 – 2.50</td>
<td>0.75 – 2.0</td>
<td></td>
</tr>
<tr>
<td>Mean ± SD.</td>
<td>1.82 ± 0.51</td>
<td>1.32 ± 0.57</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>1.50</td>
<td>.50</td>
<td></td>
</tr>
<tr>
<td>p_1</td>
<td>0.002*</td>
<td>&lt;0.001*</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>3rd Month</th>
<th>6th Month</th>
</tr>
</thead>
<tbody>
<tr>
<td>Min. – Max.</td>
<td>1.70 – 2.75</td>
<td>1.25 – 2.36</td>
<td></td>
</tr>
<tr>
<td>Mean ± SD.</td>
<td>2.30 ± 0.35</td>
<td>1.72 ± 0.39</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>2.35</td>
<td>1.75</td>
<td></td>
</tr>
<tr>
<td>p_1</td>
<td>0.003*</td>
<td>0.001*</td>
<td></td>
</tr>
</tbody>
</table>

| t | 2.024 | 1.509 |
| p | 0.066 | 0.157 |

*: Statistically significant at p ≤ 0.05

On the third month, the mean probing depth scores for the study group was 1.82 ± 0.51 while the mean probing depth scores for the control group was 2.30 ± 0.35. This difference in the probing depth score between the control and study group was found to be statistically not significant. (p= 0.066).

On the sixth month, the mean probing depth scores for the study group was 1.32 ± 0.57 while the mean probing depth scores for the control group was 1.72 ± 0.39. This difference in the probing depth score between the control and study group was found to be statistically not significant. (p=0.157).

Table 2: Comparison between the two studied groups according to marginal bone height

<table>
<thead>
<tr>
<th></th>
<th>3rd Month</th>
<th>6th Month</th>
</tr>
</thead>
<tbody>
<tr>
<td>Min. – Max.</td>
<td>0.15 – 0.37</td>
<td>0.20 – 0.57</td>
</tr>
<tr>
<td>Mean ± SD.</td>
<td>0.22 ± 0.07</td>
<td>0.44 ± 0.13</td>
</tr>
<tr>
<td>Median</td>
<td>0.20</td>
<td>0.50</td>
</tr>
<tr>
<td>p_1</td>
<td>&lt;0.001</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>3rd Month</th>
<th>6th Month</th>
</tr>
</thead>
<tbody>
<tr>
<td>Min. – Max.</td>
<td>0.12 – 0.20</td>
<td>0.30 – 0.50</td>
</tr>
<tr>
<td>Mean ± SD.</td>
<td>0.17 ± 0.03</td>
<td>0.44 ± 0.13</td>
</tr>
<tr>
<td>Median</td>
<td>0.18</td>
<td>0.30</td>
</tr>
<tr>
<td>p_1</td>
<td>&lt;0.001</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

| T | 1.663 | 0.814 |
| P | 0.122 | 0.431 |

*: Statistically significant at p ≤ 0.05

The distance from the top of the implant platform and the first point of bone-implant contact mesially and distally were used to represents the bone defect.

Measurement of the bone density around the implant: Image J software was used to evaluate radiographic bone density mesial and distal to each implant.

Statistical analysis of the data
Comparison between different groups regarding categorical variables was tested using Chi-square test. For normally distributed data, comparison between two independent populations was done using independent t-test, also paired t-test was used to analyze two paired data. Significance of the obtained results was judged at the 5% level.

Table: Fig. 6: Periapical x-ray for implants placed in control group; a) Immediate postoperative, b) After 3 months, c) After 6 months.
On the third month, the mean marginal bone level value for the study group was 0.22 ± 0.07 while the mean marginal bone level value for the control group was 0.17 ± 0.03. This difference in marginal bone level value between the study and control groups was not statistically significant. (p=0.122).

On the sixth month, the mean marginal bone level value for the study group was 0.44 ± 0.13 while the mean marginal bone level value for the control group was 0.44 ± 0.13. This difference in marginal bone level value between the study and control groups was no statistically significant. (p=0.431).

Mean peri-implant bone density values and standard deviation immediately post-operative, at 3 months and at 6 months were shown in (Table 3).

| Table 3: Comparison between the two studied groups according to bone density |
|---------------------------------|------------------|------------------|------------------|
|                                | Bone density      |                  |
|                                | Immediate         | 3rd Month        | 6th Month        |
| Study                          | postoperative     |                  |
| Min. – Max.                    | 85.10 – 101.51    | 86.64 – 106.38   | 90.33 – 107.02   |
| Mean ± SD.                     | 94.88 ± 5.45      | 96.74 ± 6.12     | 98.35 ± 5.28     |
| Median                         | 97.20             | 98.23            | 99.25            |
| $p_1$                          | 0.014*            | 0.001*           |
| Control                        |                  |                  |
| Min. – Max.                    | 81.48 – 91.94     | 85.91 – 103.32   | 93.22 – 105.0    |
| Mean ± SD.                     | 93.88 ± 3.96      | 95.95 ± 5.65     | 98.80 ± 3.58     |
| Median                         | 94.95             | 97.55            | 98.95            |
| $p_1$                          | 0.020*            | 0.003*           |
| $T$                            | 2.747*            | 0.251            | 0.184            |
| $P$                            | 0.701             | 0.806            | 0.857            |

$T$: Student t-test
$p_1$: p value for Paired t-test for comparing between Immediate postoperative with each other periods in each group
*: Statistically significant at $p \leq 0.05$

Immediately post-operatively, the mean peri-implant bone density value for the study group was 94.88 ± 5.45 while the mean peri-implant bone density value for the control group was 93.88 ± 3.96. This difference in the peri-implant bone density value between the study and control groups was statistically not significant (p=0.701).

On the third month, the mean peri-implant bone density value for the study group was 96.74 ± 6.12 while the mean peri-implant bone density value for the control group was 95.95 ± 5.65. This difference in peri-implant bone density value between the study and control groups was statistically not significant (p=0.806).

On the sixth month, the mean peri-implant bone density value for the study group was 98.35 ± 5.28 while the mean peri-implant bone density value for the control group was 98.80 ± 3.58. This difference in peri-implant bone density value between the study and control groups was statistically not significant (p=0.857).

**DISCUSSION**

The success of osseointegrated dental implants depends on whether there is a sufficient volume of healthy bone at the recipient site at the time of implant placement. The placement of an implant at a site with a thin crestal ridge (e.g., post extraction ridge) could result in a significant buccal dehiscence. Thus, it seems prudent to prevent alveolar ridge destruction and make efforts to preserve it during extraction procedures (16).

Alveolar ridge preservation is a surgical procedure aimed at retaining maximum bone and soft tissue after a tooth has been removed. By maintaining the original ridge morphology, there will be a minimal need for augmentation procedures thereby allowing the resultant restoration to be placed in an aesthetically and functionally ideal position (17).

Simion et al (18) reported that success rates are satisfactory when placing implants in previously grafted bone. In a retrospective study of 607 titanium plasma sprayed implants placed in regenerating bone (with DFDBA), 97.2% of maxilla implants and 97.4% of mandible implants were successful for an average of 11 years.

This study was designed to assess whether it could be advantageous to place implants immediately after tooth extraction or if it would be preferable to preserve the socket, wait for bone healing and then place the implant.

Seven implants were placed after 6 months of socket preservation (study group) while the other implants were placed immediately after teeth extraction (control group).

As regarding patient selection, all patients were free from any systemic diseases such as diabetes mellitus, renal and endocrinial disturbances, blood dyscrasias and osteoporosis. All patients in the current study were non-smokers. Nicotine, which is the major component of tobacco, is cytotoxic and prevents differentiation of osteoblasts like cells to osteoblasts thus reducing alveolar bone quality (19).

Regarding the surgical procedure, all included patients were subjected to delicate surgery using delayed implant placement protocol for the study group and immediate placement protocol for the control group. In the study group a crestal incision and elevation of full thickness mucoperiosteal flap was performed to provide adequate access. In the control group atraumatic extraction technique used to preserve the buccal plate of bone. In both groups a low speed high torque hand piece was used for the preparation of the implant bed, and the drilling was performed under profuse irrigation using cold normal saline for proper cooling and to avoid overheating of the bone tissues which would compromise osseointegration in accordance to Strbac et al (20). This also matches findings obtained by Lee et al (21), and Augustin et al (22).

In the present study, a two - stage implantation procedure was selected to allow for prolonged direct bone-implant interface and unimpeded healing of the augmented ridge before implant exposure to functional load (23).
In this study we used easy graft material (pure beta tricalcium phosphate) as a bone graft substitute which was prepared by mixing the granules with biolinker then was packed around the implant in the coronal gap between the fixture and the socket wall. This agreed with Ormianer et al. (2006) (24). They assessed the survival of 1065 immediately placed dental implants in augmented alveolar bone sites in 338 patients. Beta tricalcium phosphate was used to augment the alveolar ridge level, fill spaces between the implant and socket wall. 97.6% of the implants survived during the observation period of 12 to 48 months.

In the present study, wound closure was performed very carefully using 3/0 black silk suture material in order to prevent postoperative infection and inflammation, epithelial down growth and bone loss of the alveolar crest during the healing period as recommended by Becker and Becker in 1990 (25).

Postoperative medications including antibiotics, mouth washes, analgesics and anti-inflammatory drugs were prescribed to all patients. Oral hygiene instructions were given to all patients early in their treatment and reinforced during the subsequent appointments so as to decrease the possibility of plaque accumulation and inflammation around the implants. This enhances the success rate of implant osseointegration and prosthetic rehabilitation (26).

Regarding implant mobility, no clinical mobility was detected in any of the implants throughout the follow up period. This was confirmed by radiographic evaluation that revealed intimate bone implant contact and absence of peri-implant radiolucency.

All cases showed a modified gingival score of 0 throughout the evaluation period indicating absence of peri-implant mucositis, which is a criteria of implant success as peri-implant mucositis may lead to progressive bone destruction (peri-implantitis) and ultimately to implant failure as reported by Esposito et al. (27).

Regarding the mean peri-implant probing depth in the present study there was a decrease throughout the whole evaluation period for both groups. Similar results were reported by Al-Ansari and Morris (28).

Regarding to peri-implant bone level; there was no statistically significant difference between two groups all over the evaluation period. This could be attributed to the effect of the easy graft material as an osteoconductive bone graft.

Both groups showed increase in peri-implant bone density from the immediate postoperative period to the end of the 6 months of the evaluation period, which indicates osseointegration of all implants.

**CONCLUSIONS**

Within the limits of this study the overall conclusion that can be drawn from this study is that there is no significant difference in marginal bone level and bone density around implants placed after socket preservation compared to those placed immediately after tooth extraction.

It is apparent from this study that maintenance of an extraction socket for future implant therapy does not exclude immediate implant placement, but knowledge and experience are needed to determine the best treatment modality.

**STATEMENT OF CONFLICT OF INTEREST**
The authors declare that they have no conflicts of interest.

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EVALUATION OF DYNAMIC COMPRESSION MINIPLATES IN TREATMENT OF MANDIBULAR ANGLE FRACTURES USING TROCAR

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Abstract:
Introduction: Mandibular angle fractures (MAFs) account for 23% to 42% of all mandibular fractures. The frequent involvement of the angle in mandibular fractures can be attributed to its thin cross-sectional bone area and the presence of a third molar.

Objectives: The aim of this study was to evaluate clinically and radiologically the use of two dynamic compression miniplates in treatment of mandibular angle fractures using Trocar instrumentation.

Materials and methods: The study was conducted on ten patients diagnosed with a single mandibular angle fracture indicated for open reduction and internal fixation. The fracture was exposed through transoral vestibular incision and reduced under general anaesthesia. Third molar tooth in the fracture line was extracted. Temporary Maxillomandibular fixation was done using 26 gauge stainless steel wires with arch bars. One dynamic compression miniplate was fixed on the external oblique ridge. The second miniplate was fixed on the lower border of the mandible using Trocar instrumentation.

Results: Pain, edema and trismus decreased significantly over the follow up period. Maximum interincisal mouth opening and bone density along the fracture line increased significantly over the follow up period. Five patients had parasthesia of the inferior alveolar nerve preoperatively. The parasthesia decreased progressively from the fourth week. Postoperative panorama radiographs showed adequate reduction and increase in bone density along the fracture line. No postoperative complication occurred.

Conclusion: Two 2.0 mm dynamic compression miniplates are suitable for treatment of mandibular angle fractures using Trocar instrumentation.

Key words: Dynamic compression miniplates, mandibular angle fractures, Trocar.

INTRODUCTION
Mandibular angle fractures (MAFs) account for 23% to 42% of all mandibular fractures (1). The frequent involvement of the angle in mandibular fractures can be attributed to its thin cross-sectional bone area and the presence of a third molar (2). The treatment of these mandibular fractures has changed dramatically in recent years. Traditional 6-week treatment of closed reduction with Maxillomandibular fixation (MMF) or open reduction with wire osteosynthesis and MMF has given way to Open Reduction and internal fixation (ORIF) osteosynthesis techniques with early mobilization and restoration of jaw function, improved airway control, nutrition, patient comfort and hygiene, and an early return to work (3). In treatment planning for a mandibular fracture, there are two schools of plate osteosynthesis, one advocating compression osteosynthesis and the other favoring the miniplate osteosynthesis (4).

The technique of rigid internal fixation was developed and popularized by Arbeitsgemeinschaft für Osteosynthesefragen (AO) in Europe in the 1970s. It showed primary bone healing under conditions of absolute stability (5). It has been shown that two segments of cortical bone brought into direct contact and fixed with absolute stability will heal without the formation of an external callus. Primary bone healing is rarely achieved with rigid fixation due to incomplete reduction of the fracture. The (AO) Swiss Association for internal fixation (ASIF) introduced the idea of axial compression into the limb fractures. As applied to the mandible, the idea of compression osteosynthesis was fortified with dynamic compression miniplates (DCP) (4, 6).

During function the balanced side of the mandible is subject to bending, which exerts tensile forces on alveolar part of the mandible (7). Rigid internal fixation must neutralize all forces (tension, compression, torsion and shearing) developed during function. Multiple fixation techniques were developed to achieve this goal including lag screw, and miniplate osteosynthesis (8).

The reduced size of miniplate system offers several advantages over the larger mandibular compression plate. Small incisions and minimal soft tissue dissection are necessary for their placement. Miniplates can be easily contoured in three dimensions. Special plate-bending pliers are used to achieve passive adaptation of the plate to the bone. Due to their small size, they will not be palpable extraorally and hence will not require a second operation for plate removal. Mini DCPs are a smaller version of the standard mandibular compression plates. The use of mini DCPs merges the principles of miniplate osteosynthesis and compression osteosynthesis (9).

Several studies have been done using conventional miniplates for fixation of the fracture of the angle of the mandible by extra oral approach (4, 10). Treatment of mandibular angle fractures by using Trocar instrumentation to fix dynamic compression mini plates offers the advantages of compression and miniplate osteosynthesis. The transbuccal technique involves the use transoral incision...
for fracture reduction and Trocar instrumentation for fixation of the DCPs. This study set out to evaluate the use of two 2.0 mm DCPs in treatment of mandibular angle fractures by Trocar instrumentation.

**MATERIALS AND METHODS**

This was a prospective clinical and radiographic study. It received clearance by the institutional ethics committee of the Faculty of Dentistry Alexandria University. It was conducted on patients selected from the emergency room in main Hospital-Alexandria University and treated in Oral and Maxillofacial Surgery Department. They were then followed up in the outpatient clinic.

**Patients**

A sample of ten patients who met the inclusion criteria were selected purposefully and a written informed consent obtained before treatment.

**Inclusion criteria**

- Patients with recent isolated fracture angle of the mandible.
- Dentate or partially edentulous.
- Adult patients with age range between 18 to 50 years old.
- Male and female patients

**Exclusion criteria**

- Patients who were medically or immunologically compromised.
- Patients with comminuted fractures.
- Patients presenting with infection at the fracture site.
- Smokers

**Materials used for intervention**

1- Trocar set (Jeil Medical Corporation, Seoul, Korea).
   Titanium mini dynamic compression plates and screws. (Figure 1)
2- Erich arch bars, 24 gauge Stainless Steel wire, and Wire cutter.
3- Complete Oral and Maxillofacial trauma surgical set.

**Preoperative Preparation**

The selected patients were evaluated by taking history and conducting thorough clinical and radiographic examination. Information about events immediately after injury such as loss of consciousness, bleeding from the ear, nose, mouth or any other part of the body, emergency treatment and medicine received was recorded. The patient’s past dental and medical history including previous treatment, chronic diseases, anticoagulants, drug allergies, transfusion, smoking and alcohol use were recorded.

On extraoral inspection, presence or absence of a swelling, ecchymosis, facial asymmetry or deformity, laceration especially on the chin area, or deviation of the mandible during opening and closing of the mouth were noted. Palpation was done starting medially and proceeding laterally with the fingertips of both hands. Any step deformity, alteration in bony contour, tenderness, and bony crepitus indicated the site of fracture. Condylar movements during opening and closing of the mouth were checked to rule out dislocation or any derangement. Anterior open bite, deviation of mandible and malocclusion were checked. The degree of mouth opening was checked as well as tongue movement.

Intraorally, bimanual palpation of the buccal and lingual sulci was done for the presence of tenderness and step deformity. The area innervated by the mental nerve was checked for numbness by pricking using a sharp dental probe. The left side was compared to the right to decide presence of anaesthesia or parasthesia. Presence of blood stained saliva, lingual haematoma or ecchymosis in the buccal sulcus pointed to the likely position of the fracture. The position of the teeth thus bent, missing or filled or over erupted was noted, and then the patient was told to close the mouth in a relaxed centric occlusion to check for malocclusion.

Standard Orthopantomogram (OPG) and a Posterior Anterior (PA) radiographs were done pre-operatively (Figure 2 (a) and (b) respectively). A final diagnosis and indication for ORIF was made. Prophylactic antibiotic were administered in the form of Amoxicillin/Clavulanic acid (Augmentin 625 mg, GlaxoSmithKline, Hungary) orally three times daily for three days.

**Fig. 1:** Trocar set, Titanium mini dynamic compression plates and screws.

**Fig. 2:** a. Preoperative OPG x-ray,
   b. Preoperative posterior anterior view x-ray (PA)
Operative procedure

General anesthesia was given to all patients by Nasotracheal intubation. The oral cavity was disinfected and then all the extraoral areas. The patient was draped with sterile towels. Management of the tooth in the fracture line was done according to each case. Ivy loops or arch bars were fixed to the teeth present according to the case. The fracture was exposed through intraoral extended vestibular incision which was made three to five millimeters from the mucogingival margin. A full mucoperiosteal flap was elevated and hemostasis achieved by use of diathermy. The fracture segments were reduced manually and held temporarily into perfect anatomic position in normal occlusion. MMF was secured to line up the teeth into the normal occlusion of the patient. Fixation of the fractured segment using two 2.0 mm dynamic compression miniplates was done. The first plate was placed Transorally on the external oblique ridge following Champy’s line of ideal osteosynthesis (12) and fixed by use of monocortical screw. A stab incision was made perpendicular to the fracture and trocar with cannula secured in place by holding the Handle. The intraoral retractor was attached to the handle and the intraoral mucogingival flap retracted (Figure 3a).

The second miniplate was placed at the lower border of the mandible transorally. It was secured using transbuccal Trocar instrumentation to fix bicortical screws (Figure 3b). The MMF was removed and the fixation tested manually. The trocar was removed.

Postoperative follow up

The patients were monitored for 24 hours then reviewed after one, two, four, six and twelve weeks post-operatively. Antibiotics were continued post-operatively for 5 days. Analgesics and anti-inflammatory drug in the form of diclofenac potassium 50 mg tablets ( Cataflam50 mg, Novartis company) was given three times daily for five days. Anti-edematous drug in the form of α-chymotrypsin ampoules intramuscular injection (α- Chymotrypsin 5 mg, Amoun, Egypt) was given once daily for three days. Pain was measured using verbal Numeric rating Scale (VNS) (13) as follows: The patient was shown a ruler labeled with numbers from zero to nine. The patients were explained the pain experience represented by each number. No pain was indicated by 0, mild pain indicated by 1, 2 and 3, moderate pain indicated by 4, 5, and 6, and severe pain indicated by 7, 8, and 9. The patients were told to point on ruler where the patient’s pain experience could be. The number was recorded as level of pain experience for that day.

Edema was evaluated by using the index finger to press on the swelling as deep as possible then approximate the depth in millimeters and the time the indentation takes to return to normal. The result was recorded using a scale used in patients with edema (14) where;

+1 (Trace) indicated slight indentation, rapid return to normal
+2 (Mild) indicated 4 mm indentation, rebound in few seconds
+3 (Moderate) indicated 6 mm indentation, rebound after 10-20 seconds
+4 (Severe) indicated 8 mm indentation and needs >30 seconds to return to normal. (Figure 5)

The maximum interincisal mouth opening was measured using calipers. The intra oral incision was examined and followed up throughout the postoperative period for signs of infection including redness, tenderness and pus discharge. Sutures were removed after seven days. The stab incision healed with discernible scar (Figure 3d).

The state of occlusion was checked throughout the postoperative period to ensure the normal occlusion of the patient in terms of molar relation and midline centralization returned to the way it was before injury (Figure 3c).

All the patients were assessed preoperatively and postoperatively for subjective symptoms related to the inferior alveolar and mental nerve by asking them about any alteration in sensation in the lower lip and the mandibular teeth on the affected side. Then objective examination of the mandibular teeth and lower lip was done using a dental probe to detect changes in the distribution of the inferior alveolar nerve compared with the contra lateral side.

Postoperative OPG x-rays (Figure 4(a) and (b)) were used to assess the mean bone density at the fracture line using image J (Java-based image processing program) (16).

This was done for each OPG view as an aid to monitor and evaluate healing fracture bone mineral density (BMD). Every postoperative OPG was studied using image J.

The collected data were analyzed using the Scientific Program for Statistical Solutions (SPSS) version 17.0 (37). Qualitative variables were described using numbers and percentages. Quantitative data were described using measures of central tendency. The distribution of Quantitative variables was tested for normality. For normally distributed data comparison between different periods using Analysis of Variance (ANOVA) with repeated measures and Post Hoc test was assessed using Bonferroni adjusted test. For ordinal data comparison between the different periods, a Wilcoxon signed rank test was applied.
Significance tests for the results are quoted as two-tailed probabilities. The obtained results were judged at 5% level of significance.

**Fig. 4:** a. Immediate postoperative OPG x-ray  
b. Postoperative OPG x-ray after 12 weeks

### RESULTS

#### Biodata

The patients included nine males and one female. Their age ranged between 16-50 years with a mean of 26.3 ± 10.133 years. The period of time that elapsed from day of injury to ORIF ranged between 1-4 days with a mean of 2.4 ± 1.07. The age of the patients was analyzed using grouped data with an age interval of ten (10) years. Fifty percent of the patients were in the age group of 20.5 to 30.5 (Table 1).

#### Table 1: Distribution of gender, age and the period of time from injury to open reduction and internal fixation Etiological factors

<table>
<thead>
<tr>
<th>Clinical sign</th>
<th>No</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>9</td>
<td>90.0</td>
</tr>
<tr>
<td>Female</td>
<td>1</td>
<td>10.0</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;20.5</td>
<td>3</td>
<td>30.0</td>
</tr>
<tr>
<td>20.5 – 30.5</td>
<td>5</td>
<td>50.0</td>
</tr>
<tr>
<td>30.5 – 40.5</td>
<td>1</td>
<td>10.0</td>
</tr>
<tr>
<td>40.5 – 50.5</td>
<td>1</td>
<td>10.0</td>
</tr>
<tr>
<td>Min. – Max.</td>
<td>16.0 – 50.0</td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>26.3±10.133</td>
<td></td>
</tr>
<tr>
<td>Number of days before operation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Min. – Max.</td>
<td>1.0 – 4.0</td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>2.4±1.07</td>
<td></td>
</tr>
</tbody>
</table>

The most common etiological factor was road traffic accident (RTA) 40% followed closely by interpersonal violence (IPV) at 30%. Sports injuries were experienced by two patients (20%).

#### Preoperative clinical findings

Fractures of the right mandibular angle constituted 60% as compared to the left (40%). All patients presented with facial asymmetry due to swelling, trismus and tenderness on the affected side. 80% of patients had malocclusion and a tooth in the fracture line. 60% of the patients presented with mouth deviated to the affected side. 50% of the patients presented with inferior alveolar /mental nerve paraesthesia or anaesthesia (Table 2).

#### Table 2: Preoperative clinical data

<table>
<thead>
<tr>
<th>Clinical sign</th>
<th>No</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swelling/Edema</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>Ecchymosis</td>
<td>3</td>
<td>30</td>
</tr>
<tr>
<td>Tenderness</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>Mouth deviation</td>
<td>6</td>
<td>60</td>
</tr>
<tr>
<td>TMJ Pain</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Crepitus</td>
<td>6</td>
<td>60</td>
</tr>
<tr>
<td>Lingual Hematoma</td>
<td>4</td>
<td>40</td>
</tr>
<tr>
<td>Malocclusion</td>
<td>8</td>
<td>80</td>
</tr>
<tr>
<td>Presence of tooth in fracture line</td>
<td>8</td>
<td>80</td>
</tr>
<tr>
<td>Parasthesia of IAN/Mental Nerve</td>
<td>5</td>
<td>50</td>
</tr>
<tr>
<td>Trismus</td>
<td>10</td>
<td>100</td>
</tr>
</tbody>
</table>

#### Postoperative clinical findings

Postoperatively pain experience decreased in all cases across the follow up period. Decreasing in pain intensity scores across the follow up period was statistically significant as P value was 0.005. (P ≤ 0.05) (Figure 5).

#### Fig. 5: Comparison of pain experience over the follow up period.

No bandage was used postoperatively to reduce edema. The edema resolved on treatment such that by the end of one week only 60% (6 cases) had mild edema. After two weeks 80% of the patients had trace levels or no edema at all. By the end of the fourth week edema had resolved in majority of the patients. By the end of twelve weeks all patients were free of swelling and facial asymmetry. Decreasing edema after one week, four weeks, six weeks and twelve weeks follow up period was found to be statistically significant as P value was 0.004 (P ≤ 0.05). (Figure 6)

#### Fig. 6: Comparison of edema over the follow up period.
The mean maximum interincisal mouth opening reduced from the preoperative level one day postoperative. Then it increased from the first week to the twelfth week in all cases. The percentage increment from the first, second, fourth, sixth to the twelfth week was 26.8%, 52.6%, 77.0%, 86.6% and 132.0% respectively. Increase in maximum interincisal mouth opening from the first week throughout the follow up period was found to be statistically significant as P values were ≤ 0.05 (P≤ 0.05).

The measurements for maximum interincisal mouth opening was grouped and used to classify trismus according to scale for trismus (15). Using this scale, 60% of the cases had moderate trismus and the other (40%) had severe trismus prior to operation. One day post operation the number of patients with severe trismus increased to 60% and the other just had moderate trismus (40%). The severity of the trismus reduced during the follow up period. The decrease in trismus over the follow up period compared to the preoperative period was found to be statistically significant as P value was ≤ 0.05. By the sixth week, over 80% of the patients could open their mouth more than 30.0 mm which is essentially normal. The transoral incision healed well in all cases. The skin stab incision healed uneventfully as well.

All patients maintained their normal occlusion throughout the postoperative period. Majority of the patients (90%) had Angle’s class I molar relation.

Five patients were found to have inferior alveolar and mental nerve parasthesia preoperatively. No patient developed iatrogenic parasthesia postoperatively. The parasthesia progressively reduced during the follow up period and by the twelfth week only one case still had incompletely resolved parasthesia of the lower lip. The motor activity of the facial nerve was assessed on all patients pre- and postoperatively. All patients had no facial nerve abnormality.

Eight patients presented with a third molar tooth in the fracture area. All patients had the molar tooth extracted intraoperatively as was indicated in each case due to infection, communication with the mouth or severe mobility. Extraction of the third molars did not displace the fracture nor make the reduction difficult in all the cases.

### Table 3: Comparison of differences in mean pixel densities immediately, six and twelve weeks postoperatively.

<table>
<thead>
<tr>
<th>Difference in pixel density</th>
<th>Post-operative</th>
<th>6 Week</th>
<th>12 Week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Min. – Max.</td>
<td>9.0 – 42.0</td>
<td>8.0 – 29.0</td>
<td>3.0 – 14.0</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>26.60±10.06</td>
<td>18.20±6.94</td>
<td>9.20±3.33</td>
</tr>
<tr>
<td>Median</td>
<td>28.0</td>
<td>19.0</td>
<td>9.50</td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td></td>
</tr>
<tr>
<td>% of change</td>
<td>31.2</td>
<td>64.0</td>
<td></td>
</tr>
</tbody>
</table>

p: Stands for adjusted Bonferroni p-value for ANOVA with repeated measures for comparison between preoperative with each other period

*: Statistically significant at p ≤ 0.05

The accuracy of reduction was evaluated on the OPG done immediately post-operation. The reduction was accurate in all patients. Patients were advised to use 0.2% chlorhexidine mouthwash.

The mean difference in pixel density decreased in all radiographs. The decrease in mean difference in pixel density from immediate postoperative, six weeks and twelve weeks was found to be statistically significant with a p value of 0.001 (P ≤ 0.05) (Table 3)

**DISCUSSION**

There are two ways of miniplate osteosynthesis in treatment of mandibular angle fractures using the intraoral incision. It can either be a single non-compression miniplate technique proposed by Michelet et al (18) and supported by Champy et al (12) or two miniplates as proposed by Kroon et al (19), Choi et al (20) and Levy et al (21). This study set out to evaluate the use of two 2.0mm dynamic compression miniplates in treatment of mandibular angle fractures using Trocar instrumentation. Majority of the patients were males (90%). The age ranged from 16 to 50 years with a mean of 26.3 years. Fifty percent (50%) of the patients were aged between 20.5 and 30.5 years. This result was in agreement with other studies by Gamal Eldin (2014) (22). The high incidence of fractures in this age group is associated with the high population of young people in Egypt who are the most active.

The mean waiting time before ORIF was 2.4± 1.07 days with a range of 1 to 4 days. This period was used for stabilization of the patients from trauma and reduction of edema. This result is in agreement with Ellis E III and Karas (1992) in their study on the treatment of MAFs using two 2.0mm minidynamic compression plates. They reported that the time from injury to open reduction and internal fixation ranged between a few hours to 7 days, with a mean of 2.5 days (23).

In this study, road traffic accident (RTA) was the main etiological factor (40%) followed closely by interpersonal violence (IPV) at (30%). The other factor was sports activities and a fall. This result is in agreement with many studies which show RTA as the main etiological factor of mandibular fractures in adults followed by interpersonal violence (24). However it differs with other studies that have found interpersonal violence (IPV) as the main cause of MAFs. Terffre et al (1991) (25) found that the etiology of maxillofacial fractures vary markedly from one country to another and even within the same country but RTAs were the main etiology. Killman et al (2003) (26) found that the most common cause of mandibular angle fracture was assault (n = 47 [69.1%]), followed by motor vehicle crashes (16.2%) and sports-related injuries (10.3%).

Preoperative antibiotics were given to all patients. This was in agreement with the findings of Zallen and Curry (1975) (17), who showed that dentate patients with compound fractures who receive antibiotic prior to ORIF resulted in a low (6%) infection rate compared to high (50%) for those who do not use preoperative antibiotics. In this study, no postoperative infection occurred.

In this study two, 2.0 mm mini DCPs were used on each
patient. One was placed on the external oblique ridge. The other was fixed on the lower border and fixed after reduction of the fracture to anatomical alignment and securing MMF. Mishira et al (1998) (27) found the use of two DCPs to give good anatomic reduction of the MAF segments and allowed full range of mandibular movement. Therefore, this technique followed the AO/ASIF principles by providing rigid fixation and stimulating primary bone healing.

The third molar tooth in the area of fracture was extracted intraoperatively due to different indications for each case. This was in agreement with Shetty et al (28) and Ellis III (2009) (29).

All the patients were reviewed after every two weeks postoperatively for twelve weeks. Pain, edema, trismus, occlusion, surgical incision, paraesthesia, and occurrence of infection were evaluated.

The mean pain intensity score decreased from mild pain in the first week to no pain by the sixth week. The decrease was experienced by all patients and can be explained by the proper reduction and fixation done that was rigid enough not to allow interfragmentary mobility. Furthermore, the soft tissue injury was minimal.

Regarding edema, by the first week 60% of the patients had mild edema and the rest had trace amounts. The edema decreased significantly such that by the sixth week 80% of the patients had no edema at all. The decrease in edema reflected the degree of soft tissue manipulation as significantly less traumatic. This is in agreement with the study done by Kale et al (2010) (30).

Maximum interincisal mouth opening (MIMO) was used to classify trismus into severe, moderate, mild and normal (15). Majority of the patients had moderate to severe trismus preoperatively (MIMO ≤ 30mm). The trismus decreased significantly during the postoperative weeks so that by the sixth week majority (80%) of the patients just had mild trismus (30mm ≤ MIMO ≤ 45mm).

Majority of the patients (90%) had class I molar relation. One patient had class III molar relation. MMF was done intraoperatively before ORIF in all cases. No patient remained with MMF postoperatively. All patients maintained normal occlusion throughout the follow up period. Maintaining normal occlusion postoperatively was attributed to the strength and stability of the two dynamic compression miniplates against the forces of mandibular movement. This was in agreement with the finding of Ellis III (1999) (31).

The inferior alveolar nerve was evaluated preoperatively and postoperatively. 50% of the patients were found to have paraesthesia preoperatively. No incidence of iatrogenic sensory disturbance was found postoperatively for those who did not have prior to ORIF. The paraesthesia decreased progressively so that by the twelfth week only one patient still had paraesthesia of the mental nerve. Paraesthesia due inferior alveolar nerve is attributed to displacement of bony fragments after trauma rather than the surgery itself. Most are neuropraxias which heal completely. The same result was reported by Rahpeyma et al (2014) (32). No incidence of postoperative marginal mandibular nerve disturbance occurred in all the cases. The technique used to determine the zone for placement of the Trocar differed with the one proposed by Guisle et al in (2012) (33). They reported the safety zone as the region made by three lines at the angle of the mandible. In our study we made the skin puncture perpendicular to the fracture in the relaxed skin crease.

In this study fracture healing was evaluated radiographically by taking digital panoramic images of the patients immediately, six and twelve weeks postoperatively. The images were analyzed using image J computer program for pixel density at the fracture line compared the differences with an equal sized area adjacent to the fracture line.

In this study, the preoperative OPG was the baseline. The pixel density at the fracture line was found to increase progressively from one day to twelve weeks postoperative. This was shown by the progressive decrease in mean difference in pixel density over the follow up period. This was in agreement with Doblar et al (2004) (34) on primary bone healing.

In this study, no patient showed any complication during the follow up period. This could be attributed to the small sample size (n=10), strict inclusion and exclusion criteria, good reduction and fixation by dynamic compression miniplates.

These results are in disagreement with studies done by Ellis and Karas (1992) (23) and Ellis and Walker (1996) (35). Both studies reported the complication as 29% and 16% respectively.

However, the results of this study are in agreement with Kale et al (2010) (30) who found the transbuccal technique to be superior to the extraoral approach.

Compressive fixation systems are biomechanically superior to adaptive systems and provide good immediate functional stability to reduce mandibular angle fractures. In this study dynamic compression miniplates were used to provide the compression, fixation and good stability of the fractured bone. This result is in agreement with the findings of Shetty et al (1995) (36).

In this study we found the combined transbuccal instrumentation with intra-oral incision technique to be safe and effective. Furthermore, the technique provided good reduction and fixation without any mobility at the fracture line. There was no visible scar on the skin and no injury to the marginal mandibular nerve.

CONCLUSIONS
1. Two 2.0 mm dynamic compression miniplates are suitable for treatment of mandibular angle fractures using Trocar instrumentation.
2. The use of Trocar instrumentation in management of mandibular angle fractures is a suitable technique for healing because there was no extraoral scar as happens in submandibular approach.
3. Bone and soft tissue healing was found to be acceptable.
4. The difficulties experienced in the use of Trocar instrumentation are acceptable in comparison to the complications that can occur in using extra oral submandibular technique.
CONFLICT OF INTEREST
The authors declare that they have no conflicts of interest.

REFERENCES

Effect of Triclosan Toothpaste on Gingivitis and Plaque Accumulation in Children Wearing Fixed Orthodontic Appliances: A Randomized Clinical Trial

Bayoumi M1 BDS, Hanno A2 PhD, Sharaf A2 PhD.

Abstract:
Introduction: The greatest preventive challenge in dentistry is the control of dental biofilm and consequently avoiding dental caries and gingival inflammation. As an adjunct to the mechanical oral hygiene measures, chemical antimicrobial agents seem to offer great benefits in the control of plaque formation and gingivitis, especially in high risk patients wearing orthodontic appliances.

Objective: The aim of the study was to evaluate the effect of Triclosan (0.3%) Colgate Total® on gingivitis and plaque formation in patients wearing fixed orthodontic appliances.

Materials and methods: Fifty children wearing fixed orthodontic appliances with an age range of 9 to 13 years were randomly assigned into 2 groups (test and control). The test group received the Colgate Total® toothpaste containing 0.32% sodium fluoride and 0.3% Triclosan. The control group received maxfresh® toothpaste containing 0.22% sodium fluoride. All patients were examined clinically to assess their gingival condition using the Plaque index (PI), Gingival Index (GI), Sulcular Bleeding Index (SBI) at follow up periods of 1, 2 and 3 months. Each group shifted to use the alternative agent after a washout period of 10 days to prevent the carryover effect of Triclosan.

Results: Showed that the Triclosan toothpaste users experienced significant reduction in plaque accumulation and gingivitis compared to the fluoride toothpaste users in the first and second follow up periods. The results also showed significant difference compared to the baseline.

Conclusion: Triclosan toothpaste proved to have marked antiplaque and antigingivitis effects. Triclosan toothpaste proved to counteract the aggravated gingival effects that can be introduced by the fixed orthodontic appliances in children at the age of puberty.

Keywords: Triclosan, toothpaste, plaque, gingivitis, children, orthodontic appliances

INTRODUCTION

One of the main concerns of dentistry nowadays is the improvement of the individual’s facial appearance, since it is considered one of the major factors in determining one’s integration into society (1). Orthodontic treatment is a crucial part of the complete dental rehabilitation, which includes improvements in dental health, function, appearance and eventually self-esteem. Although this kind of treatment in turn leaves countless positive effects on the patient’s personality and appearance, it can cause harm to the patient’s gingival conditions after the placement of a fixed appliance (2). Fortunately, gingival inflammation is usually temporary and does not lead to attachment loss (2-4). In addition, gingival hyperplasia is usually noticed around orthodontic bands, leading to pseudo-pocketing giving a false picture of a periodontal disease. The condition usually resolves within weeks after finishing the treatment provided that proper oral hygiene practices are followed throughout the orthodontic treatment period, otherwise the condition worsens (5). Bleeding usually is experienced due to the micro-ulcerations that occur in the epithelium that lines the gingival sulcus (6).

Orthodontic appliances, especially fixed bonded appliances, can complicate the oral hygiene maintenance process by introducing large numbers of plaque retentive irregular surfaces in the oral environment (7, 8). An increased gingival irritation can be attributed to the mechanical irritation caused by the subgingival placement of the orthodontic bands, which may also result in the translocation of bacterial plaque to the subgingival region during the physiological tooth movement (9).

Young patients approaching the age of puberty are occasionally less interested in oral hygiene which in the presence of fixed orthodontic appliances becomes a more difficult routine (10). Adequate mouth hygiene and cleanliness of the teeth are related to the frequency of brushing and the adequate removal of bacterial plaque from the tooth surface (11). Despite of the documented relationship between poor oral hygiene and gingival inflammation, it is still advocated that self-performed plaque control measures are not entirely efficient to control gingival inflammation (12). The addition of antimicrobial agents to conventional toothpastes has the ultimate goal of increasing the effect of control or elimination of microorganisms involved in many oral microbial infections in the human mouth to control gingival diseases (13).

Triclosan is a low-toxicity, non-ionic, chlorinated bisphenol that is compatible with toothpaste components, such as fluoride and surfactants. Triclosan has been reported to promote inhibition of cyclooxygenase/lipoxygenase pathways and exhibits anti-inflammatory effects (14, 15). Considering the information available on the material, Triclosan has been proven to be safe and effective as a broad-spectrum antibacterial agent. It has also been proven to be compatible with two of the most used salts in dentistry, sodium fluoride and sodium monofluorophosphate (16).

Colgate Total, which contains Triclosan, stands as the only toothpaste approved by the US Food and Drug Administration as well as the American Dental Association (ADA) to fight plaque and gingivitis, therefore it is mostly recommended by dentists and hygienists to be used in the US (17). Since too many commercial oral cleansers with
different antibacterial agents are available in the market, this study aimed to test the hypothesis that combining the benefit of mechanical plaque removal with the potential antibacterial effect of the 0.3% Triclosan toothpaste would be of superior benefit compared to fluoride toothpaste when used by children undergoing orthodontic treatment, and whom are considered at high risk to gingival disease.

**MATERIALS AND METHODS**

This study was planned to be a randomized clinical trial based on a crossover design to evaluate the effect of Triclosan toothpaste compared to conventional fluoride toothpaste. The required sample size was calculated using Epi-info software and accordingly an estimated sample of 25 children per group was considered adequate. A power of 80% was used to detect a clinically meaningful reduction in plaque index from baseline to 3 months among the study groups (18) with precision of 5%, α= 0.05, effect size = 0.85.

Fifty children with an age range of 9-13 years were selected from the Pediatric Dentistry Department as well as the Orthodontic Department, Faculty of Dentistry, Alexandria University. Exclusion criteria were:

2. Medically compromised or intellectually disabled patients.
3. Children with special health care needs affecting their motor function.
4. Children with severe gingivitis more than grade 2 (gingival index according to Loe and Silness) (19).

The sample was randomly divided into 2 groups using the Fish Bowl technique to achieve random allocation to the study groups.

Group I: Colgate Total® toothpaste containing the 0.32% sodium fluoride plus the active ingredient 0.3% Triclosan (test group). *

Group II: Colgate Maxfresh® toothpaste containing 0.22% sodium fluoride (control group) **

Each child was informed to brush twice daily with the assigned toothpaste and provided with a follow up chart each visit where the dentist and the parent assessed the compliance of the child. The children colored in the follow up chart each time they brushed their teeth.

After a washout period of 10 days (20), the participants in each group were reallocated to the counterpart group and used the alternative agent (crossover procedure). Each candidate in each group was supplied with an orthodontic toothbrush recommended by the orthodontist at the beginning of the study. After the washout period and the crossover, the patients were supplied with another toothbrush to be used in the second 3 month period.

In order to assess the gingival conditions, the following indices were used:

- Plaque index (PI) (21), Gingival Index (GI) (22, 6), and Sulcular Bleeding Index (SBI) (6).

Baseline assessment of plaque and gingivitis was carried out using the previously mentioned indices. Each participant was reevaluated for plaque, gingivitis and gingival bleeding levels at 1, 2 and 3 month intervals. Based on the half-life period of ingested Triclosan in the body (23), a washout period of 10 days was given to each group during which children were instructed to use the control toothpaste before they shifted to the alternative one. Reevaluation of the final outcome was carried out using the same indices at follow up intervals of 1, 2 and 3 months.

The present study was performed after receiving the approval of the Research Ethics Committee in the Faculty of Dentistry, Alexandria University. Parents of the selected children signed an informed consent after explaining the nature of the study, that their children will receive 2 different toothpastes throughout the follow up periods (1 every 3 months) and how the test material can offer better gingival health.

**Statistical analysis:**

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. Qualitative data were described using number and percent. Quantitative data were described using range (minimum and maximum) mean, standard deviation and median. Comparisons between different groups regarding categorical variables were tested using Chi-square test. The distributions of quantitative variables were tested for normality using Kolmogorov-Smirnov test, Shapiro-Wilk test and D’Agstino test. If data revealed normal data distribution, parametric tests were applied. If data were abnormally distributed, non-parametric tests were used. Comparisons between the two studied groups were done using Mann Whitney test while comparisons between the different periods were done using Friedman test and pair wise comparison was assessed using Wilcoxon signed ranks test. Significance of the obtained results was judged at the 5% level.

**RESULTS**

Children who participated in the study had an age range of 9-13 years with a mean age of 11.58 ± 1.36 years. Out of the total sample, 37.8% were males and 62.2% were females. Following randomization, there was no statistically significant difference between the two groups regarding age and gender, (P=0.254) and (P=0.918) respectively.

In group 1, the comparisons in the first follow up period regarding the Plaque index (PI) revealed statistically significant difference between the mean values of baseline, first, second and third month using the Friedman test (P=0.017). When each of the first, second and the third month mean values were compared to the baseline, statistically significant differences were also revealed [(P=0.003), (P=0.012) and (P=0.002) respectively]. There were reductions in the percentage of change from the baseline value by 40.7% in the first month, 33.3% in the second month and 37.8% in the third month indicating a marked decrease in the plaque formation in the first month. The reduction fluctuated during the following months.

In group 2, comparison of the baseline, first, second and third month mean values using the Friedman test revealed no statistically significant difference (P=0.149). Comparing the mean values of the first, second and third month with the baseline value revealed statistically significant difference only in the third month [(P=0.419), (P=0.120) and (P=0.048)
respectively]. The decrease in the percentage of change was only 8.8% in the first month, 14.7% in the second month and 22.1% in the third month. The results indicated gradual decrease in the amount of plaque formation in the first, second and third months respectively.

When the 2 groups were compared in the first, second and third months, only statistically significant difference was seen in the first month (P=0.016), whereas no statistically significant differences between groups were evident in the second and third months [(P=0.169) and (P=0.232) respectively] as shown in figure 1.

In group 1, the comparisons in the first follow up period regarding the Gingival Index (GI) revealed a statistically significant difference between the baseline, first, second and third month mean values using the Friedman test (P<0.001). When each of the first, second and the third month mean values were compared to the baseline, statistically significant differences were revealed [(P=0.001), (P=0.002) and (P=0.003) respectively]. There was a decrease in the percentage of change from baseline value by 31.7% in the first month, 26.8% in the second month and 34.1% in the third month. This indicated maximum improvement in the gingival condition in the third month and a slight drop in the second one.

In group 2, comparison of the baseline, first, second and third month mean values using the Friedman test revealed no statistically significant difference (P=0.286). Comparing each of the first, second and third month mean values to the baseline value, no statistically significant differences were revealed [(P=0.739), (P=0.285) and (P=0.405) respectively]. The first month showed an increase in the percentage of change with a value of 2.3% indicating a worsened gingival condition, whereas the second and third months showed slight reduction in the percentage of change with values of 9.3% and 7.0% respectively indicating slight improvement in the gingival condition.

When comparing both groups in the first, second and third months, statistically significant differences were found [(P<0.001), (P=0.029) and (P=0.004) respectively] as shown in figure 2.

In group 1, the comparisons in the first follow up period regarding the Sulcular Bleeding Index (SBI) revealed a statistically significant difference between the baseline, first, second and third month mean values using the Friedman test (P<0.001). When each of the first, second and the third month mean values were compared to the baseline, statistically significant differences were revealed [(P=0.084), (P=0.015) and (P=0.004) respectively]. There was a decrease in the percentage of change with values of 22.2% in the first month, 31.1% in the second month and 56.7% in the third month which also indicates improvement in the bleeding condition of the gingiva throughout the 3 months.

Comparisons of both groups in the first, second and third months, revealed statistically significant differences (P=0.006), (P=0.008) and (P=0.050) respectively as shown in figure 3.
In the second follow up period, the test group comparisons regarding the PI revealed a statistically significant difference between the baseline, first, second and third month mean values using the Friedman test (P<0.001). When each of the first, second and the third month mean values were compared to the mean value after the washout period, statistically significant differences were revealed [(P<0.001) at each month]. There was a decrease in the percentage of change with values of 45.9% in the first month, 43.4% in the second month and 63.1% in the third month respectively indicating a decrease in plaque formation during the follow up period with maximum effect in the third month.

In the control group, comparison of the baseline, first, second and third month mean values using the Friedman test revealed no statistically significant difference (P=0.109). When each of the first, second and the third month mean values were compared to the group mean value after the washout period, statistically significant difference was evident in the second month [(P=0.109), (P=0.004) and (P=0.115) in the first, second and third months respectively]. Also there was an increase in the percentage of change with the values of 19.3% in the first month, 27.7% in the second month and 18.1% in the third month respectively. This indicated increase in plaque formation with maximum increase in the second month.

Comparisons of both groups in the second follow up period regarding the PI revealed statistically significant difference [(P=0.006), (P<0.001) and (P<0.001) in the first, second and third month respectively] as shown in figure 4.

When each of the first, second and the third month mean values were compared to the group mean value after the washout period, statistically significant differences were revealed [(P<0.001) in each month]. There was an increase in the percentage of change with the values of 77.3% in the first month, 77.3% in the second month and 68.2% in the third month respectively. This indicated deterioration of the gingival condition in the first and second month followed by slight improvement in the third month.

Comparisons of groups regarding the GI revealed statistically significant differences in the second and third months only [(P=0.065), (P=0.001) and (P= 0.004) in the first, second and third months respectively] as shown in figure 5.

In the second follow up period, the test group comparisons regarding the SBI revealed a statistically significant difference between the baseline, first, second and third month mean values using the Friedman test (P<0.001). When each of the first, second and the third month mean values were compared to the baseline value after the washout period, statistically significant differences were revealed [(P<0.001) in each month]. There was a decrease in the percentage of change with values of 67.5% in the first month, 80.0% in the second month and 85.0% in the third month respectively indicating marked improvement in the bleeding condition of the gingiva.

In the control group, comparing the baseline, first, second and third month mean values using the Friedman test revealed no statistically significant difference (P=0.137). When each of the first, second and the third month mean values were compared to the baseline value after the washout period, no statistically significant differences were seen [(P=0.071), (P=0.088) and (P=0.513) in the first, second and third months respectively]. There was an increase in the percentage of change with the values of 47.4% in the first month, 42.1% in the second month and 15.8% in the third month respectively indicating deterioration in the bleeding condition of the gingiva except for the third month which showed slight improvement.

Comparisons of groups regarding the SBI revealed statistically significant differences in the first, second and third months [(P=0.007), (P<0.001) and (P=0.003) respectively] as shown in figure 6.
Table 1 shows comparison between the third month of the first and second follow up period in the same group. In group 1, children used the Triclosan toothpaste in the first follow up period and after the crossover they shifted to use the control toothpaste. Results showed significant differences regarding only the GI and SBI [(P=0.008) and P=(0.032) for the PI, GI and SBI respectively]. The percentage of change from the third month of the first follow up period to the third month of the second one increased by 16.7%, 37.0% and 83.3% for the PI, GI and SBI respectively.

In group 2, children used the control toothpaste in the first follow up period and after the crossover they shifted to the Triclosan toothpaste. Results showed significant differences [(P=0.001) in the PI, GI and SBI respectively]. The percentage of change from the third month of the first follow up period to the third month of the second one decreased by 57.5%, 35.0% and 69.2% for the PI, GI and SBI respectively.

**DISCUSSION**

Since orthodontic treatment has been increasingly performed due to the social demands, the present study was planned to explore a new convenient method to prevent this potential problem, which is gingivitis during and after orthodontic treatment. This study aimed at evaluating the effect of 0.3% Triclosan toothpaste on gingivitis and plaque formation which appear after fixed orthodontic appliance construction.

In the current study, a general trend of constant improvement in the Triclosan toothpaste users with statistically significant differences from the baseline throughout the follow up periods regarding the PI, GI and SBI. Similarly, the control group showed slight improvement in the plaque accumulation and gingivitis that did not reach significant levels. The improvement in the SBI was significant. This may be due to the fact that mechanical plaque removal also contributed to the better gingival condition.

Regarding the PI, improvement was seen among the test toothpaste users in both the first and second follow up periods. However, some fluctuations were evident in both periods. This may be attributed to the fact that participants experienced a sense of habituation and loss of interest in the strict oral hygiene regimen. Participants may have thought that they achieved their goal in maintaining good oral
hygiene after the marked improvement and appraisal by the examiner. After reinforcing oral hygiene instructions, participants were remotivated and showed improved compliance throughout the following months through assessment of the follow up chart.

Similar fluctuations in the results were shown regarding the GI and SBI during the first follow up period. In the second follow up period, the GI and SBI showed a constant improvement in their gingivitis and plaque levels. This may be attributed to the fact that shifting to the test toothpaste and exposing them to the chemotherapeutic effect of Triclosan caused pronounced improvement in their oral condition. They probably would have benefited from their experience in the first follow up period during which they were under strict oral hygiene practices.

Regarding the control toothpaste, the mean values of the PI, GI and SBI showed slight improvement in the plaque formation and gingival conditions in the first follow up period. The results showed slight fluctuation in the mean values of the GI. The mean values of the SBI showed marked improvement than PI and GI in the first follow up period. This indicated that although no therapeutic agent exits, mechanical plaque removal (brushing twice daily) under strict supervision improved the gingival condition especially bleeding gums. Brushing twice daily is considered the most common method of plaque control and has been recommended by the American Dental Association as a regimen for good oral hygiene (24).

In the second follow up period, the results showed increase in the mean values of the PI, GI and SBI. The increase showed nearly the same pattern in the 3 indices, a marked increase in the first month followed by minimum improvement in the second and third months. This indicated deterioration in the oral hygiene and the gingival conditions following shifting from the test to the control toothpaste. Although the conditions improved in the third month, the differences between the test and control groups were statistically significant. Moreover, when comparing the third months of the first and the second follow up periods the Triclosan showed greater improvement in the oral hygiene, gingivitis and gingival bleeding conditions.

The present results were confirmed in many literature reviews. Reviews done by Davis et al in 2004 (25), Gunsolley in 2006 (26), and Blinkhorn et al in 2009 (27) concluded that Triclosan toothpaste reduces formation of supragingival plaque and incidence of gingivitis when compared to conventional fluoride toothpaste.

Similarly, the same results were proven by DeVizio in 2008 (28) and by Singh et al in 2010 (29) in their six week clinical investigation. Singh et al concluded that the toothpaste containing 0.3% Triclosan, 2.0% PVM/MA copolymer and 0.243% sodium fluoride is efficacious in reducing gingivitis and supragingival plaque. They also provided evidence in their study that the this toothpaste provides a greater level of antiplaque and antigingivitis effect than does the toothpaste containing 0.454% stannous fluoride, sodium hexametaphosphate and zinc lactate.

However, the study of He et al in 2013 (30) showed that not all patients display the same response to the toothpaste.

In their study, they proved that patients already using 0.3% Triclosan toothpaste with residual gingivitis showed reduction in the gingival inflammation and bleeding when shifting to the 0.454% stannous fluoride toothpaste compared to those who continued using the 0.3% Triclosan toothpaste.

On the other hand, Bhavesh et al in 2011 (34) found that both Triclosan and Chlorhexidine equally inhibited the colonization of Streptococcus mutans on the orthodontic components and thus the same incidence of plaque formation and gingivitis.

Although Chlorhexidine has significant effect on plaque formation and gingivitis, the use of this therapeutic agent is not advocated for long periods. The use of Triclosan seems to be more useful and safer in patients who are exposed to the risk of gingivitis for the periods needed during their orthodontic treatment.

CONCLUSIONS
1. Triclosan toothpaste proved to have marked antiplaque and antigingivitis effects.
2. Triclosan toothpaste proved to counteract the gingivitis aggravated by the fixed orthodontic appliances in children at the age of puberty.

CONFLICT OF INTEREST
The authors declare that they have no conflicts of interest.

REFERENCES
EVALUATION OF COLLOIDAL SILVER GELATIN SPONGE (GELATAMP) IN PATIENTS RECEIVING ANTICOAGULANT AFTER TOOTH EXTRACTION (CLINICAL STUDY)

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Abstract:
Introduction: Patients with a history of heart attack or stroke may take anticoagulant. Because these are anticoagulant medications, bleeding time after dental treatments, may be prolonged. Gelatamp is made of 95% foam gelatin sponge and 5% finely dispersed colloidal silver. Gelatamp has the great advantages of both haemostatic and bactericidal effect.

Objectives: To evaluate clinically the use of Gelatamp to avoid postoperative dry socket and bleeding in patients receiving anticoagulant therapy without altering the medication dosage.

Materials and methods: This study was conducted on fifty patients indicated for teeth extraction divided into two equal groups. Study group consisting of 25 patients who received their medicine as usual and extraction was done followed by insertion of gelatamp in the socket and supported with heavy pack. Control group consisting of 25 patients who stopped their medicine until INR reached 1.6 and Prothrombin activity more than 60% after that extraction was done and the socket was supported with heavy pack. The patients were monitored for 24 hours in both groups.

Results: Adequate socket healing was detected in all patients of both groups.

Conclusion: Gelatamp is an effective material as local hemostatic agent after extraction for anticoagulant patients (within the therapeutic range of INR) without interruption of their medicine.

Key words: Anticoagulant, Tooth extraction, Gelatamp, Local hemostatic agent, Cardiac patients.

INTRODUCTION

Many patients taking Coumarin derivatives, such as warfarin, present to the oral and maxillofacial surgeon needing to have teeth extracted. The surgeon is faced with the choice of altering or stopping warfarin and risking thromboembolism or leaving the patient on the warfarin and risking uncontrolled bleeding (1).

The goal of anticoagulant therapy is to prevent clot formation or expansion and Warfarin is the most common drug used in this therapy. Warfarin is an antagonist of vitamin K, an necessary for synthesis of clotting factors II, VII, IX and X, as well as the naturally occurring endogenous anticoagulant proteins C and S (2).

Warfarin will affect the factor with the shortest half-life first and the factor with the longest half-life last. The half-lives for factors VII, IX, X, and II are 6, 24, 40, and 60 hours, respectively Factor VII is affected first, and this increases the prothrombin time (PT). Factors IX, X, and II will be affected later and increase the partial thromboplastin time (PTT) (3).

If warfarin therapy is stopped, it would take about four days for INR to reach 1.5 in almost all patients and with this INR, any surgery can be safely performed (4).

After warfarin therapy is restarted, approximately three days will be needed for the INR to reach 2.0. Therefore, if warfarin is withheld for four days before surgery and treatment is restarted as soon as possible after surgery, patients would have a sub-therapeutic INR for approximately two before surgery, and two days after surgery increasing the risk of thromboembolism (5).

The activity of anticoagulants is expressed using the international normalized ratio (INR). For an individual not taking anticoagulant or antiplatelet drugs, the normal coagulation profile is an INR of 1.0. The INR must be measured prior to dental procedures, ideally this should be done within 24 h before the procedure (6).

The level of INR suitable for the patient depends on the condition of the patient. The recommended INR level according to the American College of Chest Physicians is between 2.0 and 3.0 for most conditions (7).

The ideal oral surgery hemostatic agent should be safe, well tolerated, bacteriostatic, preformed for operator convenience, packaged, sterile, single-use, remain where applied, dissolve in the first week post surgery. It should also be used in patients taking antithrombotic drugs (8).

Gelatamp (gelatine sponge with colloidal silver); is similar to Gelfoam which is absorbable gelatin sponge available as a sterile sponge like dressing (9). This sponge holds many times its weight in blood and provides a stable scaffold for clot formation, thus, the mode of action of the Gelfoam is believed to be related to formation of a mechanical matrix that facilitates clotting. Gelfoam is thought to act intrinsically by promoting the disintegration.
of platelets causing a subsequent release of thromboplastin, this is in turn simulates the formation of thrombin in the interstices of the sponge. It may be soaked in thrombin or epinephrine solution to enhance its haemostatic properties (10).

Silver is promoted within alternative medicine in the form of colloidal silver, though it has never been proven safe and effective. The silver ion (Ag+) is bioactive and in sufficient concentration readily kills bacteria in vitro. Silver also kills bacteria in external wounds in living tissue and therefore physicians use wound dressings containing silver sulfadiazine (Ag-SD) or silver nano-materials to treat external infections (11, 12). Wound dressings containing silver are increasing in importance due to the recent increase of antibiotic-resistant bacteria (13).

Gelatamp is effective against wide range of microorganisms, which are found in the oral cavity. It has been found to be very effective against bacteria, which are resistant to antibiotics. The finely dispersed colloidal silver provides a large active surface for the continuous release of silver ions. As silver does not dissolve easily it is not washed out of the gelatin sponge but is continually released as the sponge is resorbed. This gives Gelatamp a depot antimonial effect throughout the resorption process. Gelatamp has the great advantages of both hemostatic and bactericidal effect. It remains in the alveolus and completely resorbed within 4 weeks (14).

The aim of this study was to evaluate clinically the use of Gelatamp to avoid postoperative dry socket and bleeding in patients receiving anticoagulant therapy without altering the medication dosage.

MATERIALS AND METHODS
Selection of patients
This study was conducted on 50 patients who were selected, and operated in the Oral & Maxillofacial Surgery Department, Faculty of Dentistry, Alexandria University, Egypt. Each patient had been informed about the nature of this study and gave an informed consent.

The patients under anticoagulant therapy (coumarin derived) and indicated for teeth extraction were included in this study. These 50 patients were divided into two equal groups:
1- Study group: Consisted of 25 patients who received their medicine as usual and extraction was done followed by insertion of gelatamp in the socket and supported with heavy pack. The patients were then monitored for 24 hours.

2- Control group: Consisted of 25 patients who stopped their medicine until INR reached 1.6 and Prothrombin activity more than 60% after that extraction was done and the socket was supported with heavy pack. Suturing was done when needed and the patients were monitored for 24 hours. Warfarin was stopped 5-7 days before extraction and the INR was followed up at the 5th and 7th days when the INR reached ≤1.6, extraction was done. After the procedure, all patients were found to be at low bleeding risk and restarting warfarin was performed immediately.

Inclusion Criteria of selection
- Patients’ age ranged between (25 - 60) years old of both sexes.
- Patient were sent for medical consultation by the physician for assessment of:
  - The International Normalized Ratio (INR values inside therapeutic range ≤ 3.0).
  - Bleeding time (3.75 ± 1.05 minutes).

Exclusion criteria of selection
- Patients with liver disease.
- Patients with renal failure.
- Uncontrolled diabetics.
- Heavy smokers.
- Patients with hematological diseases.

Material
Gelatamp (Gelatamp gelatine sponge with colloidal silver Colteñe/Whaledent Ltd. President Suite Kendal House Victoria Way Burgess Hill West Sussex, RH15 9NF/U.K.) is made of foam gelatine and finely dispersed (colloidal) silver. Silver forms silver ions in moist conditions. The porous foam structure absorbs its own weight in blood several times over, promotes thromboocyte aggregation due to the large surface and fills the wound cavity. Gelatamp remains in the alveolus and is completely resorbed within 4 weeks. (Figure 1)

Fig. 1: Showing gelatamp.

Surgical phase
Study group
- Local anesthesia without epinephrine (Mepecaine 3%, 1.8ml, Alexandria Co. for pharmaceuticals & Chemical industries Alexandria-Egypt).
- Non surgical extraction of tooth using the appropriate maxillary and mandibular forceps. (Figure 2)

Fig. 2: Socket after tooth extraction.
The socket was examined for any tooth or bone fragments.
- Gelatamp was inserted and supported with heavy pack: it is supplied sterilized and ready for use. The size of the small sponge can be adjusted to fit the wound cavity. Two Gelatamp sponges can be used for larger wounds. Care must be taken that the sponge is not compressed. Pressure on the sponge will destroy its structure and prevent the collection of blood within it. (Figure 3)

![Gelatamp delivery into the socket](image3)

**Fig. 3:** Showing gelatamp delivery into the socket.

- Biting on sterilized gauze to make sure the material in its place.
- Post operative instructions were given to the patient.

**Control group**
- Local anesthesia without epinephrine (Mepecaine 3%, 1.8ml, Alexandria Co. for pharmaceuticals & Chemical industries Alexandria-Egypt).
- Non surgical extraction of tooth (Figure 4)
- Pack pressure on the socket until bleeding stopped.

![Socket after tooth extracted in the control group](image4)

**Fig. 4:** Showing socket after tooth extracted in the control group.

**Postoperative phase**
- It was confirmed that the bleeding was controlled after extraction.
- In case the bleeding did not stop adequately after gelatamp application, suturing was used to approximate the wound margins.
- The patients were under observation over 24 hours. The postoperative evaluation was done clinically as follows 1) Postoperative bleeding: Hemostasis evaluated immediately after extraction by determining clot formation time by clinical observation.

Bleeding was assessed postoperatively by bleeding scale (15):
- Grade 0: Very low (almost no bleeding).
- Grade 1: Low (slight oozing of blood from the socket which usually stops by its self or after pressure is applied).
- Grade 2: Normal. (Clinically significant).
- Grade 3: High. (Bleeding occurs after clot has significantly formed).
- Grade 4: Very high (excessive bleeding that could not be controlled by local hemostatic agents or stitches).

2) Postoperative pain:
Pain was evaluated though VAS (visual analogue scale) at first, third and seventh days postoperatively, taking pain scores from 0 to 5.(16) Patients were asked about the pain severity according to (VAS) as follow:
- 0 No pain
- 1 Slight pain
- 2 Mild pain
- 3 Severe pain
- 4 Very severe pain
- 5 Extremely severe pain

3) Postoperative healing:
Adequate socket healing was evaluated clinically at the first and seventh postoperative days including the following parameters:
- Presence or absence of broken down blood clot.
- Presence or absence of sign and symptom of dry socket.
- Presence or absence of inflammation and infection.

**Statistical Analysis**
- The data were collected and entered into the personal computer. Statistical analysis was done:
- Number and percentage of each group (study group and control group).
- Chi square test and Monte Carlo test used to compare between laboratory investigation of both groups.
- Fisher Exact test for comparing between immediate and each other period.

**RESULTS**
In this study, fifty patients (age range of all the fifty patients varied from 30 – 60 years old) were divided equally into 2 groups:
- Group 1 (study group) consisting of 25 patients and included 14 males and 11 females.
- Group 2 (control group) consisting of 25 patients and include 16 males and 9 females.

The posterior teeth were the most common teeth extracted. The study group included six maxillary anterior teeth, eight maxillary posterior teeth, four mandibular anterior teeth and seven mandibular posterior teeth. On the other hand, the control group included four maxillary anterior teeth, eight maxillary posterior teeth, three mandibular anterior teeth and ten mandibular posterior teeth.
The INR in the study group ranged from 1.61 - 2.00 in 3 patients, 2.01 - 2.5 in 16 patients, 2.51 – 3 in 6 patients. In the control group the INR of all the patients ranged from 1-1.6.

The bleeding time was in normal range in the both groups (3.75 ± 1.05 minutes). The Prothrombin activity in study group ranged 20 – 50% while in control group ranged from 60-80%.

1) Postoperative Bleeding
Table 1 shows the comparison between the two groups regarding postoperative bleeding grades at different times:

1- Immediately after tooth extraction before gelatamp was delivered in the study group and before pack pressure was applied in the control group, it was found that there was a significant difference in bleeding between the two groups, with higher bleeding scores detected in the study group.

2- After 5 minutes, 30 minutes, 2 hours, first and seventh day postoperative it was found that there was no significant difference between the two groups.

| Table 1: Comparison between the two studied groups according to bleeding |
|---|---|---|---|---|---|---|---|---|---|
|  | After extraction | After 5 minutes | After 20 minutes | 2 hours | 1st day | 7th day |
| --- | No. | % | No. | % | No. | % | No. | % | No. | % |
| Study | Grade 0 | 10 | 76.9 | 25 | 100.0 | 25 | 100.0 | 24 | 96.0 | 25 | 100.0 |
| Grade 1 | 3 | 8.3 | 24.0 | 0 | 0.0 | 0 | 0 | 1 | 4.0 | 0 | 0.0 |
| Grade 2 | 5 | 12.5 | 20.0 | 0 | 0.0 | 0 | 0 | 6 | 24.0 | 0 | 0.0 |
| Grade 3 | 0 | 0.0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 |
| Grade 4 | 0 | 0.0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 |
| Control | Grade 0 | 10 | 75.0 | 25 | 100.0 | 25 | 100.0 | 24 | 96.0 | 25 | 100.0 |
| Grade 1 | 3 | 7.5 | 22.5 | 0 | 0.0 | 0 | 0 | 1 | 3.4 | 0 | 0.0 |
| Grade 2 | 4 | 10.0 | 10.0 | 0 | 0.0 | 0 | 0 | 0 | 0 | 0 | 0.0 |
| Grade 3 | 0 | 0.0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 |
| Grade 4 | 0 | 0.0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 |

The bleeding time was in normal range in the both groups (3.75 ± 1.05 minutes). The Prothrombin activity in study group ranged 20 – 50% while in control group ranged from 60-80%.

2) Postoperative Pain
Table 2 shows the comparison between the two groups regarding visual analogue scale at different periods of follow up. From this table, it was found that there was no significant difference between the two groups at different times (first, third and seventh days postoperatively).

| Table 2: Comparison between the two studied groups according to pain |
|---|---|---|---|---|===|===|===|===|
| 1st day | 3rd day | 7th day |
| --- | No. | % | No. | % | No. | % |
| Study | Grade 0 | 19 | 76.0 | 24 | 96.0 | 25 | 100.0 |
| Grade 1 | 6 | 24.0 | 0 | 0 | 0 | 0 | 0 |
| Grade 2 | 0 | 0.0 | 1 | 4.0 | 0 | 0 | 0 |
| Grade 3 | 0 | 0.0 | 0 | 0 | 0 | 0 | 0 |
| Grade 4 | 0 | 0.0 | 0 | 0 | 0 | 0 | 0 |
| Control | Grade 0 | 18 | 72.0 | 25 | 100.0 | 25 | 100.0 |
| Grade 1 | 7 | 28.0 | 0 | 0 | 0 | 0 | 0 |
| Grade 2 | 0 | 0.0 | 0 | 0 | 0 | 0 | 0 |
| Grade 3 | 0 | 0.0 | 0 | 0 | 0 | 0 | 0 |
| Grade 4 | 0 | 0.0 | 0 | 0 | 0 | 0 | 0 |

2) Postoperative Healing on seventh postoperative Day:
Adequate socket healing was detected in all patients of both groups without any sign or symptoms of dry socket. (Figures 5,6)

DISCUSSION
In patients who receive oral anticoagulant treatment, dental extractions are a common procedure. In the past, some authors have proposed that the anticoagulant treatment be stopped for several days before the dental extraction (17); others have proposed reducing the dose of anticoagulant intake for several days before extraction (18). In recent years, it has been suggested that extractions be carried out without any interruption or diminution of the anticoagulant treatment but with emphasis on the efficiency of the local hemostasis (19).

In our study the patients were divided equally into two groups. The patients of the first group (study group) were maintained on their medicine and the patients of the second group (control group) stopped their medicine before extraction. Published reports have shown an increase in the number of patients attending dental units worldwide who are on warfarin therapy (20). For many years, controversy has surrounded the correct management of patients on warfarin therapy requiring minor oral surgery (MOS) procedures (21). Over the last two decades, various clinical protocols were suggested for managing such patients, which included withdrawal of warfarin, reducing the dose, substitution of heparin for warfarin, and continuation of the normal dose of warfarin (22).
Patients treated with oral anti-vitamin K anticoagulants require periodic monitoring, based on the prothrombin time (PT). Since this parameter is somewhat imprecise, use of the INR (international normalized ratio: proportion between patient PT and control PT, standardized and corrected) is currently advised (23).

In the study group the INR was ≤ 3. It consisted of 25 patients (14 males and 11 females), in whom gelatamp delivery to the socket was performed to stop bleeding after tooth extraction.

In the control group the INR was ≤ 1.6 and the prothrombin activity was > 60%. This group consisted of 25 patients (16 males and 9 female) in whom the socket was only supported with heavy pack to stop bleeding after tooth extraction.

Immediately after teeth were extracted we found the bleeding in the study group was higher than in the control group and it was significant because the study group did not stop their medicine so the INR in the study group was higher than in the control group.

Prothrombin time in the study group was higher than in the control group and it was significant because the level of INR in the study group was higher than in the control group.

Regarding postoperative bleeding after five minutes of tooth extraction and after gelatamp was delivered to the socket in the study group and pack pressure in the control group the result was not significant. However the score of bleeding of patients in the study group was better than that in the control group. After two hours, in both group, no patients had bleeding.

On the first day postoperative, there was one patient in the study group who had bleeding grade 1, while in the control group, 3 patients had bleeding grade 1. There was no significant difference between the two groups. The four patients said they experienced bleeding after the medicine was taken at night and the bleeding stopped by applying pressure on the socket using wet gauze for 30 minutes.

On the seventh postoperative day, no patients in the study group had bleeding but in the control group, one patient had bleeding grade 1, the patient said that the bleeding started at night after the anticoagulant was taken. The cardiovascular specialist advised the patient to stop the medicine for 2 days and the bleeding eventually stopped.

In recent years a growing number of investigators have adopted a more conservative approach, preferring not to interfere with drug treatment (i.e., without suspending the medication several days before dental treatment or modifying the dosing scheme), and controlling bleeding after the dental procedure by means of local hemostatic measures (24).

In the present study, results confirm the use of wet gauze with normal saline, gelatamp and suture to control bleeding that is the same as the results obtained by Beirne and Koehler in 1996 (25).

Wahl in 2000 (26), proposed that if INR is in the normal range, there is no need to stop warfarin for dental extraction and if INR is more than the normal range, the dentist should decide on the type of treatment considering the type of dental problem.

Bajkin et al (27) likewise found no significant differences on comparing two groups of approximately 110 patients each (anticoagulation being suspended three days before surgery in one group and maintained in the other).

As indicated by these results, no significant differences were observed in the prevalence of bleeding in one group versus the other – thus supporting the opinion of the above authors that it is not necessary to either suspend or reduce anticoagulation in the context of minor surgery, since local hemostatic measures suffice to minimize bleeding.

In the present study, we evaluated the risk between stopped medicine (risk of thromboembolism) and continuing medicine (risk of bleeding). It has been stated and shown in numerous studies that the risks of arterial and venous thromboembolism can be significantly reduced (by up to 80%) by anticoagulation therapy (28).

Regarding the postoperative pain, in the first day postoperative 6 patients in the study group had pain VAS1 and 7 patients in the control group had pain VAS1. On the third day postoperative, one patient in the study group had pain VAS2 and the patient who complained from the pain was found to have a sharp bony edge painful upon touching so it was smoothed and irrigated and the patient presented without pain after 2 days.

The present study showed that from the first to the seventh postoperative day there was no significant difference regarding pain between the two groups as shown in the result. This finding is in match with Malmquist et al (29) who found there was no significant difference in pain between using local hemostatic agent or not after extraction.

In this study, there was no reported incidence of postoperative infection in all patients and the wound healing progressed well in both groups. This is supported by studies which suggested that the level of coagulation and anticoagulation has no significant impact on wound healing after dental extraction (30).

**CONCLUSIONS**
From the results of this study we can conclude that:
- Gelatamp is an effective material as local hemostatic agent after extraction for anticoagulant patients without interruption of their medicine (within the therapeutic range of INR).
- There is no need to stop the anticoagulant drug before extraction to reduce the risk of thromboembolism.

**CONFLICT OF INTEREST**
The authors declare that they have no conflicts of interest.

**REFERENCES**
EVALUATION OF POLYMER BUR FOR CARIOUS DENTIN REMOVAL IN PRIMARY TEETH

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Abstract:

**Introduction:** The development of a self-limiting caries removal technique would be of great clinical importance. Smart bur II is a relatively new bur in the dental market and its manufacturer is claiming that it is the ultimate bur for selective caries removal.

**Objectives:** The aim of this study was to evaluate caries removal time and efficacy of Smart bur II in comparison with conventional carbide bur.

**Material and methods:** Twenty-three children, each with bilateral primary canine showing comparable class V carious lesions were selected for this study. They were randomly divided into two groups. Group I (n=30): caries was removed using the Smart bur II. Group II (n=30): caries was removed using the conventional carbide bur. The efficacy of caries removal was evaluated by “tug-back” sensation. Time needed for caries removal in both groups was recorded in seconds. An additional group of seven extracted carious primary canine were randomly selected for the in vitro study. Teeth were cut into 2 halves through center of the lesion, one half was subjected to caries removal using Smart bur II as in group I and conventional carbide bur was used in the other half as in group II. The specimen were prepared and topographic features of dentin after caries removal was evaluated using the scanning electron microscope.

**Results:** The comparison of caries removal efficiency between smart bur II and carbide bur showed that the smart bur II completely removed caries in 11 cases accounting for 36.6% and incompletely removes caries in 19 cases accounting for 63.4%. While the carbide bur completely removed caries in 28 cases accounting for 93.3% and incompletely remove caries in 2 cases accounting for 6.7%. Caries removal time ranged between 192 seconds to 380 seconds for smart bur II, while caries removal time ranged between 198 seconds to 361 seconds for carbide bur (control group). The mean ± SD caries removal time was 271.16 ± 26.78 for Smart bur II and 235.16 ± 27.37 for carbide bur. The results of both cariess removal time were significantly different at p ≤ 0.05 and p ≤ 0.05 respectively.

**Conclusions:** The smart bur II had significantly lower caries removal efficiency when compared to conventional carbide bur. The smart bur II required significantly longer caries removal time when compared to conventional carbide bur.

**Keywords:** Polymer bur, conventional carbide bur, caries removal efficiency, caries removal time.

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INTRODUCTION

Over the last decades, dental research has notably improved restorative techniques and materials with the purpose of producing, as reliably as possible, the characteristics and appearance of lost dental tissue. Moreover, the development of adhesive restorative systems minimized the need for resistance form or additional retention and enabled cavities to be prepared without excessive reduction and extension into sound tooth structure (1).

Dentin caries can be divided into two layers. The superficial or outer layer (infected dentin) is contaminated with bacteria, which dissolves the mineralized tissue of dentin and damages the collagen matrix so that remineralization becomes impossible. This layer must be completely removed during caries excavation. However, the inner layer (affected dentin) is invaded by bacteria, which dissolves the mineralized tissue, but the cross-banded ultrastructure of the collagen matrix remains. If these bacteria and their metabolic products which are the main cause of caries are removed, the inner layer of dentin caries can remineralize (2).

Traditional methods of caries removal, such as burs and spoon excavators, tend to remove affected as well as infected dentin, because it is difficult clinically to distinguish between the two. However, total removal of all caries may not be necessary to control progression of the lesion, provided that the restoration is sealed adequately from the oral environment (3). Hence, mechanical caries excavation may have the disadvantage of leaving residual caries or preparing over extended cavities (4). Recent developments in caries removal have therefore involved removal of only soft infected dentin (5).

Moreover, the use of the drilling as the conventional caries removal and cavity preparation method, other than being painful, can cause deleterious thermal (6) and pressure effects on the pulp (7), thus lowering the regenerative potential of the pulp-dentin complex.

Because conventional carbide burs may result in excessive loss of sound tissue, alternative techniques have been researched for their caries removal efficacy (8). Chemomechanical systems utilize solutions or gels to selectively dissolve carious dentin. These methods however are quite time consuming (9). Another selective method for removal of carious dentin is air abrasion. Removal of carious dentin can be controlled by varying the hardness and sizes of the abrasive particle, the cross sectional area of the fluid stream, and the shape of the abrasive particles. The drilling method is preferred for deep caries removal due to air abrasion’s inability to remove soft carious dentin (10).

The sonoabrasion technique provides the removal of the carious tissue using diamond-coated oscillating tips. This method, however, tends to under prepare cavities. (11) These approaches apparently have not superseded conventional methods, including burs and hand excavation, among dental practitioners.

Boston in 2000 (12), has described a polymer bur that only removed softened and infected dentin but not the...
affected dentin. The cutting elements of the bur were made of a softer polyamide polymer material different than the traditional carbide bur. This minimally invasive excavation has the advantage of fewer dentinal tubules being cut and, thereby, less pain sensations being triggered compared to using conventional burs. Polymer bur instruments look like conventional burs, but they are not manufactured from metal, instead, they are manufactured from a special polymer material. The cutting edges are not spiral-like but shovel-like straight. The polymer material has a Knoop Hardness of 50 and was developed with the aim to be harder than carious, softened dentin (Knoop Hardness 0–30) but softer than healthy dentin (Knoop Hardness 70–90). The manufacturer aim was to remove carious dentin selectively, whereas, healthy dentin is not affected. The polymer cutting edges will wear down in contact with harder materials, such as healthy dentin, and will go blunt (13).

Allen et al. in 2005 (14) showed that using polymer burs without local anesthesia was accepted by patients. Silva et al. in 2006 (15) found that dentin surfaces of permanent teeth prepared by polymer burs exhibited significantly lower bond strengths than with carbide burs. An in-vitro study by Hauman and Kuzmanovic in 2007 revealed that polymer burs remove significantly less sound dentin than stainless steel burs and concluded that polymer burs do not cut affected, sound dentin (16).

However, Silva et al. in 2006 evaluated tooth surfaces prepared by polymer burs with transmission electron microscopy and found incompletely removed infected dentin in extracted permanent molars (15).

Due to these advantages and few studies reported, the purpose of this study was to determine the polymer bur’s efficacy for caries removal in the primary teeth in comparison with the conventional carbide bur.

The null hypothesis was that, the smart bur II is as efficient as the conventional carbide bur in carious dentin removal.

**MATERIALS AND METHODS**

The study consisted of a clinical trial and an in vitro study.

Thirty bilateral primary carious canines in children aging 4-8 years were selected from the Pediatric Dentistry and Dental Public Health out-patient clinic at the Faculty of Dentistry, University of Alexandria after securing necessary consents.

The selected children fulfilled the following criteria: Children aged 4-8 years. Children definitely positive or positive according to Frankl Behavior Rating Scale (17). The teeth inclusion criteria were as follows: Class V dentinal carious lesions accessible to rotary instrument (18), caries with medium or soft consistency according to Bjornadal (19), and asymptomatic vital teeth with no proximal caries as evidenced by bite wing radiograph. Teeth exclusion criteria were as follows: Carious lesions with clinical or radiological signs and symptoms of pulpal involvement (interfere with sleeping, history of spontaneous throbbing pain, sensitivity to percussion, gingival redness, swelling or fistula).

Pre-operative examination was done to assure proper case selection, including: (History taking, clinical examination and radiographic examination.)

The researcher was trained and calibrated on the use of smart bur II, intra-examiner reliability was then assessed using Kappa statistics. The Kappa value was 0.8 revealing high strength of agreement (20).

The sixty teeth were randomly assigned to two groups of 30 teeth each as follows:

**Group I:** (n=30) caries was removed using the smart bur II (SS White).

**Group II:** (n=30) the control group, caries was removed using the conventional carbide bur (SS White).

Treatment was carried out according to the following steps: (No local anesthesia was given, unless required, partial isolation was done using cotton rolls and saliva ejector, caries removal was performed using either of the two methods)

In group I (test group) removal of carious dentin was carried out employing smart bur II mounted on a low speed handpiece without water spray. Caries removal proceeded until the smart bur II becomes dull after repeated contact with healthy dentin (21).

In group II (control group) carious dentin was removed using a low speed handpiece without water spray (21), and different sized Tungsten carbide burs, appropriate to cavity size.

Caries removal was terminated when no dentin discoloration was observed visually, under adequate light (22).

Following cavity preparation, each tooth was examined by visual inspection and tactile sensation using a mirror and an explorer to assess caries removal efficiency. Caries was considered to be removed when the explorer did not stick in dentin and did not give a tug-back sensation. The efficiency of caries removal was graded as complete or incomplete and numerically scored 0 or 1 respectively (23).

For both groups, the duration taken for caries removal for each sample was recorded in seconds using a stopwatch. Time taken for caries removal was calculated from the actual start of caries removal until complete carious dentin removal (24).

A total of seven freshly extracted carious deciduous teeth fulfilling the tooth criteria in the clinical trial, were collected for the in vitro study. Each tooth was sectioned longitudinally through the center of the carious lesion into two halves. In one half, caries was removed using the smart bur II, test group (Group I). In the other half, caries was removed using the conventional carbide bur, control group (Group II).

In both groups, caries was removed following the same steps as in the clinical study.

After caries removal, all specimens were dehydrated by passing through ascending grades of ethyl alcohol, 50%, 70%, 95%, then absolute alcohol. Specimens were then vacuumed and gold sputter coated with gold-palladium layer prior to examination (25). The topographical features of the dentin was examined using scanning electron microscope (SEM).

Data concerning caries removal efficiency and time required for caries excavation were tabulated and fed to the
computer and analyzed using statistical software (SPSS pc+ version 16.0). Comparison between the two groups was done using Fisher’s exact test for caries removal efficiency. Two-Tailed paired T test was used for time required for caries excavation. Significance of the obtained results was judged at 5% significance level.

RESULTS

A. Results of Clinical Study:
The present study included 23 patients, they had thirty bilateral primary carious canines, to compare the caries removal efficiency and time of smart bur II to that of carbide bur. From the 23 patients participating in the study, 14 (60.8%) were females and 9 (39.2%) were males. Patients’ ages ranged between 4-7 years with mean ± SD age of 5.39 ± 0.94 years.

The study included 16 carious bilateral primary maxillary canines, and 14 carious bilateral primary mandibular canines (a total of 30 bilateral primary canines).

Table (1) shows the comparison of caries removal efficiency between smart bur II and carbide bur showed that the smart bur II completely removed caries in 11 cases accounting for 36.6% and incompletely removed caries in 19 cases accounting for 63.4%. Whereas, the carbide bur completely removed caries in 28 cases accounting for 93.3% and incompletely removed caries in 2 cases accounting for 6.7%. Fisher’s exact test revealed significant difference between both groups (P ≤ 0.05).

Table 1: Comparison of caries removal efficiency between smart bur II and carbide bur.

<table>
<thead>
<tr>
<th>Tug back score</th>
<th>Smart bur II n (%)</th>
<th>Carbide bur n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>11 (36.6%)</td>
<td>28 (93.3%)</td>
</tr>
<tr>
<td>1</td>
<td>19 (63.4%)</td>
<td>2 (6.7%)</td>
</tr>
<tr>
<td>total</td>
<td>30 (100%)</td>
<td>30 (100%)</td>
</tr>
</tbody>
</table>

*: Statistically significant at p ≤ 0.05.

Table (2) shows the comparison of caries removal time between Smart bur II and carbide bur. Caries removal time ranged between 192 to 380 seconds for smart bur II (test group), whereas caries removal time ranged between 198 to 361 seconds for carbide bur (control group). The mean ± SD caries removal time for smart bur II was 271.16 ± 26.78 (test group) and for carbide bur was 235.16 ± 27.37 (control group). Two-Tailed paired T test revealed significant difference between both groups (P ≤ 0.05).

Table 2: Comparison of caries removal time between smart bur II and carbide bur.

<table>
<thead>
<tr>
<th></th>
<th>Smart bur II (seconds)</th>
<th>Carbide bur (seconds)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Min-max</td>
<td>192-380</td>
<td>198-361</td>
</tr>
<tr>
<td>Mean</td>
<td>271.1667</td>
<td>235.1667</td>
</tr>
<tr>
<td>(SD)</td>
<td>26.78</td>
<td>27.372</td>
</tr>
</tbody>
</table>

P <0.0001*

*: Statistically significant at p ≤ 0.05.

B. Results of scanning electron microscope:
Examination of the dentin surface at the floor of the cavity following caries removal using the smart bur II revealed the following:

Most of the specimens showed an irregular globular surface, almost completely covered by smear layer (Figure 1).

Fig. 1: Scanning electron micrograph (SEM) of dentin surface following caries removal by smart bur II (Mag. x2000)

The smear layer gave the dentin floor a cloudy appearance with very few, barely detected dentin tubules orifices (Figure 2).

Other cavities appeared with obvious fissures within their floor, certain areas showed clearly exposed dentinal tubules openings with little amount of smear debris haphazardly scattered (Figure 3).

Fig. 2: Scanning electron micrograph (SEM) of dentin surface following caries removal by smart bur II (Mag. x7500)

Fig. 3: Scanning electron micrograph (SEM) of dentin surface following caries removal by Smart bur II. (Mag. x2000)
Examination of the dentin surface at the floor of the cavity following caries removal using the carbide bur revealed the following:

Most of the specimens showed an irregular porous surface (Figure 4), with almost complete removal of the smear layer (Figure 5). Obvious cracking and scratches were seen traversing the floor of the cavity. The openings of dentinal tubules were evidently exposed with fissures and grooves passing from them into the adjacent peritubular and intertubular dentin (Figure 6). Bacterial deposits were barely detected in carbide bur specimens.

![Fig. 4: Scanning electron micrograph (SEM) of dentin surface following caries removal by carbide bur. (Mag. x 2000)](image)

![Fig. 5: Scanning electron micrograph (SEM) of dentin surface following caries removal by carbide bur. (Mag. x 7500)](image)

![Fig. 6: Scanning electron micrograph (SEM) of dentin surface following caries removal by carbide bur. (Mag. x 2000)](image)

DISCUSSION

As the principles of minimal invasive approach indicate the need to remove only dental tissue to the extent that is strictly necessary for treatment. The development of a self-limiting caries removal technique would be of great clinical importance.

Smart bur II is a relatively new bur in the dental market and its manufacturer is claiming that it is the ultimate bur for selective caries removal. The current study found an interest in comparing caries removal efficiency and time needed for caries removal between Smart bur II and conventional carbide bur in primary teeth. Also the present study assessed the topographic features of dentin after caries removal with the smart bur II compared with the conventional carbide bur in primary teeth. The study consisted of two parts, a clinical trial and an in-vitro study. In both studies self-control study design was employed.

The clinical trial included 30 primary canines, inclusion and exclusion criteria aimed to ensure that all studied teeth were vital, with no pulpal involvement. Therefore, no pulpal treatment was needed. To ensure standardization, each primary canine was used as test control. Class V cavity design was considered the most appropriate cavity design for comparing caries removal efficiency and time between smart bur II and conventional carbide bur in primary teeth. Class V cavity does not have any macro-mechanical undercut eliminating the need of access gaining before employing smart bur II. In the present study, sample characteristics concerning lesion location and consistency were comparable to exclude any variable that could affect the final results.

Rubber dam was not used in the present study to avoid any possible discomfort that could be associated with clamp placement, since treatment was initiated without local anesthesia. Also, according to manufacturer’s instruction, smart bur II doesn’t necessitate complete isolation. Allen et al in 2005 stated that when dentin cutting is limited to the superficial layer of infected dentin, sparing the odontoblast reaction zone, caries removal can be completed without the need for local anesthesia (26).

In the present study the visual and tactile criteria were adopted because it is the most widely used clinical criterion to evaluate complete caries removal (27).

The results of the present study revealed significant difference between smart bur II and conventional carbide bur in both caries removal efficiency and time needed to remove caries. Smart bur II was significantly less efficient in caries removal and required more time for caries removal. El Nasri et al in 2015 evaluated the efficacy of caries removal by hand excavation (ART), chemomechanical caries removal agent (carisolve) and polymer bur (smart bur II). The results of El Nasri study showed that smart bur II had significantly lower caries removal efficiency when compared to either carisolve, or hand excavation (ART), the lower caries removal efficiency of smart bur II reported by El Nasri is in agreement with our results (28).

Celiberti et al in 2006 assessed caries removal effectiveness of 4 different dentin excavation methods, one of them was polymer bur in primary molars. The study revealed that polymer bur and Er:Yag laser left the largest amount of decayed tissue unexcavated in agreement with our results. However, both hand excavator and chemomechanical caries removal showed effective caries removal (29).

The results of the present study regarding time needed for caries removal were in agreement with Prabhakar et al in 2009 (30), and Allen et al in 2005 (26). The polymer bur
caries excavation time was significantly longer when compared to conventional bur in both of the previous studies. The former studies attributed the longer excavation time of polymer bur to the lower hardness number of the polymer bur, the path taken by the instrument and the need to change the bur when it becomes dull following contact with sound dentin. Vijay et al in 2012 concluded that polymer bur was more time consuming than conventional burs, but at the same time the polymer bur was more conservative and selective in removing carious dentin (31).

The in vitro study included 7 primary canines. The methodology of the in vitro part of current study was conducted to simulate the clinical situation as closely as possible. Thus natural primary teeth were employed, following the same teeth selection criteria used in the clinical trial. To ensure standardization each primary canine was used as test control. The scanning electron microscope was employed to evaluate the topographic characteristics of the dentin surface following the smart bur II and the conventional bur caries removal.

The scanning electron microscope showed different topographic characteristics of the dentin surface in both tested groups. The dentin surface following the smart bur II caries removal showed irregular globular surface, almost completely covered by smear layer. However, the dentin surface following conventional bur caries removal showed an irregular porous surface with almost complete removal of the smear layer.

The topography of the prepared dentin surface influence the bonding of the adhesive restorative materials. After mechanical removal of caries with rotary instruments a smear layer is formed (32). The smear layer is an amorphous layer of organic and inorganic debris which is formed on the dentin surface after accomplishment of cavity preparation and removal of the carious tissue. It adheres firmly to the dentin surface from where it cannot be removed by the ordinary water spray and prevents resin from adhering to dentin, thus the smear layer has to be removed or modified prior to the placement of the restoration (33).

The topographic study also showed very few, barely detected dentinal tubules orifices, with numerous bacterial deposits on dentin surface following the smart bur II caries removal. Whereas the openings of dentinal tubules were evidently exposed, with clearly obvious peritubular and intertubular dentin, and bacterial deposits were barely detected on dentin surface following the conventional bur caries removal. This probably indicates that the conventional bur removes both the infected and affected dentin reaching to the underlying sound dentin, while the smart bur II removes only the infected dentin and preserves the affected dentin.

A possible limitation of the present study was comparing caries removal with smart bur II to a single type of minimal invasive caries removal methods (conventional carbide bur), on the other hand comparing smart bur II to different minimal invasive caries removal methods might have revealed a wider range of results. However, further studies with special attention to restorative and adhesive characteristics following the use of smart bur II are needed.

CONCLUSIONS
Within the limitations of the present study, the following was concluded:

• In comparison to the conventional carbide bur the smart bur II had less caries removal efficiency when compared to conventional carbide bur.
• In comparison to the conventional carbide bur the smart bur II required longer caries removal time when compared to conventional carbide bur.
• The dentin floor topography varied between the tested materials indicating more dentin removal by carbide bur.

CONFLICT OF INTEREST
The authors declare that they have no conflicts of interest.

REFERENCES
EXPRESSION OF BONE MORPHOGENETIC PROTEIN-2 UNDER SIMVASTATIN THERAPY AFTER CYCLOSPORIN -A- INDUCED ALVEOLAR BONE LOSS IN RATS

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Abstract:
Introduction: Cyclosporin-A has been used as an immunosuppressant to prevent the rejection of organ transplants. However, alveolar bone loss is an important negative side-effect of this drug. Simvastatin, a hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor, is known to inhibit cholesterol biosynthesis. It has advanced effects on bone formation in vivo and in vitro. So, we evaluated the expression of BMP 2 after administration of simvastatin in cyclosporin -A-associated alveolar bone loss in rats.

Objective: To evaluate the effect of simvastatin and cyclosporin -A- on Alveolar bone by investigating the expression of Bone Morphogenetic Protein -2 (BMP-2) using Immunohistochemical and Image analysis investigation methods.

Materials and methods: 24 adult male albino rats were divided into 3 groups: Group I: control group; 4 rats. Group II: cyclosporine -A- group; 10 rats (10 mg/kg) daily subcutaneous injection. Group III: Cyclosporin -A- /Simvastatin group; 10 rats, simvastatin was taken orally daily (20mg/kg/day). Two rats from the control group and 5 rats from each of the studied experimental groups (group II & III) were sacrificed on days 15 and 30 consecutively, and examined using Immunohistochemical method, and Image analysis.

Results: Immunohistochemical results revealed strong expression of BMP 2 in osteoblasts, osteocytes after simvastatin administration and weak expression in CsA. The same results were statistically significant in Immunohistochemical Optical Density (IOD) results. Histomorphometrical analysis of bone volume showed a significant increase in bone volume in simvastatin group than CsA group, and significant decrease in CsA than control.

Conclusion: we can conclude that Simvastatin counteract the adverse effect of CsA induced alveolar bone loss by induction of BMP 2 in osteoblasts and osteocytes that induced new bone formation.

Keywords: Simvastatin, Cyclosporin A, bone loss, Bone Morphogenetic Protein 2.

INTRODUCTION
Bone is a rigid and dynamic organ that is characterized as a type of specialized connective tissue. Biochemically, it is defined by a mixture of inorganic elements and an organic matrix. It is composed of 50 to 70% mineral, 20 to 40% organic matrix, 5 to 10% water, and 3% lipids (1).

Bone matrix is mostly composed of type I collagen, about 90%, with trace amounts of types III and V. and 10% ground substances including: proteoglycan, glycoproteins, and non-collagenous proteins, that are embedded in the extracellular matrix as (bone morphogenetic proteins, insulin like growth factor-1 and 6, colony stimulating factors, cytokines, adhesion molecules) (2).

Bone remodeling is the process by which bone is renewed to maintain bone strength and mineral homeostasis. The remodeling process resorbs old bone and forms new bone to prevent accumulation of bone micro damage. Remodeling begins before birth and continues until death (3).

The remodeling cycle is composed of four sequential phases which are: quiescent, activation, resorption, reversal, and formation phases (4).

Bone Morphogenetic Protein (BMPs) are a group of proteinaceous growth factors in the TGF-β superfamily (5). The discovery of BMPs in the pioneering work by Urist in 1965 (6) was a landmark in the development of bone tissue engineering. The classical role for BMPs is considered to be the induction of (ectopic) cartilage and bone formation (6).

According to the WHO, Osteoporosis is defined as “a systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue with a consequent increase in bone fragility and susceptibility to fracture”. It is a common and costly disease with a considerable annual health system burden all over the world. Morbidity and mortality related to this disease and its associated fragility fractures are also increasing (7).

In osteoporosis, disruption of the resorption and formation processes in the bone remodeling cycle results in net bone loss and, therefore, in a lower bone mass and increased risk of fracture (8).

Osteoporosis affects the jawbones, whose structure may be impaired by other conditions resulting in bone loss. One of these is periodontitis (PD), a chronic infection-mediated condition modulated by different genetic and environmental conditions.
factors, characterized, in advanced forms, by loss of the soft tissue attachment to teeth and resorption of alveolar bone (9). Osteoporosis is a significant adverse reaction in transplant recipients. Recently, an increasing number of immunosuppressive programs use glucocorticoid-free regimens, but other immunosuppressants, such as calcineurin inhibitors (e.g. cyclosporine A), are also associated with the pathogenesis of transplantation-related osteoporosis (10).

Cyclosporin-A (CsA) is a cyclical polypeptide of 11 amino acids, one of which is unique to the cyclosporins. First isolated as an antifungal agent, it has been shown to have marked immunomodulatory properties. These properties have meant that the drug can be used as an immunosuppressant to prevent the rejection of transplants, both of organs (kidney, liver, and pancreas) and bone marrows, as well as in the control of autoimmune diseases (11). One of the proposed side effects of cyclosporine is bone loss after long-term use. Bone loss was seen in trabecular bone more than cortical bone (12).

Cyclosporine A inhibits osteoblast differentiation, osteocalcin production, and collagen synthesis, reducing the bone replaced in each remodeling cycle (13). Simvastatin is a chemically modified derivative of lovastatin. It is a butanoic acid with the empirical formula C25H38O5 (14).

Simvastatin is a reversible competitive inhibitor of the enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase), so, like the other statins, is an inhibitor of the mevalonate pathway and consequently cholesterol synthesis (15).

Simvastatin, a synthetic statin has a number of pleiotropic effects as well. In addition to its anti-resorptive actions, it has been found to exert anabolic effects on bone (16). Local stimulation of BMP-2, a major bone growth regulatory factor, can lead to new bone formation. Mundy et al. (1999) (17) identified that lovastatin, and simvastatin, mevastatin, and fluvastatin increased gene expression for BMP-2 in osteoblasts.

The biologically significant, anti-inflammatory and antioxidant properties of simvastatin are other pleiotropic effects of interest from a periodontal therapeutic standpoint (18).

One of the most important pleiotropic effect of statins are anticancer agents because of their ability to trigger apoptosis in a variety of tumor cells in a manner that is sensitive and specific to the inhibition of HMG-CoA reductase (19).

MATERIALS AND METHODS
Materials
1. Experimental animals:
The study was performed after gaining the approval of the Research Ethics Committee, Faculty of Dentistry, Alexandria University.

Twenty-four adult male albino rats weighting 90-100 grams were selected to be used in this study. These animals were obtained from the Institute of Medical Research, Alexandria University.

Animals were housed in specially designed wire mesh bottom cages. They were supplied a regular diet throughout the whole experimental period which last for 30 days.

Rats were divided randomly into three groups:
Group I: control (consisted of 4 rats) was injected with 1 ml saline.
Group II: Cyclosporin -A (Sandimmun®; supplied in form of vials. Each vial contained 50 mg of Cyclosporine A).
The group consisted of 10 rats, (20) which were subcutaneously injected with Cyclosporin -A., subcutaneous daily injection (10 mg/kg body weight, once a day).
Group III: cyclosporin A/Simvastatin group consisted of 10 rats. This group was treated with both Cyclosporin -A and Simvastatin (Zocor®; supplied in form of tablets. Each tablet contained 20 mg of simvastatin). Simvastatin was taken by oral daily doses (once a day) at 20 mg/kg (21).

Two rats from the control group and 5 rats from each of the studied experimental groups (group II & III) were sacrificed on days 15 and 30 consecutively after commencement of the daily treatments.

Methods:
1. Immunohistochemical technique:
Immunohistochemical procedures were carried out using an indirect immunoperoxidase system. Tissue slices were blocked on the coated slides and treated with rabbit polyclonal antibodies to BMP-2 for detecting the immunostaining by Avidin Biotin Complex (ABC) method (22).

2. Image analysis:
Morphometric analysis was carried out in alveolar bone of Haematoxylin and Eosin-stained section. Images were viewed and recorded using the Olympus microscope equipped with digital camera, using computer program Matlab software (Image J, the MATHWORKS, Inc., and USA). The image of each section of all groups was captured using x40 objective lens (Barr = 50 µm) with numerical aperture of a high resolution (16 bit digital camera, 1280x1024 pixel) for counting osteoclast and osteoblast cells and calculating bone volume, and immunochemical Optical Density (IOD).

Statistical analysis (23):
Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. Quantitative data were described using range (minimum and maximum), mean, standard and median. The distributions of quantitative variables were tested for normality using Kolmogorov-Smirnov test, Shapiro-Wilk test and D’Agstino test, also Histogram and QQ plot were used for vision test. For normally distributed data, comparison between more than two population were analyzed F-test (ANOVA) to be used and Post Hoc test (LSD). For abnormally distributed data, comparison between more than two independent population were done using Kruskal Wallis test. Significance of the obtained results was judged at the 5% level.
RESULTS
The results obtained from this study included both immunohistochemical results and image analysis.

1. Immunohistochemical findings
Immunohistochemistry stains BMP 2 protein in the apical region of the alveolar bone of the albino rats was done on the control and the two experimental study groups.

In the present study the BMP 2 immunoreactivity appeared as granules and diffuses brown color in cytoplasm and cell membrane of osteoblasts, osteocytes and bone matrix.

The control sample of the BMP 2 immunoreactivity was negative due to the absence of the polyclonal antibody of BMP 2 immunoreactivity.

Group A (15 days):

Group IA (Control): (fig 1)
The immunointensity of the cells were moderate (+2) to strong (+3). The bone matrix appeared with several spreading granules (appeared in incremental lines) representing the presence of BMP 2 in the matrix itself.

Group IIA (CsA 15 days): (fig 2)
The immunoreactive stains of BMP 2 protein were weak (+1) to moderate (+2) on osteoblasts, and osteocytes. The bone matrix appeared with less expression and diffuse granules (This group revealed a decrease immunoreactive expression of BMP 2 protein than group IA) lesser than group IA).

Group IIIA (CsA& Simvastatin 15 days): (fig 3)
The osteoblast and osteocytes were moderate (+2) to strong (+3) positive expression of immunoreactive stains of BMP 2 protein. The bone matrix appeared with several spreading granules (appeared in incremental lines) representing the presence of BMP 2 protein expression in the matrix itself.

Group B (30 days):

Group IB (control): (fig 4)
The immunoreactive of BMP 2 protein expression of the osteoblast cells, and osteocyte were moderate (+2) to strong (+3). The bone matrix appeared with several spreading granules (appeared in incremental lines) representing the presence of BMP 2 protein expressed in the matrix itself.

Group IIB (CsA 30 days): (fig 5)
There was a decreased of the immunoreactive stain of BMP 2 protein expression in the osteoblast cells, osteocytes were given a negative (0) to weak (+1).
Simvastatin Effect on Cyclosporin-A-Induced Bone Loss

Fig. 5: Paraffin section micrograph of rat alveolar bone administrated (CsA 30 days) in the apical portion. (ABC –DAB  Bar=50µm x 400).

Group IIIB (CsA& Simvastatin 30 days): (fig 6)
The long duration of the administrative simvastatin plus CsA showed an increase of the BMP 2 protein expression. The osteoblast and osteocytes were moderate (+2) to strong (+3) immunoreactive stain. The bone matrix appeared with several spreading granules, representing the presence of BMP 2 protein in the matrix itself. The osteoblasts appeared plump and strongly immunopositive arranged on the bone surface.

2. Image Analysis:
   a. Bone volume:
      Group A (after 15 days)
      Table (1) showed mean values of bone volume per total volume in the apical region of the control (IA), CsA (IIA), and (CsA &Simvastatin) (IIIA), after 15 days, the values were (0.21 ± 0.02, 0.12 ± 0.02, 0.26 ± 0.05), respectively.

      There was a statistically significant decrease in the bone volume in group CsA group (IIA) in comparison with control group (IA) (p1= 0.002). But, a statistically significant increase in the bone volume in group (CsA &Simvastatin) (IIIA) in comparison with CsA group (IIA) (p3 <0.001).

      In contrast, there was no statistically significant difference between control group (IA) and group (CsA &Simvastatin) (IIIA) (p2= 0.071).

Fig. 6: Paraffin section micrograph of rat alveolar bone administrated (CsA& Simvastatin 30 days) in the apical portion. (ABC –DAB  Bar=50µm x 400)

Table 1: Comparison between the control and the two studied groups according to bone volume.

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (n=5)</td>
<td>B (n=5)</td>
<td>A (n=5)</td>
<td>B (n=5)</td>
</tr>
<tr>
<td>Bone volume</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Min. – Max.</td>
<td>0.17–0.23</td>
<td>0.19–0.28</td>
<td>0.09–0.15</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>0.21 ± 0.02</td>
<td>0.28 ± 0.04</td>
<td>0.12 ± 0.02</td>
</tr>
<tr>
<td>Median</td>
<td>0.22</td>
<td>0.21</td>
<td>0.13</td>
</tr>
<tr>
<td>P1</td>
<td>0.002*</td>
<td>&lt;0.003*</td>
<td>0.071</td>
</tr>
<tr>
<td>P2</td>
<td>&lt;0.004*</td>
<td>&lt;0.004*</td>
<td></td>
</tr>
<tr>
<td>P3</td>
<td>&lt;0.004*</td>
<td>&lt;0.004*</td>
<td></td>
</tr>
</tbody>
</table>

Table (1): Comparisons between the different studied groups according to Bone volume

Group B (after 30 days):
Table (1) illustrated the comparison between control (IB), CsA (IIB), and (CsA &Simvastatin) (IIIB) regarding bone volume in the apical region, where the values were (0.23 ± 0.04, 0.09 ± 0.02, 0.29 ± 0.06) respectively.

It was noticed that (CsA &Simvastatin) (IIIB) exhibited the highest bone volume followed by the control group (IB), where the lowest bone volume related to CsA (IIB).

There was statistically significant decrease between CsA (IIB) and control (IB) (p1 <0.001), also a high significant increased between CsA (IIB), and (CsA &Simvastatin) (IIIB) (p1 <0.001).

On the other hand, after Simvastatin treatment there was a statistically significant difference increased in (CsA &Simvastatin) (IIIB) compared to control (IB) group (p2= 0.040).

b. Immunohistochemical Optical Density (IOD) of BMP 2:
   Group A (15 days)
The mean value of IOD for the BMP 2 were 117.62 ± 5.83, 98.07 ± 9.92, and 121.51 ± 1.68 for group IA (control), IIA (CsA), and IIIA (CsA& Simvastatin), respectively.

From table (2), there was a statistically significant decrease in IOD between group IA (control) and IIA (CsA), where p value is p1<0.001. Also, there was a statistically significant increase in IOD of BMP-2 for group IIIA (CsA& simvastatin) than group IA (control), where p value is p3<0.001.

There was no statistically significant difference in IOD between group IA (control) and group IIIA (CsA& simvastatin).

   Group B (30 days)
The mean value of IOD for the BMP 2 in group B were 116.37 ± 6.53, 95.37 ± 8.73, and 95.37 ± 8.73 for group IB (control), IIB (CsA), and IIIB (CsA& Simvastatin), respectively.

There was a statistically significant decrease in IOD
between group IB (control) and IIB (CsA), where p value is p1<0.001. Also, there was a statistically significant increase in IOD of BMP-2 for group IIB (CsA& simvastatin) than group IB (control), where p value is p3<0.001.

There was no statistically significant difference in IOD between group IB (control) and group IIB (CsA& simvastatin).

Table 2: Comparison between the control and the two studied groups according to IOD of BMP 2 in the alveolar bone.

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>A</th>
<th>B</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=5)</td>
<td>(n=5)</td>
<td>(n=5)</td>
<td>(n=5)</td>
<td>(n=5)</td>
<td>(n=5)</td>
</tr>
<tr>
<td>Max.</td>
<td>110.0</td>
<td>109.8</td>
<td>102.7</td>
<td>102.5</td>
<td>104.6</td>
<td>105.6</td>
</tr>
<tr>
<td>Min.</td>
<td>89.3</td>
<td>89.5</td>
<td>82.7</td>
<td>82.5</td>
<td>85.7</td>
<td>85.9</td>
</tr>
<tr>
<td>Mean ± SD.</td>
<td>96.0 ± 8.8</td>
<td>96.0 ± 8.8</td>
<td>90.9 ± 7.9</td>
<td>91.5 ± 7.8</td>
<td>113.1 ± 5.4</td>
<td>113.1 ± 5.5</td>
</tr>
<tr>
<td>Median</td>
<td>96.0</td>
<td>96.0</td>
<td>90.9</td>
<td>91.5</td>
<td>112.1</td>
<td>112.1</td>
</tr>
<tr>
<td>p1</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>0.332</td>
<td>0.533</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p2</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p3</td>
<td>0.724</td>
<td>0.627</td>
<td>0.348</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

p1: p value for Post Hoc Test (LSD) comparing Group I and each of the other groups.
p2: p value for Post Hoc Test (LSD) comparing Group II and Group IIIB.
p3: p value for Student t-test comparing between A and B in each group.

*: Statistically significant

**DISCUSSION**

It is estimated that 200 million women have osteoporosis worldwide, for these reason it is considered as a major public health problem (24).

Both osteoporosis and periodontal diseases are bone resorptive diseases; it has been hypothesized that osteoporosis could be a risk factor for the progression of periodontal disease (9).

Immunosuppressive drugs that are used mainly after organ transplantation to prevent rejection as cyclosporine A, are associated with post transplantation-related osteoporosis (10).

Cyclosporin A (CsA) immunosuppressive properties result primarily from inhibition of T lymphocyte activation. CsA suppresses the immune response by inhibiting evolutionary conserved signal transduction pathways. CsA binds to their intracellular receptors, immunophilins, creating composite surfaces that block the activity of specific targets. For CsA/cyclophilin the target is calcineurin. Inhibition of the action of calcineurin results in a complete block in the translocation of the nuclear factor of activated T cells (NF-AT), resulting in a failure to activate the genes regulated by the NF-AT transcription factor. These genes include those required for B-cell help such as interleukin (IL-4) and CD40 ligand as well as those necessary for T-cell proliferation such as IL-2 (25).

In the present study, we used an established and well characterized animal model to evaluate, firstly, the role of simvastatin in the prevention of cyclosporine A-induced alveolar bone loss on the absence of inflammation and, secondly, estimate the expression of BMP-2 after simvastatin therapy indicating bone formation.

In this study, the duration of induction of alveolar bone loss by administration of immunosuppressive doses of cyclosporine A for 30 days caused alveolar bone loss, in agreement with previous reports (26, 27). They observed an increased bone resorption, represented by a significant increase in osteoclast-like cell number, accompanied by a reduction in the bone volume.

Cyclosporine A (Sandimmun®) was given subcutaneously daily (10 mg/kg body weight, once a day). According to Wassef et al (20), this dosage provides plasma peak and therapeutic concentrations of 1000 and 750 ng/mL, respectively. This dosage of cyclosporine A and period of treatment were previously shown to cause alveolar bone loss in rats (28).

Simvastatin (Zocor) was given oral daily doses (once a day) at 20.0 mg/kg. This method has been used successfully in previous studies in this animal model (21). The dose was within the range found to be safe and effective for increasing bone density in rats, whereas literature posology reports include both weekly and daily administrations (29).

In addition, we showed that administration of simvastatin counteracted the deleterious effects of cyclosporineA on bone turnover in the absence of inflammation. These results are compatible with those reported by Ohno et al (29), who showed that treatment with cerivastatin (a synthetic statin) improves cyclosporine A-induced high-turnover osteopenia in transplanted bone, mainly through the inhibition of bone resorption. Many studies also stated the same results (26, 27, 30).

Immunohistochemical analysis was done to study the expression of BMP 2 in all groups. The results of this study showed a significant increase in the BMP 2 after simvastatin administration, approximately near that in the control, while it showed a significant decrease in CsA treated group.

BMPs are synthesized as large precursors, which are processed and proteolytically cleaved to yield biologically active substance. BMPs have been demonstrated in osteoblasts, and osteoprogenitor cells, and surprisingly in osteoclasts (31).

McCullough et al conducted a study to examine the immunohistochemical distribution and intensity of staining of BMPs in various cell types found in the ectopic bone to define the role of BMPs in bone formation. The results was moderate to intense cytoplasmic immunostaining for BMP-2 in osteoprogenitor cells, most osteoblasts showed at least moderate staining for BMP-2 and There was moderate to intense staining for BMP-2 in most osteocytes found within the trabecular bone matrix (32).

In the present study, we found that the level of expression of BMP 2 in bone matrix, osteoblasts, and osteocytes in CsA group is significantly lower than that in the control. Luppen et al supported our result. They propose that glucocorticoid (GC), another immunosuppressive drug inhibited Bmp2, which was secondary to the inhibition of...
the osteoblast phenotype (33).

In simvastatin group, the level of expression of BMP-2 was significantly higher than in the CsA group, but to a near extent similar to the control. In agreement with Garrett et al (34).

BMP-2 has been shown to enhance collagen synthesis. In addition, BMP-2 induces a significant increase in cellular alkaline phosphatase activity at doses ranging between 20 and 200 mg/ml (35).

In agreement with Ohnaka et al, who stated that Pitavastatin, a newly developed statin, increased the expression level of mRNA for BMP-2 (36).

In bone volume histomorphometric analysis, we can thus state that CsA at a dose of 10 mg/(kg body weight day) leads to a significant decrease in bone volume per total volume (BV/TV). These results were supported by Nasser et al (37).

Animal studies have demonstrated acute increases in bone remodeling consisting of both osteoclast activation and, in some circumstances, increases in osteoblastic bone formation in CsA treated animals. There is limited but persuasive evidence that T cells may mediate the action of CsA (25).

While in simvastatin treated group the bone volume per total volume (BV/TV) were significantly increase. Junqueira et al supported our results and stated that there is a statistical significant differences were observed between the ovariectomized animals of the control and simvastatin treated groups, in histomorphometric analysis of bone volume proving that treatment with simvastatin improved the bone regeneration of the ovariectomized rats (21).

Finally, it can be recommended to use statins in the treatment of osteoporotic patients, especially who receive immunosuppressive drugs.

CONCLUSIONS
Simvastatin counteracts the adverse effect of CsA induced alveolar bone loss by induction of BMP-2 in osteoblasts, and osteocytes that induces new bone formation. Also Simvastatin has a pleotropic effect as an anti-inflammatory, antioxidant, and anticancerous agent.

CONFLICT OF INTEREST
The authors declare that they have no conflict of interest.

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EXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTOR DURING HEALING OF EXTRACTION SOCKETS IN DIABETIC RATS

El Hady T¹ BDS, Karam S² PhD, El Sawa A² PhD, Saad N³ PhD

Abstract:
Introduction: Vascular endothelial growth factor (VEGF) is one such candidate. It functions as an endothelial cell mitogen, chemotactic agent, and inducer of vascular permeability. Other angiogenic growth factors such as basic fibroblast growth factor (bFGF) and transforming growth factor β (TGF-β) have been described, but VEGF is unique for its effects on multiple components of the wound healing cascade, including angiogenesis and recently shown epithelization and collagen deposition. VEGF is produced by many cell types that participate in wound healing: endothelial cells, fibroblasts, smooth muscle cells, platelets, neutrophils, and macrophages.

Objectives: To evaluate the expression of VEGF during healing of extracted sockets in diabetic rats.

Materials and methods: 24 adult male rats aged about 6 months and weighing about 250 gms were divided into 2 groups; Group I (12 rats) non diabetic and Group II (12 rats) diabetic. For study group, the rats were fasted overnight and diabetes was induced by a single intra peritoneal injection of streptozotocin 60 mg/kg body weight in 0.1 M citrate buffer. All animals, were exposed to surgical wounds (extracted lower right first molar). They were sacrificial as follows 4 rats from each group at intervals of 3 days, 7 days, 21 days after extraction for immunohistochemical study.

Results: In the present study, immunohistochemical expression of VEGF was detected as brown cytoplasmic reaction. All the examined cases showed positive results for VEGF with different scores.

Conclusions: these results demonstrated the expression of VEGF in diabetic rats during healing of extraction sockets significantly higher than control group in late periods.

Key words: VEGF, Extraction wound healing, diabetes, immunohistochemistry.

INTRODUCTION

Wound healing is a sequence of cellular and biological response directed toward restoring tissue integrity and functional capacity following injury (1).

The inflammatory stage occurs during the first week of healing. White blood cells enter the socket to remove contaminating bacteria from the area and begin to break down any debris, such as bone fragments, that are left in the socket (2).

In the second week the healing continues to get organized through fibroplasia and new blood vessels that begin to penetrate towards the center of the clot. Trabeculae of osteoid slowly extend into the clot from the alveolus, and osteoclastic resorption of cortical margin of the alveolar socket is more distinct (1). In smaller sockets the epithelium may have become fully intact by this point (2).

By the third week the extraction socket is filled with granulation tissue and poorly calcified bone forms at the wound perimeter (1).

Diabetes mellitus is a group of metabolic diseases in which a person has high blood glucose either because the body does not produce enough insulin, or because cells do not respond to the insulin that is produced by the pancreas. This resulting high blood sugar produces the classical symptoms of polyuria: frequent urination polydipsia (increased thirst) and polyphagia (increases hunger) (3).

Modelling and establishment of new blood vessels is critical in wound healing and takes place concurrently during all phases of the reparative process. In addition to attracting neutrophils and macrophages, numerous angiogenic factors secreted during the haemostatic phase promote angiogenesis (4).

Resident endothelial cells are responsive to a number of angiogenic factors, including FGF, VEGF, PDGF, angiogenin, TGF-β and TGF-α. A fine balance is kept by the action of inhibitory factors, such as angiostatin and steroids (5).

Inhibitory and stimulatory agents act on proliferating endothelial cells directly as well as indirectly, by activating mitosis, promoting locomotion and by stimulating the host cells to release endothelial growth factors (6).

Under hypoxic conditions, molecules are secreted from the surrounding tissue, promoting proliferation and growth of endothelial cells. In response, a four-step process takes place: (i) production of proteases by endothelial cells for degradation of the basal lamina in the parent vessel in order to crawl through the extracellular matrix, (ii) chemotaxis, (iii) proliferation, and (iv) remodeling and differentiation. FGF and VEGF play central regulatory roles in all of the processes (7).

Initially, there is no vascular supply in the wound centre, so viable tissue, which is limited to wound margins, is perfused by uninjured vessels and by diffusion through damaged interstitium (8). Capillary sprouts from the surrounding edges invade the wound clot and, within a few days, a microvascular network composed of many new capillaries is formed (9).

Chemotaxis is the ability of cells to move along a chemical gradient (10). This biochemical mechanism enables cells to reply properly to environmental stimuli that determine proliferation, differentiation and migration.
Chemotactic agents act on cell surface receptors to direct the cell migration that is involved in angiogenesis during wound healing (11).

Migration is the consequence of chemotactic activity and is necessary for angiogenesis (12).

Vascular endothelial growth factor (VEGF) is one such candidate. It functions as an endothelial cell mitogen, chemotactic agent (13), and inducer of vascular permeability (14). Other angiogenic growth factors such as basic fibroblast growth factor (bFGF) and transforming growth factor β (TGF-β) have been described, but VEGF is unique for its effects on multiple components of the wound healing cascade, including angiogenesis and recently shown epithelization and collagen deposition (15).

MATERIALS AND METHODS
A clinical trial was conducted on ten adult patients of both Experimental animals: All experimental animal procedures were approved by faculty institutional ethics committee. Twenty four adult male rats weighing about 200-250 gms, and aged about 6 months were used in this study. These animals were obtained from the Institute of Medical Research Alexandria University. Animals were housed in specially designed wire mesh bottom cages, three animals per cage. All the animals were supplied a regular and the same diet adlibitum throughout the whole experimental period. The study included the following groups:

Group I: (12 rats).
Group II: (12 rats) with induced diabetes.

Methods
Preoperative phase
Induction of diabetes:
For study group, the rats were fasted overnight and diabetes was induced by a single intraperitoneal injection of streptozotocin 60 mg/kg body weight in 0.1 M citrate buffer (16).

Streptozotocin (STZ) was freshly prepared immediately before injection, and it was kept in cold store and refrigerator temperature (2-8°C) away from light. If it is not used fresh, streptozotocin solution can exhibit reduced ability to induce diabetes (17).

At the time the animals were entered the study, their body weight and blood glucose level were recorded then they were measured 48 hours after the administration of streptozotocin, then every two weeks throughout the time of the experiment.

Operative phase
Anaesthesia
Each animal received general anaesthetical solutions of 10% (40 ml/kg body weight) and zylazine 2% (5 mg/kg body weight)

Surgical procedures
The surgical site was disinfected using iodine swab, extraction of mandibular right 1st molar of all animals was carried out.

Animals euthanasia:
Four animals in each group were sacrificed at 3, 7, and 21 days following tooth extraction. Jaws were dissected, and specimens were fixed in 10% formalin saline for 7 days to be prepared for Immunohistochemical study.

Immunohistochemical evaluation:
Immunostaining for VEGF using anti-rabbite polyclonal antibody, clone Po-A (ready to use) was done according to kit manual. Antigen retrieval using citrate buffer, (PH 0.6) was done for 1 hour at 40 °C (18).

Assessment of VEGF expression
The presence of brown cytoplasmic precipitate in the inflammatory cells and others indicated positive reaction. Tissue sections were examined under light microscopy at x100 magnification in order to identify five fields with the largest number of immunostained cells using x400 magnification, the counting of the immunopositive cells was performed in each one of these fields.

Immunoreaction of VEGF was classified according to Ruiz et al (19) to the following scores: 0 – no staining; 1 – weak, staining in 11 – 25 % of cells; score 2 – moderate, staining in 26 – 75 % of cells; score 3 – strong, staining in more than 76% of cells.

RESULTS
In the present study, immunohistochemical expression of VEGF was detected as brown cytoplasmic reaction. All the examined cases showed positive results for VEGF but in different scores.

3 days after tooth extraction
In all studied cases, the VEGF immune staining was detected in inflammatory cells, endothelial cells, fibroblast, osteoblast cells.

In group I, score for the VEGF expression ranged from 2 – 3 Fig. 1(A), in the median (2.0- 3.0) (Table 1).

In group II, score for the VEGF expression ranged from 2 – 3 Fig. 2 (A), in the median (2.0-3.0) (Table 1).

7 days after tooth extraction:
In all studied cases, the VEGF immune staining was detected in endothelial cells, fibroblast, osteoblast cells and residual inflammatory cells.

In group I, score for the VEGF expression ranged from 2 – 3 Fig.1 (B), in the median (2.0-3.0) (Table 1).

In group II, score for the VEGF expression ranged from 2 – 3 Fig. 2 (B), in the median (2.0-3.0) (Table 1).

21 days after tooth extraction
In all studied cases, the VEGF immune staining was detected in inflammatory cells, endothelial cells, fibroblast, osteoblast cells.

In group I, score for the VEGF expression ranged from 1 – 2 Fig. 1 (C), in the median (1.0-2.0) (Table 1).

In group II, score for the VEGF expression ranged from 2 – 3 Figs. 2(C), in the median (2.0-3.0) (Table 1).

Using Mann Whitney test to compare between the different periods Wilcoxon signed ranks test was applied, in group I, no significant changes between 3 days and 7 days p1 (1.000), whereas statistically significant changes were
observed between 3 days and 21 days p2 (0.038) and statistically significant change between 7 days and 21 days p3 (0.059).

In group II, no significant changes between 3 days and 7 days p1 (1.000), and no significant changes between 3 days and 21 days p2 (0.083) and no significant changes between 7 days and 21 days p3 (0.083).

**Fig. 1 (A):** Group I after 3 days showing moderate cytoplasmic brown colouration in ≥26 ≤75% of inflammatory cells, endothelial cells (red arrow) and osteoblast (yellow arrow) (VEGF, Avidin biotin, DAB x 400).

**Fig. 2 (A):** Group IV after 3 days showing strong cytoplasmic brown colouration in ≥76% of inflammatory cells, osteoblast cells (yellow arrow) and endothelial cells (red arrow) (VEGF, Avidin biotin, DAB x 400).

**Fig. 1 (B):** Group I after 7 days showing strong cytoplasmic brown colouration in ≥76% of endothelial cells (red arrow), fibroblast (black arrow), and residual inflammatory cells (VEGF, Avidin biotin, DAB x 400).

**Fig. 2 (B):** Group III after 7 days showing strong cytoplasmic brown colouration in ≥76% of inflammatory cells, endothelial cells (red arrow) and fibroblast cells (black arrow) (VEGF, Avidin biotin, DAB x 400).

**Fig. 1 (C):** Group I after 21 days showing weak cytoplasmic brown colouration in ≥11 ≤ 25 % of keratinocytes (blue arrow) and fibroblast cells (black arrow) (VEGF, Avidin biotin, DAB x 400).

**Fig. 2 (C):** Group III after 21 days showing moderate cytoplasmic brown colouration in ≥ 26 ≤ 75% of endothelial cells (red arrow) and fibroblast cells (black arrow) (VEGF, Avidin biotin, DAB x 400).
Table (1): Comparison between the two studied groups according to expression of VEGF during the healing of extracted socket in diabetic rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>After extraction</th>
<th>3 days</th>
<th>7 days</th>
<th>21 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td></td>
<td>3.0 (2.0 - 3.0)</td>
<td>3.0 (2.0 - 3.0)</td>
<td>1.0 (1.0 - 2.0)</td>
</tr>
<tr>
<td>Sig. bet. periods</td>
<td>p1 = 1.000</td>
<td>p2 = 0.038</td>
<td>p3 = 0.059</td>
<td></td>
</tr>
<tr>
<td>Group II</td>
<td></td>
<td>3.0 (2.0 - 3.0)</td>
<td>3.0 (2.0 - 3.0)</td>
<td>2.0 (2.0 - 3.0)</td>
</tr>
<tr>
<td>Sig. bet. periods</td>
<td>p1 = 1.000</td>
<td>p2 = 0.083</td>
<td>p3 = 0.083</td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>1.000</td>
<td>1.000</td>
<td>0.042*</td>
<td></td>
</tr>
</tbody>
</table>

Abnormally distributed data was expressed in median (Max.- Min.) and was compared using Mann Whitney test. To compare between the different periods Wilcoxon signed ranks test was applied

p1: p value for comparing between 3 days and 7 days
p2: p value for comparing between 3 days and 21 days
p3: p value for comparing between 7 days and 21 days
*
Statistically significant at p ≤ 0.05

DISCUSSION

The VEGFs and their corresponding receptors are key regulators in a cascade of molecular and cellular events that ultimately lead to the development of the vascular system, either by vasculogenesis, angiogenesis or in the formation of the lymphatic vascular system (20).

Although VEGFs’ main effects are on endothelial cells, they also bind to VEGF receptors expressed on monocytes, neurons, chondrocytes and osteoblasts (21).

Recent studies have shown that the combination of angiogenic and osteogenic factors can stimulate bone healing and regeneration (22).

Both osteogenesis and angiogenesis are integrated parts of bone growth and regeneration. Combined delivery of osteogenic and angiogenic factors is a novel approach in bone regenerative engineering. Exogenous addition of vascular endothelial growth factor (VEGF) and bone morphogenetic proteins (BMPs) together with an osteoconductive scaffold is a very promising method to enhance bone repair (23). Therefore the present study was designed to evaluate the expression of VEGF in healing of extraction socket in diabetic rats.

The present result revealed positive expression of VEGF by inflammatory cells, precursor endothelial cells, keratinocytes, and bone cells including osteoblasts and active osteocytes in different periods in all groups but in different scores. These results are in agreement with E. Fadhil et al (24), who found positive expression of VEGF by bone marrow stromal cells, adipocytes, mesenchymal stem cells, precursor endothelial cells, and bone cells including osteoblasts and active osteocytes in different periods in all groups but in different score Therefore, his data provide evidence that VEGF activity is essential for appropriate bone formation and mineralization in response to injury.

VEGF is produced by many cell types that participate in wound healing: endothelial cells (25), fibroblast (26), smooth muscle cells (27), platelets (28) and neutrophils (29).

In the present study, figure 1 (A, B) & figure 2 (A, B) show positive cytoplasm immuno staining in endothelial cell of newly formed capillaries. One of VEGF’s roles in wound healing is in stimulation of angiogenesis. Wound healing angiogenesis involves multiple steps including vasodilation, basement membrane degradation, endothelial cell migration, and endothelial cell proliferation (30).

VEGF induces endothelial cell migration in wound healing through two primary mechanisms, chemotaxis and vasodilatation. In the initial phase of angiogenesis, endothelial cells migrate before mitotic division (31).

Mechanism I: Chemotaxis—Chemotaxis is a highly regulated process involving cell adhesion molecules’ interaction with the extracellular matrix (32).

Mechanism II: Increasing vascular permeability—Another mechanism by which VEGF induces endothelial cell migration in wound healing is related to the increase in vascular permeability mediated by NO and prostacyclin. Leakage of the plasma protein fibrinogen and its subsequent conversion in the extracellular space to a fibrin gel stimulates endothelial migration (33).

In the present study, figure 1 (C) shows granulation tissue formation established healing responses. An essential feature of normal wound repair is the formation of granulation tissue, i.e. fibrovascular tissue containing fibroblasts, collagen and blood vessels, which is the hallmark of an established healing response (34).

In addition, VEGF does not only contribute to vasculogenesis in the embryonic period and angiogenesis of normal tissues such as smooth muscle, cardiac muscle, and liver but is also secreted at solid cancer and inflammatory tissues and is closely involved in pathological angiogenesis in the many diseases including cancer, chronic rheumatoid arthritis and diabetic retinopathy (35).

Presently, VEGF is used clinically as a blood test item. The circulating VEGF concentration has been reported to be significantly higher in patients with various diseases in which VEGF is associated with pathological angiogenesis than in healthy Individual (36).

Kakizawa et al (37), compared the serum VEGF concentration between diabetic patients and healthy controls and reported that it was significantly higher in diabetic patients.

In the different groups the expression of VEGF was not significantly different at 3 and 7 days, but it was significantly different at 21 days.

In diabetic retinopathy, retinal vessels are damaged by abnormal metabolism due to hyperglycemia, hypoxia is induced by the occlusion of retinal vessels, and VEGF expressed at the site of vascular damage. Thus, it has been established that VEGF promotes pathological retinal angiogenesis (38). Moreover Takayama et al reported that the serum VEGF concentration was significantly higher in patients with diabetic retinopathy than without, they suggested that VEGF expressed in the retina affects the serum VEGF concentration through peripheral blood vessels (39). Also, it has been reported that diabetic rats show marked VEGF expression at the site of surgery and associated pathological angiogenesis and vascular hyper permeability which affect the delay in wound healing after
periodontal surgery compared with normal rats (40) confirming our results of delayed healing in group III.

CONCLUSIONS
From the results of the current study, the following can be concluded:
- Expression of VEGF in diabetic rats during healing of extraction socket is higher than control group in late period.
- Diabetes is considered an important risk factor for severe infection of the wound.
- Diabetes induction impaired healing of extraction wound in comparison with non diabetes group.

ACKNOWLEDGEMENT
The authors would like to express thanks to the department of Oral Biology at the Faculty of Dentistry, Alexandria University and to their families for their support throughout the study.

CONFLICT OF INTEREST
The authors declare that they have no conflicts of interest.

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A COMPARATIVE STUDY BETWEEN POROUS TITANIUM GRANULES AND NANOCRYSTALLINE HYDROXYAPATITE IN HEALING OF MANDIBULAR DEFECTS IN DOGS
Ahmed N¹ BDS, Mahmoud T² PhD, Shafik S³ PhD, El Dibany R² PhD.

Abstract:
Background: Bone grafting is a common technique in Oral and Maxillofacial surgery to replace missing bone. Grafting materials include autografts, allografts, xenografts and synthetic bone substitutes. Two synthetic bone materials are currently available; porous titanium granules and nanocrystalline hydroxyapatite.

Objectives: This study compared the use of Porous titanium granules (PTG) versus Nanobone® in healing of mandibular defects in dogs and this was estimated by histological and histomorphometric analysis.

Materials and Methods: The study was conducted on 10 healthy experimental dogs. An osseous defect of 10 mm depth and 10 mm width was created in the premolar area of both right and left sides of the mandible. The right side defects were grafted with PTG while the left side defects were grafted with Nanobone®. Histological and histomorphometrical evaluation was carried out to monitor bone healing and quantitate the bone volume with both PTG and Nanobone® at 3, 6 and 12 weeks intervals post-operatively.

Results: The mean bone volume value with PTG was 773.4 ± 499.4 on the 3rd week, then increased on the 6th week to be 10125.3 ± 19287.3 and 2676.0 ± 1388.2 on the 12th week. The mean bone volume value with Nanobone® was 525.5 ± 332.1 on the 3rd week, then on the 6th week it became 1976.8 ± 1568.1. There was a statistically significant difference regarding the mean bone volume value between the two groups.

Conclusion: Both PTG and Nanobone® have osteoconductive properties and are effective in healing bone defects, but the histomorphometric analysis quantified the bone volume with both PTG and Nanobone® and revealed that the maximum amount of the total regenerated bone was seen in the PTG group; bone was formed within its porosities.

Key Words: Porous Titanium Granules, Nanobone®, bone defects, bone volume, histomorphometric analysis.

INTRODUCTION
Bony defects in the jaw occur for various reasons. They are caused by infection, tumors or cysts. To maintain teeth or to provide sufficient bone height for later implantation or prosthesis supply, the filling of bone defects is necessary (1).

Grafting materials include autografts, allografts, xenografts and synthetic bone substitutes. However, the majority of grafting procedures are autografts. i.e. the graft is taken from the same patient. The graft can be harvested either intraorally; from the mandibular symphysis, retromolar region or maxillary tuberosity or extraorally; from the femur, tibia, ribs or iliac crest of the pelvis (2,3).

Nanocrystalline hydroxyapatite bone graft has been introduced for augmentation procedures in intrabony defects. Its Advantages are osteoconductivity and bioresorbabilty (4). When it is used as a bone graft substitute, rapid healing of critical size defects was observed in animal experiments and in human applications (4). It binds to bone and stimulates bone healing by stimulation of osteoblastic activity (5).

Nanobone® is a nanocrystalline hydroxyapatite embedded in a porous silica gel matrix. Unlike conventional HA forms that are usually sintered at temperatures ranging from 700°C up to 1200°C, Nanobone® is produced at a temperature < 200°C. The lower processing temperature has a profound effect on the material’s porosity and surface area (6).

The biocompatibility of titanium (Ti) has been proved in recent years. Titanium particles can stimulate the activation of complement system and platelets and can increase the level of platelet-derived growth factor. This factor has been shown to promote bone growth (7). These properties of titanium are incorporated in porous titanium granules (PTG), which contain 700-1000 micrometers diameter granules, and its porous nature makes the bone infiltration through the particles possible (8-13).

Therefore, due to the scarce histological data on both PTG and Nanobone® this study was designed to compare histologically and histomorphometrically the osseous defect regeneration and bone volume value after applying PTG and Nanobone®.

MATERIALS AND METHODS
This study was conducted on 10 healthy adult mongrel dogs, about 18-24 months old, and with an average weight 10 to 15 kg. The animals were kept under the same nutritional and environmental conditions at the Physiology Department, Faculty of Medicine, Alexandria University. The dogs were divided equally into two groups (right side and left side):

Group 1: A surgical defect was created in the right premolar area of the body of the mandible and was grafted with PTG (Natix™).
**Group II:** A surgical defect was created in the left premolar area of the body of the mandible and was grafted with Nanobone®.

Nanix™ PTG (Tigran technologies AB: Medeon science park, S-205 12 Malano, Sweden) is a bone graft material made of pure titanium and available in vials. The titanium granules are between 0.7 mm and 1.0 mm in size, porous, irregular in shape, grey in color and non-resorbable. These irregularities enable the granules to interlock with bone thus enabling primary and long term stability through osteoconduction and osseointegration.

Nanobone® (Nanograft: Pharma k dental Gmbh, Germany) consists of nanocrystalline HA embedded in a silica gel matrix. It has two forms; fine (0.6mm x 2mm) and rough (1mm x 2mm). All NanoBone® technology products are produced in a sol-gel process at temperatures below 200°C. The low temperatures means that the material is not sintered and its surface is therefore highly porous with pores ranging from nanometers to micrometers in size. The autologous proteins from the blood enter the nanopores and cover the entire inner surface.

All dogs were healthy as documented by a veterinarian report. All dogs were kept on the same balanced diet consisting of milk, broth and meat throughout the whole period of the study. Each animal received a single dose of antibiotics intravenously in the form of ampicillin 25 mg/kg body weight (Epico pharmaceutical co., 10th of Ramadan City, Cairo, Egypt) just before the operation. All operating procedures were performed under general anesthesia and strict sterile conditions in an animal theatre. Each animal was generally anaesthetized via intravenous injection of thiopentone sodium 5% (Egyptian international pharmaceutical industries company (E.I.P.co), the dose of which was calculated on the basis of 30 mg/kg body weight.

With the dogs under general anesthesia, a 5 cm gingival incision was performed on the buccal side in the premolar region on both sides of the dogs mandible and the mucoperiosteal flap was elevated, an Osseous defect of 10 mm depth and 10 mm width was created on both sides of the dogs mandible by the aid of a trephine bur on electric motor under copious saline irrigation (fig.1).

Group I: The right side of the body of the mandible was packed with PTG (fig.2a).

Group II: The left side of the body of the mandible was packed with Nanobone® (fig.2b) 3/0 chromic cat gut was used to suture the flap.

Post-operatively, the animals were transferred to a clean cage to be kept under observation to assess the presence or absence of any infection, wound dehiscence or graft rejection, they were kept on soft diet consisting of bread, milk and broth during the first four weeks postoperatively, the dogs received intra-muscular injection in the form of (25-50mg/kg/dog of ampicillin) every 24 hours for 5 days post-operatively, the dogs received the same course of medication; anti-inflammatory and analgesic in the form of diclofenac potassium 25mg/day (Cataflam, Novartis pharma,cairo, Egypt) twice daily for 3 days.

![Fig 1: A photograph showing an osseous defect of 10mm width and 10mm depth created in the mandibular premolar area.](image1)

![Fig2: (a) A photograph showing grafting of the bone defect of the right side of the mandible with PTG. (b) A photograph showing grafting of the bone defect of the left side of the mandible with NanoBone®.](image2)
bone volume formed with PTG and Nanobone® on intervals of 3, 6 and 12 weeks.

**Statistical analysis of the data**
Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. Quantitative data were described using Range (minimum and maximum), mean, standard deviation and median. The distributions of quantitative variables were tested for normality using Kolmogorov-Smirnov test, Shapiro-Wilk test and D’Agostino test, also Histogram and QQ plot were used for vision test. The data were abnormally distributed so, non-parametric tests were used. For abnormally distributed data, comparison between two independent populations were done using Mann Whitney test. Significance of the obtained results was judged at the 5% level.

**RESULTS**

**Macroscopic results**
All animals tolerated the surgical procedure without complications, and they started to eat on the first postoperative day and exhibited a normal pattern of activity. Healing was uneventful without any signs of infection or wound dehiscence. All blocks obtained from both group I and group II at 3, 6 and 12 weeks were prepared for microscopic and histomorphometric evaluation.

**Microscopic results:** Hematoxylin and Eosin stain

**On the third postoperative week**

**Group I (PTG group)**
Complete filling of the defect areas by dense collagen fibers with high vascularity surrounding the PTG with fibroblasts in between and old bone bordering the defect areas. No inflammatory reaction was detected. The mean bone volume value with PTG was 773.4 ± 499.4 mm³ on the 3rd week (Table 1, Fig. 3a).

**Group II (Nanobone® group)**
Nanobone® filling the defect area with granulation tissue in between except for some areas of resorption of the graft material, as the inorganic components of Nanobone® were removed by decalcification process so it appeared as empty spaces. There is a few amount of fibrovascular tissue and old bone found at the periphery of the defect. No inflammatory cells were detected. The mean bone volume value with Nanobone® was 525.5 ± 332.1 mm³ on the 3rd week. (Table 1, Fig. 3b)

**On the sixth postoperative week**

**Group I (PTG group)**
The defect areas showed deposition of newly calcified bone spicules over the PTG surrounding irregular areas with remnants of homogenous PTG, osteoid ground substance of bone and slight fibers in between. The mean bone volume value with PTG was 10125.3 ± 19287.3 mm³ on the 6th week. (Table 2, Fig. 4a)

**Group II (Nanobone® group)**
Although, Nanobone® still occupying parts of the defect areas, newly formed bony specules were seen occupying the resorbed graft areas. Nanobone® was separated from the old bone surface with some granulation tissue within the space. The active osteoblasts were observed rimming the new bone and connected to old bone. The mean bone volume value with Nanobone® was 287.4 ± 322.5 mm³ on the 6th week. (Table 2, Fig. 4b)

**Table 1:** Comparison between PTG and Nanobone regarding bone volume after 3 weeks.

<table>
<thead>
<tr>
<th>Duration</th>
<th>PTG (n=10)</th>
<th>Nanobone (n=10)</th>
<th>Z</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Min. – Max.</td>
<td>79.5 - 1239.4</td>
<td>120.8 - 1008.5</td>
<td>0.361</td>
<td>0.393</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>773.4 ± 499.4</td>
<td>525.5 ± 332.1</td>
<td>2.883</td>
<td>0.004</td>
</tr>
<tr>
<td>Median</td>
<td>961.7 mm³</td>
<td>391.9 mm³</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Z: Z for Mann Whitney test
*: Statistically not significant at p>0.05

**Table 2:** Comparison between PTG and Nanobone regarding bone volume after 6 weeks.

<table>
<thead>
<tr>
<th>Duration</th>
<th>PTG (n=10)</th>
<th>Nanobone (n=10)</th>
<th>Z</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Min. – Max.</td>
<td>253.0 - 46689.3</td>
<td>70.2 - 890.4</td>
<td>2.883</td>
<td>0.004</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>10125.3 ± 19287.3</td>
<td>287.4 ± 322.5</td>
<td>2.883</td>
<td>0.004</td>
</tr>
<tr>
<td>Median</td>
<td>763.7 mm³</td>
<td>190.3 mm³</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Z: Z for Mann Whitney test
*: Statistically not significant at p>0.05
Fig. 4: (a) LMs after 6 weeks showing the defect areas almost filled with calcified bone spicules formed over the Tigran granules (red arrows) with areas in between containing slight fibers and remnants of homogenous tigran granules and osteoid ground substance. (green arrows). (H&E stain x100)
(b) LMs after 6 weeks showing the newly formed bone spicules replacing areas of resorbed Nanobone® and connected to the old bone surface (red arrows) with remnants of the graft in its way to resorption and still filling the defect areas with granulation tissue (green arrows). (H&E stain x100)

On the twelfth postoperative week
Group I (PTG group)
Complete filling of the defect areas with newly formed thick bone and fusion with the old bone trabeculae. Haversian systems and osteocytes were seen with clear lines of demarcation between old and new bone. There were very small areas in its way of calcification. The mean bone volume value with PTG was 2676.0 ± 1388.2 mm³ on the 12th week. (Table 3, Fig. 5a)

Group II (Nanobone® group)
The defect areas were filled with newly formed bone spicules replacing areas of resorption of the graft material and connected to the old bone. There were areas of unresorbed Nanobone® still well demarcated from the newly formed bone and surrounded by fibrous tissue. The mean bone volume value with Nanobone® was 1976.8 ± 1568.1 mm³ on the 12th week. (Table 3, Fig. 5b)

Table 3: Comparison between PTG and Nanobone regarding bone volume after 12 weeks

<table>
<thead>
<tr>
<th>Duration</th>
<th>Tigran (n = 10)</th>
<th>Nanobone® (n = 10)</th>
<th>Z</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>After 12 weeks (mm³) x10³</td>
<td>618.4 - 4410.5</td>
<td>100.7 - 3924.7</td>
<td>3.187</td>
<td>0.001</td>
</tr>
<tr>
<td>Min. – Max.</td>
<td>2676.0 ± 1388.2</td>
<td>1976.8 ± 1568.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>2463.1 mm³</td>
<td>1575.6 mm³</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Z: Z for Mann Whitney test
*: Statistically not significant at p>0.05

The mean bone volume value on the 3rd postoperative week showed no statistically significant difference when comparing PTG with Nanobone® in osseous defects regeneration (p = 0.393) (Table 1). While, the mean bone volume value on the 6th postoperative week showed a statistically significant difference when comparing PTG with Nanobone® in osseous defects regeneration (p = 0.004) (Table 2). Also, the mean bone volume value showed statistically significant difference on the 12th postoperative week when comparing PTG with Nanobone® in osseous defects regeneration (p = 0.001) (Table 3, fig. 6).

Fig. 5: (a) LMs after 12 weeks showing Complete filling of the defect area with thick, new and well calcified bone. (H&E stain x100)
(b) LMs after 12 weeks showing new bone spicules filling the defect areas replacing the resorbed Nanobone® (red arrows) and connected to the old bone (D). (H&E stain x100)

DISCUSSION
Reconstruction of maxillofacial continuity defects has always been a challenge for scientists and surgeons over the years. The main goal of the reconstruction of the maxillofacial region is to restore facial form, function, full rehabilitation of occlusion and articulation (14).

The present study was conducted to compare the effect of PTG and Nanobone® on bone healing of surgically created osseous defects in dogs. The dogs were divided equally into two groups;
Group I: A surgical defect was created in the right side of the body of the mandible and was grafted with PTG.

Group II: A surgical defect was created in the left side of the body of the mandible and was grafted with Nanobone®.

In this study, the experimental animals of choice were the Dogs due to several advantages including: being less expensive to maintain, cost effective to feed and they show patterns of bone accretion and peak bone mass profiles similar to those of human as well as true skeletal maturity (15).

The present study revealed that PTG can be considered as an appropriate bone substitute material, and it can promote bone regeneration in bone defects due to its osteoconductive properties, these results agreed with Wohlfahrt et al in 2010 (16) who stated that significantly more bone was formed in PTG grafted defects compared to those left empty. The new bone grew both through the porosities of the granules and onto the implant surfaces.

PTG belongs to a class of material that may be preferable for filling cavities because it serves as a non-resorbable scaffold that promotes a three-dimensional matrix, which can stabilize and maintain the shape of the filled area. This agreed with Marei in 2010 (17) where PTG were packed around the implant in the coronal gap between the fixture and socket wall; PTG showed good stabilization of the implant and preferable osseointegration between bone and the implant surface without being resorbed.

The histomorphometric analysis in the present study revealed that the bone volume value in the osseous defects filled with PTG was higher than those occupied with Nanobone® but no statistically significant difference could be detected between the two groups. This agreed with Wälivaara and Abrahamsson in 2013 (18) where no statistically significant difference could be detected between autogenous bone or one of the bone graft substitutes, when they were used to fill osseous defects in dogs following apicectomy of mandibular premolars. This non-significant difference might be explained by sample size limitation.

The present study proved that PTG can be used in small defects without membrane. This disagreed with Ruiz RF et al. in 2014 (19) who found that the PTG particles must be covered by a membrane, especially when grafting larger defects, to control particle dislocation, promote clot stabilization and separate the PTG graft from undesired soft tissue cells.

Macroscopically, no signs of inflammation and complete healing of the surgical sites was observed in all dogs of both groups. These findings agree with those of Gholami in 2010 (20) who reported the application of PTG does not interfere with the initial healing of the surgical wound. Bone marrow around PTG was fatty vascular but in some locations where grafted material (BIO- OSS and BIO-GEN) were used, the bone marrow was fibrovascular which provides evidence that the bone formation remodeling process in the samples containing PTG was developing correctly.

There were no signs of infection, abnormal reaction, wound dehiscence or extrusion of the material in any of the dogs. This disagreed with GabAllah in 2014 (21) where gingival inflammation, apical migration of attachment epithelium and destruction of the periodontium were detected. The signs of complete periodontium regeneration were not clearly seen when Nanobone® was tested in healing of periodontal defects in dogs in comparison with autogenous grafts and control groups.

In two studies performed by Gotz et al (22) in 2010 and Harms et al in 2012 (23), they reported a high osteoconductivity of Nanobone®. The presence of silicate ions appears to promote the process of bone formation and remodeling at the bone-Hydroxyapatite interface. In contrast to what was observed with other HA-based bone substitute materials, the rapid osseointegration of Nanobone® seemed to prevent its complete degradation. The Nanobone® particles were completely and firmly embedded within newly formed bone without a detectable fibrous interface and with no indication of an adverse host reaction to the material. This agreed with the present study that showed varying amounts of newly formed bone found through the specimens. Well-mineralized regenerated bone with lamellar parallel-fibred structure and Haversian systems surrounded the residual NanoBone® particles. These results also were in accordance with Canullo L (24) in 2009 where concluded that NanoBone® showed good histological outcomes for augmenting maxillary sinus floor with critical bone volume. There was evidence of new bone formed at (3, 6 and 12) weeks in defects filled with NanoBone®. The area under the graft gradually filled with new bone adjacent to peripheral defective area with bundles of connective tissue fibers extending to surround the NanoBone® were observed. The bone formation became apparent with increasing time of implantation. This result was in accordance with Alaa, Jaber, Kadhim and Al-Soudani (25) in 2012 where the amount of new bone formed in NanoBone® filled defects was much more than that formed in controls when it was used in healing of experimentally induced frontal bone defects in rabbits.

The present study revealed complete healing of the defects in all dogs with new bone formation without the need to use a membrane. This result was in agreement with EL Dibany and Shoukry (26) in 2014 where the combined use of Nanobone® and PRF for bone regeneration following the enucleation of large mandibular odontogenic cysts induced accelerated bone healing and improved the quality and quantity of regenerated bone also without using a membrane.

Histologically, the results of the present study were: Group I; the defect areas showed signs of complete fusion and healing of PTG with formation of bone trabeculae and immature Haversian system. Complete filling of the defect area with thick, new well calcified bone and lines of demarcation with old bone, numerous old osteons and osteocytes. Woven bone is a weak structure and does not have well-organized tissues (27) and it is the first bone tissue that is formed in the bone regeneration process (28).

Regarding Group II, bone formation was seen in direct contact with surface of NanoBone® granules, also there was
obviously fibrous connective tissue embedded in the calcified matrix. Mature connective tissue form the intergranule matrix (29,30).

CONCLUSIONS
Both PTG and NanoBone® are effective in osseous defect regeneration by forming new bone. Regarding the mean bone volume value, there was a statistically non-significant relation between PTG and NanoBone® groups on the 3rd week, while the relation is statistically significant between them at the 6th and 12th week.

The bone volume formed with PTG was more than that formed with NanoBone® throughout the follow up period.

CONFLICT OF INTEREST
The authors declare that they have no conflicts of interest.

REFERENCES