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Histological Evaluation of Osseous Defects Combined with Orthodontic Tooth Movement

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Abstract

Background: The aim of this study was to histologically evaluate the effectiveness of different times of initiating orthodontic tooth movement on enhancement of bone formation in surgical bony defects. **Methods:** In 18 male guinea pigs, 3-4 months of age, a bony defect was created in the alveolar process midway between the central incisor and mandibular 1st molar. These bony defects were implanted with bioactive glass particles and collagen membrane. According to the application of the orthodontic tooth movement, the animals were divided into three groups, each comprised of six guinea pigs. In Group I, the orthodontic tooth movement was initiated immediately after the surgical procedure. In Group II, the orthodontic tooth movement was applied 2 weeks after the surgical procedure, while in Group III no orthodontic tooth movement was applied. Section blocks for histology were made at 1, 3 and 6 weeks after the surgical procedure. **Results:** All experimental sites showed active bone formation with plump osteoblast and osteoid matrix deposition in the treated area. In Groups I and II a dense fibrous tissue formation and highly cellular coarse bone were seen at six weeks. The histomorphometric analysis showed that Group I revealed the greatest number of newly formed trabeculae: 2.4, 6.4 and 8.6 at 1, 3 and 6 weeks, respectively. In addition, Group I defects revealed a greater total surface area of newly formed bone than Groups I and III: 2.96 mm^2 at the end of the study period. **Conclusion:** The combined orthodontic/regenerative therapy seemed to enhance the process of bone formation. Bone formation was histologically observed in all test groups. Defects treated with immediate application of orthodontic tooth movement showed a statistically significant increase in trabecular count and total surface area of newly formed bone than the other experimental groups.

Key words: Surgical bony defects, combined orthodontic / regenerative therapy, bone grafts, membranes, follow-up studies

Introduction

Bone is a connective tissue and guarantees protection and support to the organ function. Bone is a dynamic tissue that constantly undergoes turnover (Proff and Romer, 2009).

In orthodontic tooth movement, sites of tension display osteogenesis over an extensive surface area, a framework consistent with modeling. However, sites of compression undergo phases of remodeling cycles (King *et al.*, 1991b). The tooth movement will occur only if the hard tissue around the tooth can undergo proper breakdown and build-up. Such remodeling requires the presence of cells able to resorb (osteoclastogenesis) and cells able to form bone (osteogenesis) (Diès *et al.*, 1996; Skoglund *et al.*, 1997).

One important consideration is how remodeling cycles are initiated. Much experimental evidence has linked bone remodeling to microdamage and to subsequent increased cellular activity. Microcracks in bone caused by fatigue or trauma may play an important role in the initiation of remodeling cycles (Galley *et al.*, 2006). Crack displacements are capable of tearing osteocyte cell processes, which may directly secrete bioactive molecules into the extracellular matrix, triggering a remodeling response (Hazenberg *et al.*, 2006). The increased prevalence of microcracks at compression sites in orthodontic tooth movement further suggests that they are important in initiating orthodontic bone remodeling (Verna *et al.*, 2004).

Much like tooth eruption, osteogenesis associated

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with orthodontics is mediated by various osteoinductive molecules. In general, most of these molecules are regulated by tensile strains and act by stimulating osteoblast progenitor cell proliferation in the periodontal ligament, with subsequent bone formation and inhibition of bone resorption. Molecules that have been linked in this way to orthodontic tooth movement include transforming growth factor-beta (TGF-β) (Brady *et al.*, 1998), various bone morphogenic proteins (BMPs) (Mitsui *et al.*, 2006), bone sialoprotein (BSP) (Domon *et al.*, 2001) and epidermal growth factor (EGF) (Guajardo *et al.*, 2000; Gao *et al.*, 2002).

Enhanced periodontal and bone regeneration by orthodontic tooth movement towards a bony defect (Geraci *et al.*, 1990; Nevins and Wise, 1990; Liou and Huang, 1998) and intrusive movement (Melsen, 1986; Cardaropoli *et al.*, 2001) have been reported. However, others did not find this effect (Polson *et al.*, 1984; Wennstrom *et al.*, 1993). Moreover, the type of bone towards which the tooth moves also plays an important role. Tooth movement into cortical bone results in fenestration or dehiscence, i.e., loss of cortical bone plate integrity (Steiner *et al.*, 1981 and Steigman *et al.*, 1993), while tooth movement in the alveolar trough results in subsiding of injury/repair cycles (Steigman *et al.*, 1993).

Recently, bioactive glass (BG), a ceramic material, has gained much attention because of its unique silica component compared to other bioceramic alloplastic graft materials. The formation of a silica-gel layer on the surface of the graft particles is thought to be responsible for the bioactivity and osteoconductivity of the material (Nishida *et al.*, 2006).

Bioabsorbable collagen membranes have been tested for their ability to promote regeneration in intrabony defects (Mattson *et al.*, 1995; Benque *et al.*, 1997; Mattson *et al.*, 1999). The collagen materials possess additional advantages over other bioabsorbable membranes (Locci *et al.*, 1997) with no specific immune reaction (Schlegel *et al.*, 1997). In an attempt to achieve periodontal regeneration, the present study included the use of bioactive glass with a bioabsorbable collagen membrane.

The rationale behind this study was to utilize the potential enhancing effect of orthodontic tooth movement on bone formation. To the best of our knowledge, the influence of the timing of the application of force has not been previously investigated. This study evaluated bony tissue responses at different times of initiation of orthodontic tooth movement.

Materials and methods

The research protocol for this study was approved by the institutional Animal Care and Use Committee of Al-Azhar University. The study was conducted on eighteen male guinea pigs, aged 3-4 months, weighing from 250-450 gram. Pre-operatively, the animals were anaesthetized with 120 mg/kg intramuscular ketamine hydrochloride (Vardimon *et al.*, 2001).

The submental region was shaved and then scrubbed with a disinfectant solution. Using a facial approach, a submental incision was made using a Bard-Parker scalpel with a #15 blade. A mucoperiosteal elevator was then used to reflect the tissues and expose the bone. By using the mental foramen as a reference point two bony defects were created using surgical bur number 2 attached to a low speed motor with an irrigation system, each midway between the mental foramen central incisor. The size of bony defects was fixed by using a sterile standard fabricated stent, which was 4 mm in depth x 4 mm in width. The surgical bony defects were thoroughly irrigated, then bioactive glass (Bio-Glass = surface activated resorbable bioactive glass, Excellence Pharma, Inc., Egypt) was compressed in the defects until the level of the material was flush with the labial cortical bone of the mandible (*Figure 1*). The collagen membrane (Biocollagen, Bioteck S.r.l Fermi, Arcugraro VI, Italy) was placed over the bioactive glass (*Figure 2*) and stabilized. The wound edges were then approximated using a tissue forceps and an atraumatic needle.

Figure 1. The bony cavity was filled with bioglass.

Figure 2. The collagen membrane was added to cover the graft material.

Figure 3. An orthodontic spring was applied to allow tipping movement.

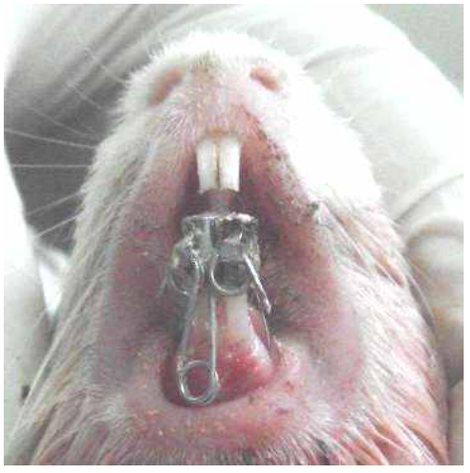

Figure 4. Separation of the teeth.

The orthodontic tooth movement was applied immediately after the surgical procedure in Group I, and the animals were sacrificed at 1, 3 and 6 weeks. The animals in Group II were subjected to the surgical procedure, after 2 weeks the orthodontic tooth movement was applied, and the animals were sacrificed at 1, 3 and 6 weeks. Group III animals were sacrificed at 1, 3 and 6 weeks after implantation of bone graft and collagen membrane in the bony cavities.

The orthodontic tooth movement was directed towards the treated defect. The orthodontic appliance consisted of standard tubes (0.018 x 0.015) placed on the central incisors and attached together by a spring to allow tipping movement of teeth (*Figure 3*). The spring was activated once per week (*Figure 4*). After sacrificing the animals, their mandibles were separated for the preparation of paraffin blocks and histological evaluation.

Histological preparation

After each period of investigation the guinea pigs were sacrificed and the site of surgery was carefully removed. The mandible was dissected from the soft tissues. The mandible was then placed in jars labeled by animal number and investigation duration. Fixation of the tissue was done using 10% formalin for 3 weeks. Decalcification of the specimens were done using ethylene diamine-tetra acetic acid (EDTA) 125 g/L distilled water and sodium hydroxide as a buffer for 3 weeks. The samples were then dehydrated in ascending grades of ethyl alcohol starting with 70% up to 100% absolute alcohol followed by methyl benzoate for one day followed by paraffin benzol for two hours. To remove the alcohol residue the samples were bathed three times in paraffin wax and placed in wax blocks of suitable size to be ready for cutting. Cutting of the samples was done using a Leitz Wetzlar microtome that obtained serial sections at 5-8 μ thick, and about 10 sections were taken from each sample.

Image analysis

The microscopic fields were randomly selected and those containing the highest number of newly formed bone were selected. Four different microscopic fields at the magnification x100 were photomicrographed for each microscopic slide. Images were captured with the aid of a digital video camera (5.1 megapixel, Olympus, Japan) mounted on a light microscope (BX60, Olympus, Japan). Captured digital images were imported to a computer system and were displaced for image analysis.

The brightness and contrast of the captured images were automatically processed prior to calculation using software (Photoshop 7.0, Adobe Corp.). Using the image analysis software (Soft Imaging System, Sis-5, Germany), the image was transformed into 8-bite for automated grey scale level of the newly formed bone trabeculae. Edges of bone trabeculae were traced, and then color-code thresholding was carried out. Finally, the color-coded bone trabeculae were cut as desired areas and other structures such as bone marrow were excluded from the field prior to calculation.

Count of the number of newly formed bone trabeculae and the total surface area of bone trabeculae was automatically calculated using Excel software (Office 2003, Microsoft Corp.®). Data were tabulated for further statistical analysis.

Results

The histological results revealed that during the six weeks period of bony cavity preparation and insertion of regenerative materials with or without orthodontic force application, several tissue changes took place among the surgically created cavities.

Group I

At one week, the histological sections of the surgical bony cavities, using H&E stain, revealed that the

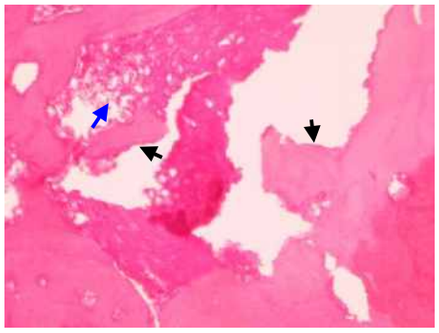

Figure 5a

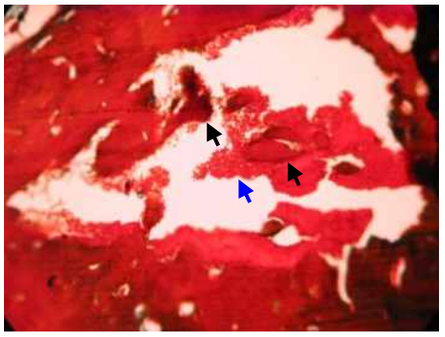

Figure 5b

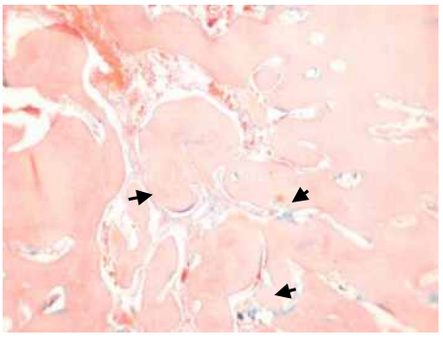

Figure 5c

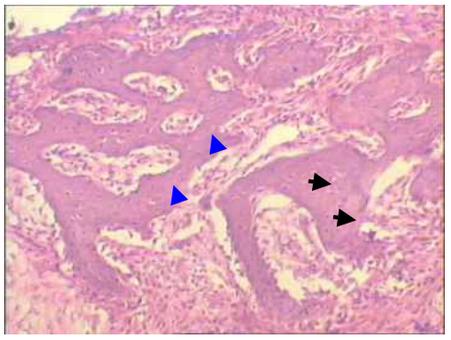

Figure 5d

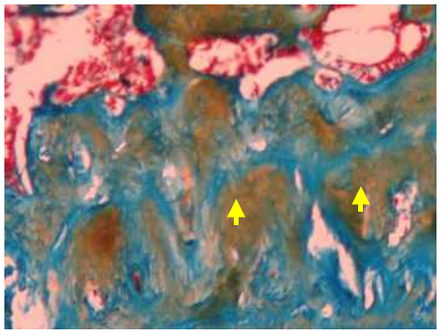

Figure 5e

Figure 5. A photomicrograph of the surgical bony cavity of Group I: a) at one week showing the presence of bone spicules (black arrow) interspersed with fibrin meshwork (blue arrow) (H&E stain x100); b) at one week showing fibrillar fibrous tissues (blue arrow) intermingled with spicules of newly formed bone (black arrow, Mallory's stain x100); c) at three weeks showing coalescence of large areas of bone trabeculae (black arrows, Mallory's stain x100); d) at six weeks showing mature mineralized bone trabeculae with osteocytes inside lacunae (black arrow). The bone trabeculae had haphazard arrangement with marrow spaces in between (blue arrow, H&E stain x100); 5 e) at six weeks period showing closely intertwining and condensing collagen fibrils (yellow arrow) and numerous dilated vascular channels (Mallory's stain x100).

original periphery of cortical plates consists of dense compact lamellar bone with the presence of irregularities. These irregularities have been rounded off by shallow depressions (Howship`s lacunae) that identify regions of bone with osteoclastic resorption. Inside the cavities, the presence of red blood cells entrapped in the fibrin meshwork in addition to a homogeneous degenerative tissue indicating necrosis and spicules of bones were seen (*Figure 5a*). Mallory`s stain revealed no difference between the aforementioned structures inside the cavities (*Figure 5b*).

At three weeks, H&E stain revealed that large areas of bone trabeculae had been formed with a scanty fibrin meshwork in between. The newly formed bone trabeculae were numerous and intermingled with each other. These features were well demarcated using Mallory`s stain (*Figure 5c*).

After six weeks, a dense fibrous tissue formation and highly cellular coarse (woven) bone were seen. The

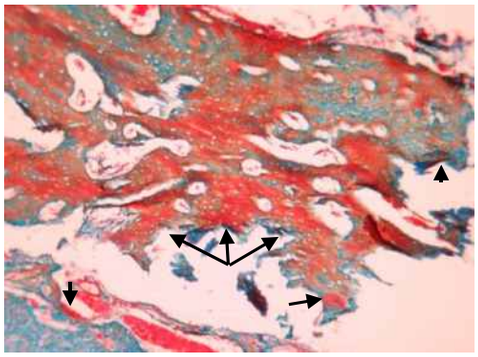

Figure 6a

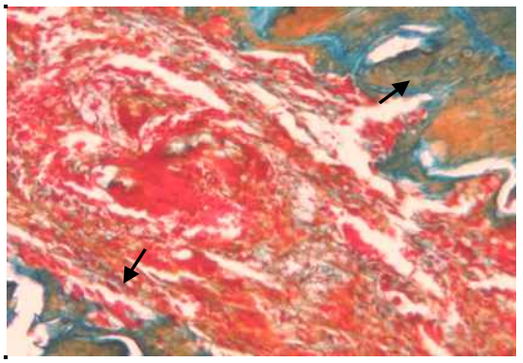

Figure 6b

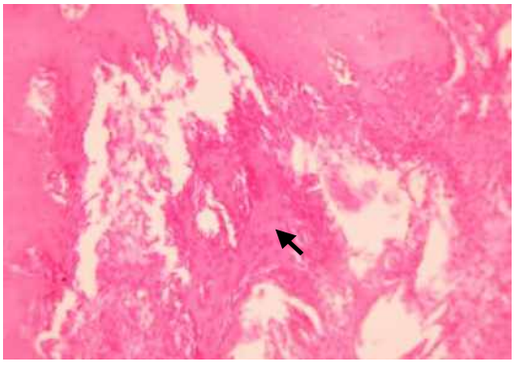

Figure 6c

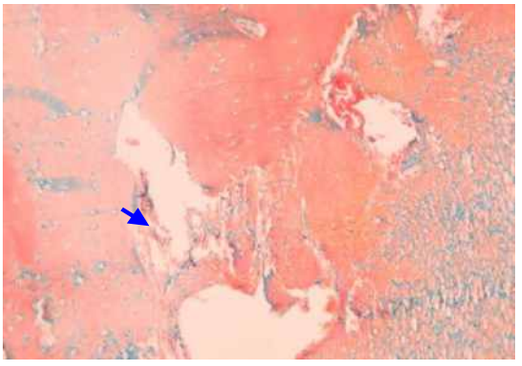

Figure 6d

Figure 6. A photomicrograph of the surgical bony cavity of Group II: a) at one week showing bone irregularities that have been rounded off by shallow depressions (Howship's lacunae, black arrow, Mallory's stain x100); b) at three weeks showing a communication of newly formed bone with the periphery of the bony cavity (black arrow, Mallory's stain x100); c) at six weeks showing a highly cellular formed bone (black arrow) nearly filling the entire cavity (H&E stain x100); d) at six weeks period showing newly formed bone interspersed by fibrous tissue (blue arrow, Mallory's stain x100);

newly formed bone trabeculae were numerous and intermingled with each other. In addition, small numbers of flattened, quiescent osteoblasts lining the bone trabeculae were seen. The latter showed haphazard arrangement (Chinese-letter pattern) with marrow spaces in between (*Figure 5d*). Mallory`s stain revealed that collagen in some areas showed closely interwining and condensing fibrils, which are seen by virtue of a deep blue color. Fibrous tissues interspersed with bone trabeculae with less fibrin meshwork were also seen (*Figure 5e*).

Group II

At one week, H&E stain revealed large islands of osteoid tissue that were dispersed in a fibrous tissue. These features were apparent with numerous marrow spaces when utilizing Mallory`s stain. A difference in staining with variability of color indicated the presence of new and old bone. The latter showed irregularities that were rounded off by shallow depressions (Howship`s lacunae) that identify regions of bone with osteoclastic resorption (*Figure 6a*).

After three weeks, H&E stain revealed the presence of numerous fragments filling the cavities intermingled with fibrin meshwork. A communication of the newly formed bone with the periphery of the bony cavity was seen. These features were distinguished when Mallory`s stain was utilized (*Figure 6b*).

At the end of six weeks, H&E stain revealed that the fibrin meshwork showed shrinkage and degeneration. The cavities were almost filled with zones of new bone, which enveloped the periphery of the cavities. In addition to the presence of coarse woven bone, formation of trabeculae of lamellar bones was seen (*Figure 6c*). Mallory`s stain revealed the same features (*Figure 6d*).

Group III

At one week, H&E stain revealed the presence of blood clots inside the cavities with formation of fibrin meshwork. Red blood cells were entrapped in the fibrin meshwork surrounded with dense fibrous tissue (*Figure 7a*). Mallory`s stain revealed that a fibrous component of granulation tissue surrounded the cavity

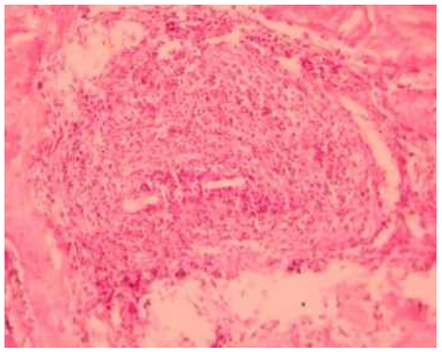

Figure 7a

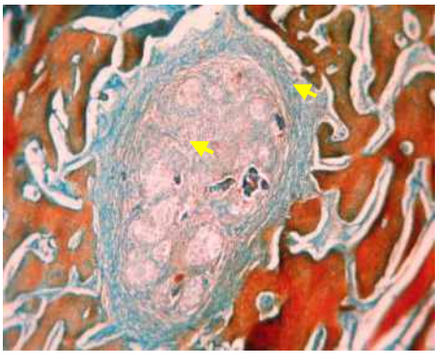

Figure 7b

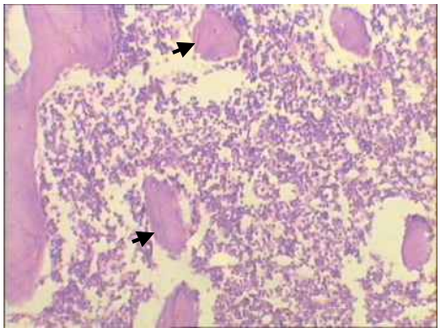

Figure 7c

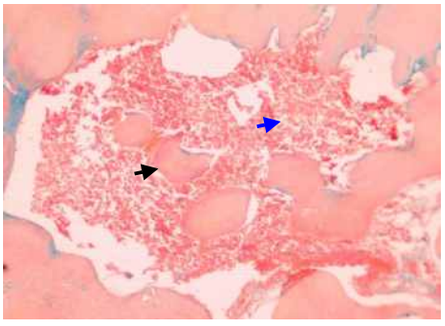

Figure 7d

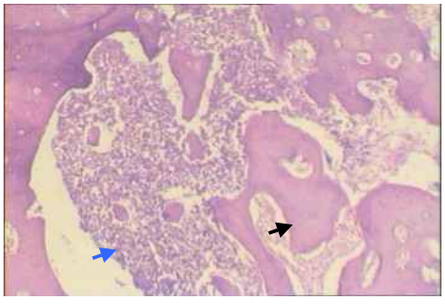

Figure 7e

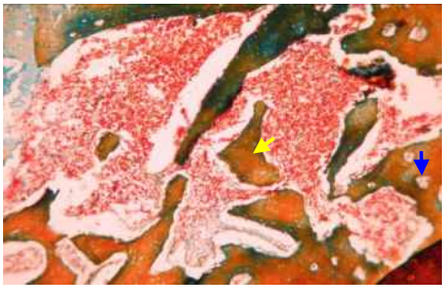

Figure 7f

Figure 7. A photomicrograph of the surgical bony cavity of Group III: a) at one week showing fibrin meshwork all over the surgically created cavity (H&E stain x100); b) at one week showing fibrous component of granulation tissue surrounds the bone graft particles (yellow arrow, Mallory's stain x100); c) at three weeks showing bony spicules (black arrow) within the bony cavity (H&E stain x100); d) at three weeks showing mature fibrous tissue (blue arrow) with newly formed bone (black arrow) within the surgically created cavity (Mallory's stain x100); e) at six weeks showing immature woven bone (black arrow) with presence of fibrous tissue in between (blue arrow, H&E stain x100); f) at six weeks showing mature collagen fibrils interspersed with fragments of newly formed bone (yellow arrow) with marrow spaces in between (blue arrow, Mallory' stain x100).

elements, including the bone graft materials (*Figure 7b*).

At three weeks, H&E stain revealed that bony fragments inside the cavities were dense and mature, replacing areas of the fibrin meshwork. They were similar to that at the original periphery (*Figure 7c*). Mallory`s stain revealed the same features (*Figure 7d*).

After six weeks, H&E stain revealed that areas of the bony cavities were filled with zones of new bone, which enveloped the periphery. In addition to the presence of coarse woven bone, formation of trabeculae of lamellar bones with marrow spaces were seen. Interestingly, the lamellar bones showed maturation and pattern of the Haversian canal systems (*Figure 7e*). Mallory`s stain revealed immature collagen fibrils interspersed with fragments of newly formed bone (*Figure 7f*).

Statistical analysis

Data were analyzed by computer with the Statistical Package for Social Science (SPSS)® ver 16.0. Analysis of

Table 1. The trabecular count and the total surface area of newly formed bone among the three groups.

Group	Trabecular count			Total surface area (mm^2)		
	1 week	3 weeks	6 weeks	1 week	3 weeks	6 weeks
I	2.4	6.4	8.6	0.17	2.72	2.96
II	2.2	5.2	8	0.95	1.64	2.33
III	0.2	2	5.4	0.011	0.49	1.90

Table 2. The mean difference values for comparison of trabecular count among the three groups (ANOVA). $p < 0.05$ was considered statistically significant.

Group		Mean difference 1 week	*p*-value	Mean difference 3 weeks	*p*-value	Mean difference 6 weeks	*p*-value
I	II	-2	0.009	-3.2	0.009	-2.6	0.008
I	III	-2.2	0.005	-4.4	0.001	-3.2	0.002
II	III	-30.2	1.0	1.2	0.56	-0.6	1

Table 3. The mean difference values for comparison of total trabecular surface area among the three groups (ANOVA). $p < 0.05$ was considered statistically significant

Group		Mean difference 1 week	*p*-value	Mean difference 3 weeks	*p*-value	Mean difference 6 weeks	*p*-value
I	II	-0.94	0.002	-1.15	0.0001	-0.43	0.92
I	III	-0.84	0.001	-2.23	0.0001	-1.05	0.06
II	III	0.775	0.01	-1.08	0.001	-0.63	0.43

variance (ANOVA) was used to compare the trabecular count and total surface of newly formed bone among the means of the three groups. *Table 1* shows the changes in the trabecular count and total surface area of newly formed bone among the three groups at 1, 3 and 6 weeks. *Tables 2* and *3* show the mean difference values for comparison of trabecular count and total trabecular surface area among the three groups.

Discussion

The present study was employed to histomorphometrically evaluate the osseous defects treated with combined orthodontic/regenerative therapy at different times of initiation of the orthodontic tooth movement. The concept of combining orthodontic tooth movement with regenerative therapy is based on the assumption that regenerative procedures could be enhanced by orthodontic tooth movement (Nemcovsky *et al.*, 1996; Diedrich, 1997; Stefania *et al.*, 2000; Vardimon *et al.*, 2001; Ogihara and Marks, 2002; Stefania *et al.*, 2002; Ogihara and Marks, 2006; Maeda *et al.*, 2007). Previously, several authors have reported the use of combined orthodontic regenerative therapy in the treatment of osseous defects. The determination of the effect of the best time to initiate the orthodontic

tooth movement has not been discussed. Therefore, to our knowledge, it appears that this is the first report to evaluate the effect of different times of initiating the orthodontic tooth movement. Immediate application of orthodontic tooth movement with regenerative surgery in treatment of intraosseous defect was for the first time selected. In this study, the selection of the period of two months (60 days) for initiation of orthodontic tooth movement in Group II was based upon several studies that evaluated periodontal regeneration from 60 to 90 days after regenerative therapy and noted advanced healing of the periodontal tissues (Caffesse *et al.*, 1993; Araujo *et al.*, 2001; Ogihara and Marks, 2006).

This study apparently showed that orthodontic tooth movement, when applied within a certain time period and of known magnitude, could be used as an adjunct factor for bone regeneration. It was also noticed that the presence of an extrinsic mechanical stimulus causes bone repair. The osteoclastic – osteoblastic coupling mechanism required for bone apposition/resorption response corresponds with the results of this study. Osteoclastic recruitment is most likely to occur not only as a sign for increased resorption activity, but also it could act as a signal for bone deposition at a site in close proximity to the resorption activity (Vardimon *et al.*, 2001). The rapid disappearance of graft particles in Groups I and II may be explained by the rapid recruitment of osteoclasts to the bony cavity that accompanied the orthodontic tooth movement.

In the present study, bioactive glass was used as the grafting material because a number of *in vivo* and *in vitro* studies have highlighted the potential for bioactive glass as an effective synthetic regenerative scaffold (Sculean *et al.*, 2002; Sculean *et al.*, 2005; Keles *et al.*, 2006). In addition, the collagen membrane was specially selected in this study owing to the following properties: it is chemotactic to fibrolasts, it provides a scaffold for periodontal ligament cell migration, it is a weak immunogen, and it can be easily manipulated and adapted (Yaffe *et al.*, 1984; Mattson *et al.*, 1999; Michele, 2002).

Regulation of bone formation by mechanical loading force seems to play a significant role in new tissue formation. The integrin-mediated signal transduction cascade is the main mechanism of mechanotransduction in cells and is associated with osteogenesis (Tang *et al.*, 2003). Cell multiplication is the first reaction in the beginning of tooth movement. Indeed, fibroblast numbers were doubled in the three days after the commencement of tooth movement (Meikle, 2006). Orthodontic tooth movement can stimulate preosteoblasts and mesenchymal cells to differentiate into osteoblasts (Faber *et al.*, 2005). Moreover, growing numbers of cytokines are known to be related to bone formation. Intense production of TGF-β1 mRNA and the translated protein contributes to angiogenesis and coincides with osteoblast migration, differentiation and the formation of extracellular matrix (Mehrara *et al.*, 1999). Expression of collagen type IV is increased in the basement membrane of newly formed blood vessels and lamin is diffusely distributed in the matrix undergoing mineralization (Campisi *et al.*, 2003). Concurrently, expression of BMP2, 4 and 7 in the connective tissue is also increased (Mehrara *et al.*, 1999).

In this animal study, the amount and type of bone formation was in agreement with the overall process previously described. The histomorphometric findings showed that cell differentiation, cell multiplication, bone formation and blood capillary hyperplasia were active with the orthodontic tooth movement. Moreover, osteogenesis can be considered as a hallmark of Groups I and II, in which the combined orthodontic regenerative therapy was used. This was previously proved to be mediated by various osteoinductive molecules that act by stimulating osteoblast osteoprogenitor cells in the periodontal ligament with subsequent bone formation and inhibition of bone resorption (Guajardo *et al.*, 2000; Domon *et al.*, 2001; Gao *et al.*, 2002; Mitsui *et al.*, 2006). In this study, bone regeneration by periodontal regenerative materials in Group II (in which the active orthodontic treatment started two weeks after regenerative surgery) showed the remodeling process as a function of orthodontic tooth movement in the presence of newly formed bone with activation and recruitment of osteoclasts followed by formation of new bone by osteoblasts at the surgically created cavity.

Groups I and II, in which combined orthodontic/regenerative therapy was used showed higher trabecular count and greater total surface area of newly formed bone than the group in which periodontal regenerative treatment alone was used during the study period. The trabecular count of newly formed bone and the total surface area were 1.5 fold greater in the bony cavities treated by combined orthodontic/regenerative surgery than bony cavity defects treated by regenerative surgery alone. Moreover, Group I (in which orthodontic tooth movement started immediately after finishing the regenerative surgery) showed a higher trabecular count and more total surface area of newly formed bone than the other groups at 1, 3 and 6 weeks.

The histomorphometric results of our study were in agreement with Vardimon *et al.* (2001); Araujo *et al.* (2001); Silva *et al.* (2006) and Nemcovsky *et al.* (2007). They reported that the orthodontic movement was not pre-requisite to the results obtained with the regenerative periodontal treatment. Moreover, bone formation in the bony cavities was greater in quantity in groups that received combined orthodontic/regenerative therapy than groups that received regenerative therapy alone.

In conclusion, the combined orthodontic

regenerative therapy resulted in favorable histological outcomes. The surgical bony defects treated with combined orthodontic regenerative therapy with immediate application of orthodontic tooth movement showed greater trabecular count and more total surface area of newly formed bone than those treated with regenerative surgery followed by delayed application of orthodontic tooth movement two weeks later.

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