Original Article

Bone inductive proteins to enhance postorthodontic stability

A pilot study

Ali H. Hassan[a]; Aziza Al-Hubail[b]; Ahmad Ali Al-Fraidi[b]

ABSTRACT

Objectives: To evaluate the use of bone morphogenetic proteins to enhance postorthodontic stability in sheep and to develop a biological method of postorthodontic retention.

Materials and Methods: First incisors were extracted in four mature and healthy sheep, and the second incisors were tipped reciprocally toward the midline and then retained. Dried bone matrix was injected into the distal periodontal space of the left second incisor. The right second incisor was left as a control. Both incisors were retained in the tipped position for 4 weeks. Then, the orthodontic appliance was removed and the teeth were left without retention. Six weeks later, the animals were killed and serial sections were prepared for histologic observation.

Results: Unlike the control, the experimental second incisor maintained its tipped position with minimal relapse. On the distal periodontal space of the experimental tooth, areas of focal fusion between newly formed bone and newly formed areas of hypercementosis were observed. In the distal periodontal space of the control tooth, osteoclastic activity was observed along most of the socket wall, and the periodontal space appeared narrow and compressed. This brought the tooth close to the boundary of the alveolar bone, confirming the relapse observed on that side.

Conclusion: This study proposes a new method of retention in which a biologically safe osteoinductive material is used to retain the teeth via induction of points of approximation between the cementum and alveolar bone. (*Angle Orthod.* 2010;80:1051–1060.)

KEY WORDS: Postorthodontic stability; BMP; Biological retainer; Retention

INTRODUCTION

Postorthodontic stability is a highly controversial issue that is difficult to ensure unless long-term permanent mechanical retention is used. Beside mechanical retention, additional procedures are also used to minimize pos orthodontic relapse, such as fibrotomy[1] and reshaping the teeth,[2,3] but none of these methods is satisfactory and reliable. In addition, long-term permanent mechanical retention represents an extra burden on patients.[4–7] The mechanism behind relapse is not fully understood and has been blamed on many factors, such as the recoil of the gingival and periodontal fibers, surrounding soft tissue, further growth, and dental factors.[8] There is a general agreement that postorthodontic retention is a highly variable and complex procedure, which cannot be ensured unless permanently in place. Therefore, finding a logical and safe solution for the unavoidable relapse represents a necessity, especially in the presence of the great advances in scientific research.

Topical administration of a bisphosphonate (risedronate), a potent blocker of bone resorption, during orthodontic tooth movements has been evaluated in rats.[9] Risedronate inhibited tooth movement in a dose-dependent manner without affecting the overall growth of the animals. These data suggested the possibility of manipulating bone remolding to temporarily minimize postorthodontic relapse.

Bone morphogenetic proteins (BMPs) are members of the transforming growth factor (TGF-B) superfamily that act as osteoinductive factors by inducing differentiation of osteoblasts from mesenchymal cells.[10] Previous studies have shown the ability of BMPs to induce bone formation in a variety of models with many clinical applications in orthopedics and in oral and

[a] Associate Professor and Consultant of Orthodontics, Director, Saudi Board in Orthodontics-Western Region, Faculty of Dentistry, King Abdulaziz University, Jeddah, Saudi Arabia.

[b] Resident, Saudi Board in Orthodontics, Faculty of Dentistry, King Abdulaziz University, Jeddah, Saudi Arabia.

Corresponding author: Dr Ali. H. Hassan, Associate Professor and Consultant of Orthodontics, Director, Saudi Board in Orthodontics-Western Region, Faculty of Dentistry, King Abdulaziz University, PO Box 122423, Jeddah 21332, Saudi Arabia (e-mail: aliresearch@gawab.com)

Accepted: March 2010. Submitted: November 2009.

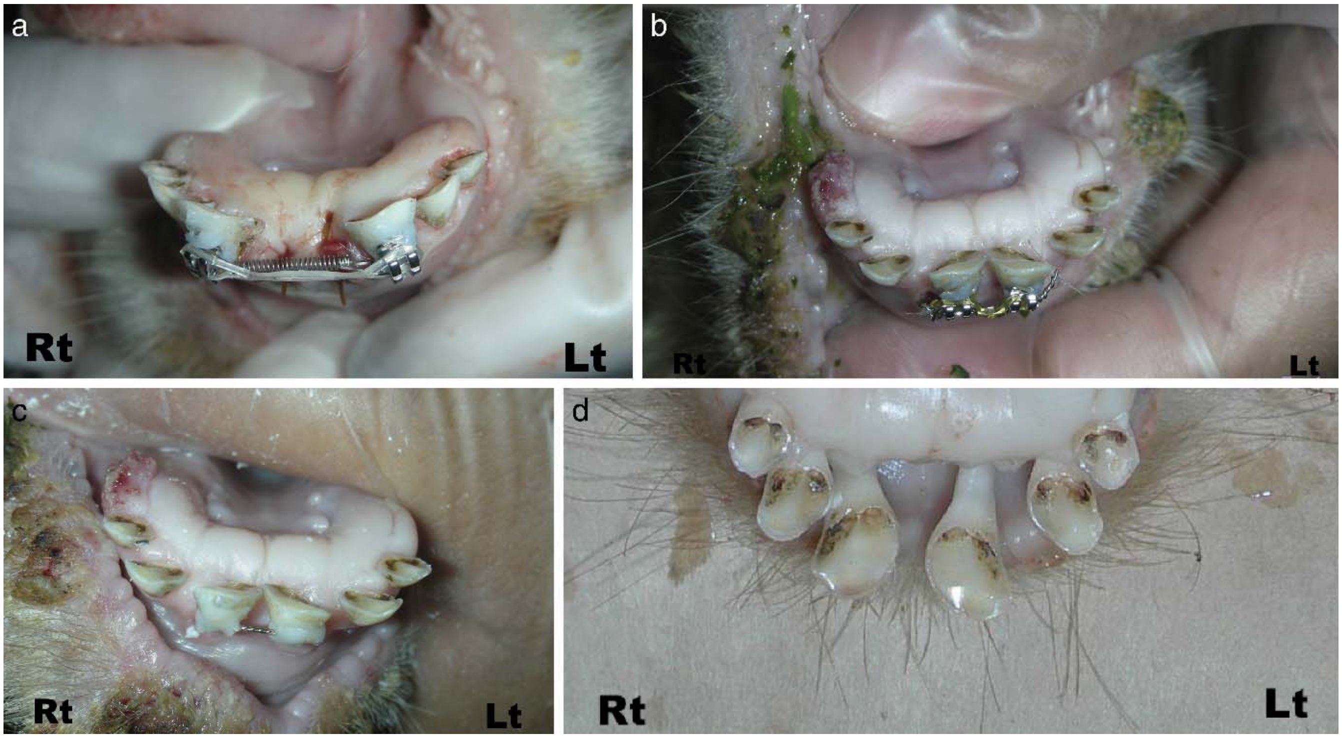

Figure 1. (a) The appliance used: brackets on lower second incisors, pulled toward the midline using power chain and a closed coil spring. (b) Complete approximation of the incisors. (c) Retention using labial fixed wire, followed by DynaGraft II injection of the distal PDL of the lower left incisor. (d) Six week after removing the fixed retainer. Note the stability of the experimental tooth (left) and the complete relapse of the control tooth (right).

maxillofacial/dental areas.[11,12] Demineralized freeze-dried bone allografts (DFDBA), which are known to contain BMPs, have been used and tested extensively for periodontal regeneration.[13] Histologic evidence of enhanced formation of bone, cementum, and connective tissue attachment has been demonstrated in human periodontal defects.[14–16]

DynaGraft II (IsoTis OrthoBiologics Inc, Irvine, Calif) is a commercially available dried bone matrix (DBM), which is a type of DFDBA. It is designed to promote bone formation by stimulating the proliferation and transformation of mesenchymal cells to osteoblasts. It has many orthopedic applications, such as augmentation or reconstruction of alveolar ridges.[17]

The theory of this research is based on (1) the need to solve the dilemma of the unavoidable relapse unless permanent mechanical retention is used, (2) the possibility of using a biologically safe osteoinductive material to regenerate bone and cementum in the periodontium, and (3) the possibility of controlling tooth movement through the use of biological materials.

The goals of this study were to evaluate the use of BMPs as contained in DBM to prevent relapse after orthodontic treatment in sheep and to develop a biological method to prevent or minimize relapse after orthodontic treatment.

MATERIALS AND METHODS

Four mature and healthy sheep weighing 25 to 30 kg and having six permanent lower anterior incisors were used according to the guidelines of the Animal Care Committee at King Abdulaziz University. The sheep were anesthetized several times during the planned experiment using ketamine (44 mg/kg intramuscularly [IM]) and acetone (5 mg/kg IM). The right and left first incisors were extracted and left to heal for 1 week. Metal brackets (Victory Series Low Profile Brackets, 3M Unitek, St Paul, Minn) were bonded to the labial surfaces of the right and left second incisors using Transbond XT Light Cure Adhesive (3M Unitek). The two second incisors were tipped reciprocally toward the midline using a combined elastomeric chain and closing coil spring (RMO, Denver, Colo) to ensure the application of heavy forces (Figure 1a). In addition, power chain was replaced on a weekly basis, three times until complete approximation of the second incisors was achieved. The displaced teeth were then retained in the new position using stainless steel ligature wire (diameter of 0.2 mm, Dentaurum, Pforzhem, Germany; Figure 1b, c).

DynaGraft II was reconstituted with phosphate-buffered saline (PBS; 0.14 mg/mL). Next, 0.2 mL of

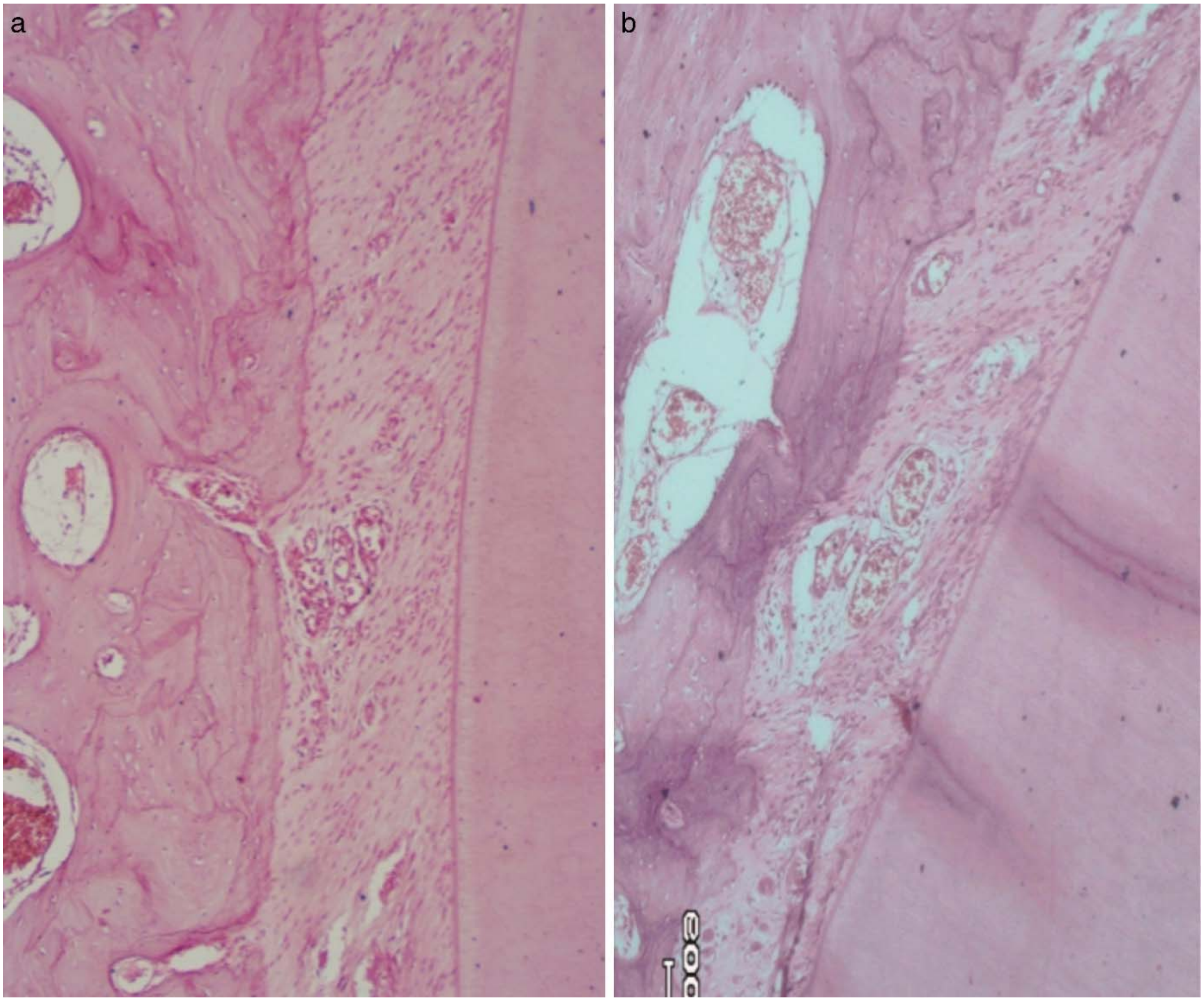

Figure 2. (a) and (b) The remodeling on the mesial socket wall of the experimental tooth (a) sheep 1 (b) sheep 2. (H&E, ×400).

the reconstituted DynaGraft II was injected into the distal periodontal space of the left second incisor (experimental side) after creating several transgingival holes using a mini screw (length, 6.0 mm; diameter, 1.8 mm; RMO). The right second incisor was used as a control with no injection. Teeth were kept in retention for 4 weeks. The appliance was then removed and the teeth were left without retention for a period of 6 weeks. The animals were then killed. Alveolar bone was harvested on each side, and a bone block carrying all of the anterior teeth was removed using carbide burs. The bone blocks were fixed immediately in 10% formaldehyde buffered with PBS at pH 7.2 for 2 weeks, rinsed under running water, and decalcified in 10% trichloroacetic acid for 1 month. Tissue blocks of the right and left second incisors were separated and processed for embedding in paraffin wax. Serial sections 5-μm thick were obtained and stained with hematoxylin and eosin (H&E) and Masson's trichrome. Blocks were sectioned longitudinally in a mesiodistal direction. Randomly selected fields of observations from each slide were viewed under a regular light microscope, and the periodontal spaces on the mesial and distal sides of the second incisors were evaluated in both groups. Histologic results were evaluated, described, and correlated with the clinical observation of the relapse.

RESULTS

Obvious clinical differences were found between the behavior of the second incisor on the experimental side and the control side. The experimental second incisor maintained its tipped position with minimal

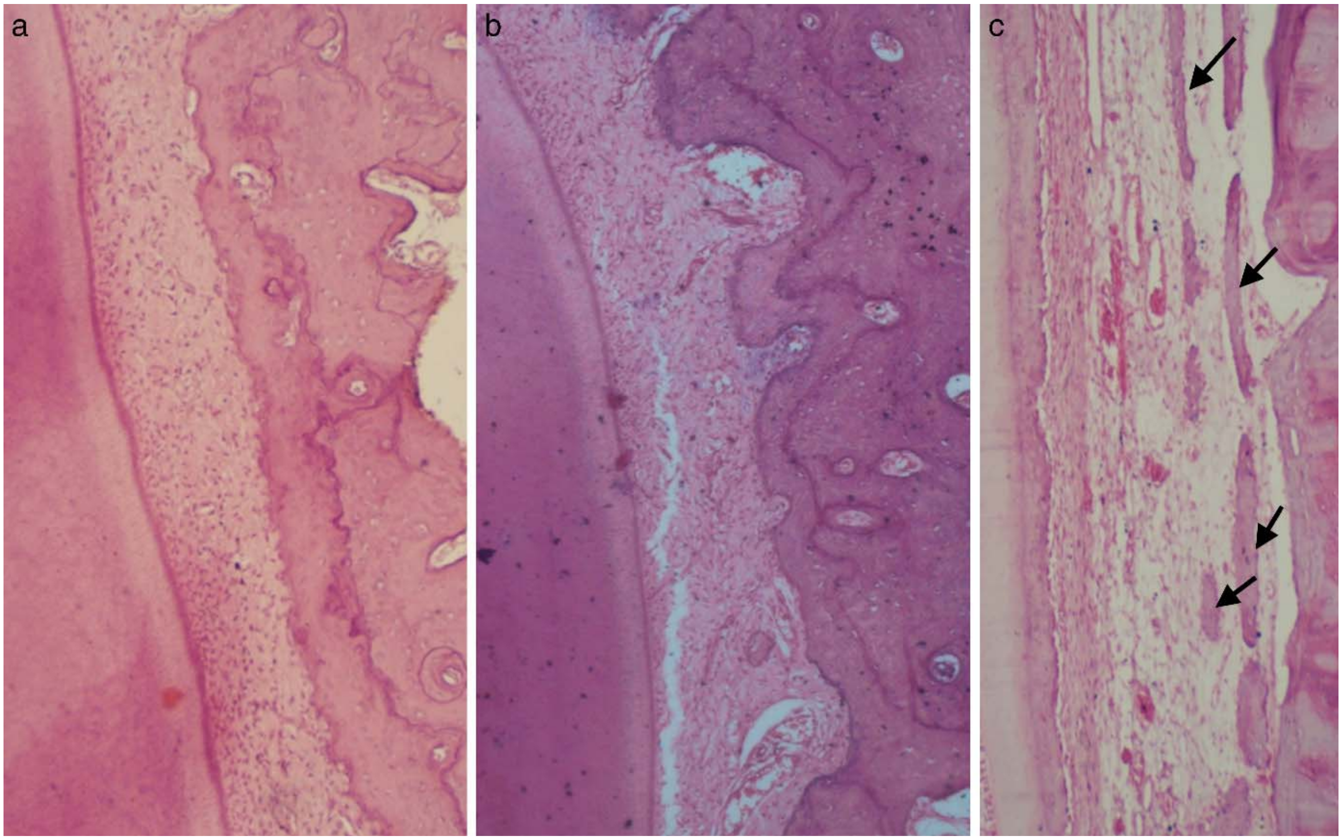

Figure 3. (a) The widened PDS was reduced in dimension via new bone formation. Distal side of the experimental tooth (H&E, ×100). (b) New bone formation on the bony socket boundary. Distal side of the experimental tooth (H&E, ×100). (c) New bone formation within the PDS. Thin plates of newly formed bone parallel to the socket wall and surrounded by osteoblasts (arrows) (H&E, ×100).

relapse during the last 6 weeks. On the other hand, the control second incisor moved back to its original position, which can be described as a complete relapse (Figure 1d).

The findings on the experimental side were microscopically different from those of the control side, confirming the clinical observations. On the mesial side of the experimental second incisor, the observations were constant and consisted of newly formed bone showing many appositional lines parallel to each other but not straight, including signs of a normal remodeling pattern that is seen in the periodontium (Figure 2a,b).

On the distal side, however, outstanding and variable observations were noticed. The periodontal space was reduced in dimension, although it was originally widened after moving the tooth in the opposite direction (Figure 3a). Mature and immature newly formed bone was seen on the bony socket boundary (Figure 3b) and within the periodontal space (Figure 3c). Thin plates of bone lined with osteoblasts and parallel in direction to the socket wall were observed (Figure 3c). Many bony specula were also observed adjacent to the bone side and spread throughout the periodontal space, closely approaching the root apices. (Figure 4a,b).

The socket boundary either appeared straight or exhibited protrusions formed by the newly formed bone, enclosing the opening of Volkmann's canals (Figure 5a, b). Osteoblasts were seen in groups associated with new bone formation and in other areas mapping new boundaries of a remodeled area of the socket wall (Figure 6).

Sites of active remodeling were observed with new bone filling areas of bone resorption. This was evident on the border of the alveolar bone (Figure 7a, b) and at the alveolar crest of the interdental septum, with many appositional lines and new periodontal fiber insertions (Figure 7c,d).

Lateral and apical hypercementosis was also observed, which was exaggerated and different from any age-related hypercementosis. It was irregular but had a specific direction toward the distal side of the socket (Figure 8a). In addition, focal fusion between the hypercementosed apices and either the small newly formed bone spicules or, more interestingly, larger masses of newly formed bone were observed (Figure 8b,c).

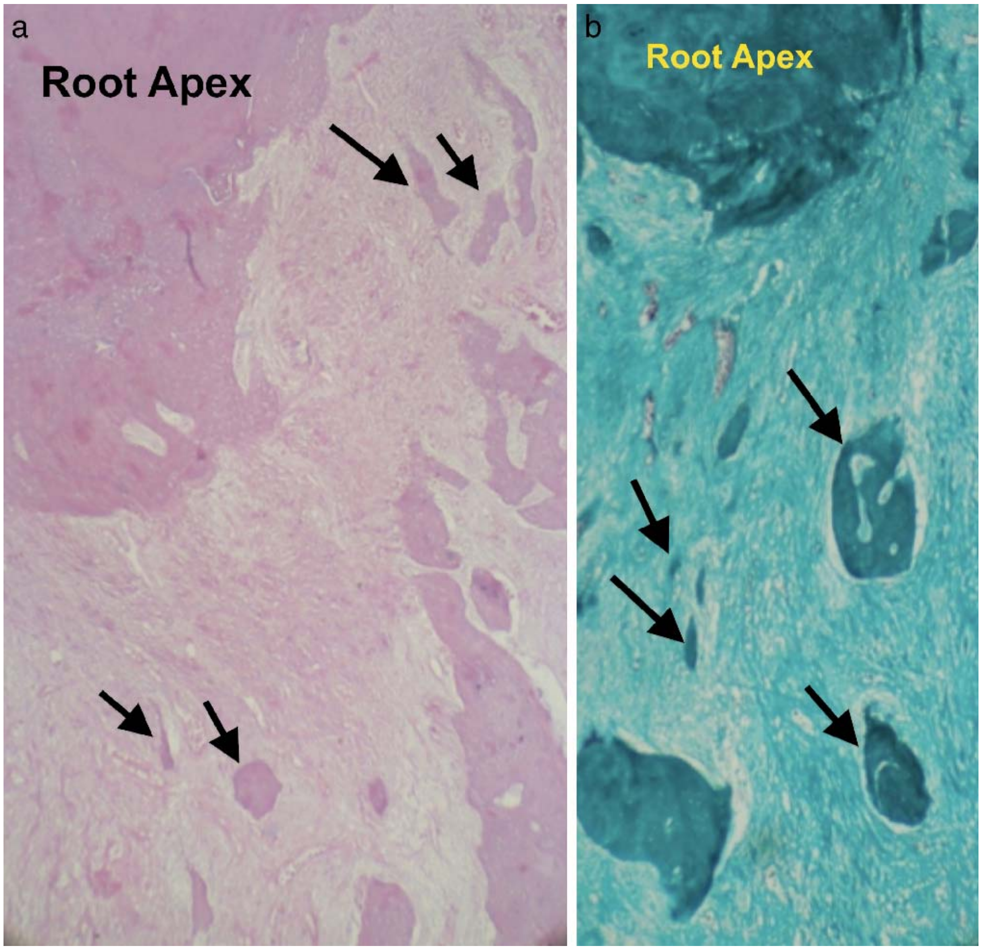

Figure 4. (a) and (b) Formation of multiple bone spicules (arrows) throughout the periodontal space and at the apical region of the socket adjacent to the hypercementosed root apex (a: H&E, ×100, b: trichrome stain, ×100).

The distal side of the control tooth showed resorption bays and osteoclastic activity along most of the socket wall. They formed a foreground over successive parallel dark lines, indicating a previous state of bone apposition (Figure 9a through c). The PDL appeared narrow and compressed bringing the tooth close to the boundary of the alveolar bone. On the mesial side, cumulative bone apposition appeared on the socket wall and, hiding behind it, irregular lines of bone resorption were observed in addition to PDL tension and osteoblastic activity (Figure 10). This was the reverse of the histologic picture seen on the distal side of the same tooth.

DISCUSSION

Retention was and still is considered a dilemma in orthodontics; it is often mismanaged by the orthodontists and disliked by the patients. Permanent retention, which several authors[18,19] cite as the only way to ensure long-term posttreatment stability, is intolerable to many patients. Developing a method of retention at the biological level is a necessity to overcome relapse and to establish a basis for further research to strengthen this weak aspect of orthodontic treatment. This pilot study is the first study to evaluate the effect of using osteoinductive proteins as biological retainers. The preliminary theory behind it is to develop points of ankylosis or at least rearrange the periodontal fibers in a way to prevent relapse.

The sheep model was used in this study because it is a convenient model, has six lower incisors anatomically and periodontally similar to human teeth, has no opposing upper incisors, and is considered a suitable model to study human remodeling and bone turnover.[20] This makes the lower incisors a good model for any experiment involving orthodontic tooth movement. However, good oral hygiene was difficult to maintain around lower incisors, which could be due to the lack of opposing teeth.

The design of tooth movement used in the present study was to tip the teeth toward the midline, using a

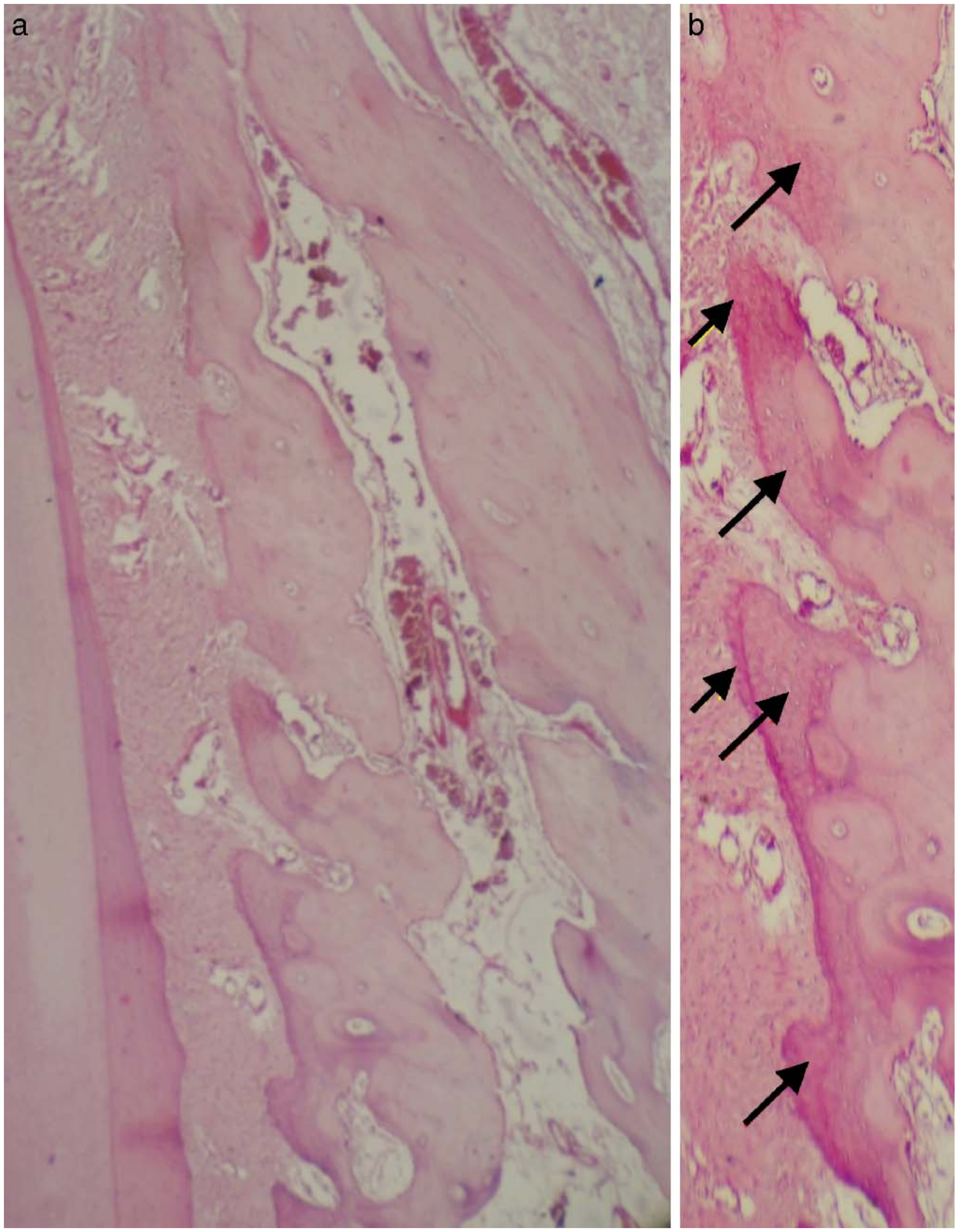

Figure 5. (a) A socket boundary consisting of newly formed bone protrusions at different magnifications (H&E, ×100). (b) Distal side of the experimental tooth; the arrows show the newly formed bone (H&E, ×400).

relatively heavy force and short periods of activation. This was to ensure that the teeth would be moved into a very unstable position to increase the chance of relapse and to assess the efficiency of the proposed method of retention more clearly. The use of such a design, however, could be responsible for the gingival recession seen on the labial surfaces of the moved teeth, which could also be attributed to the difficulty of maintaining good oral hygiene in sheep.

The method of applying the osteoinductive material used in this study was simple, safe, noninvasive, and suitable for use in the orthodontic field. The proposed method starts by creating transgingival channels, extending into the periodontium using mini screws, followed by the injection of a simple and safe osteoinductive material. At the same time, upper and lower fixed lingual retainers were used for a short time, approximately 3 months, to allow for the osteoinduction to take place. This could be more acceptable to the patients than permanent retention. Although this method seems to be promising, careful long-term evaluation should be done in animals before it is used in humans.

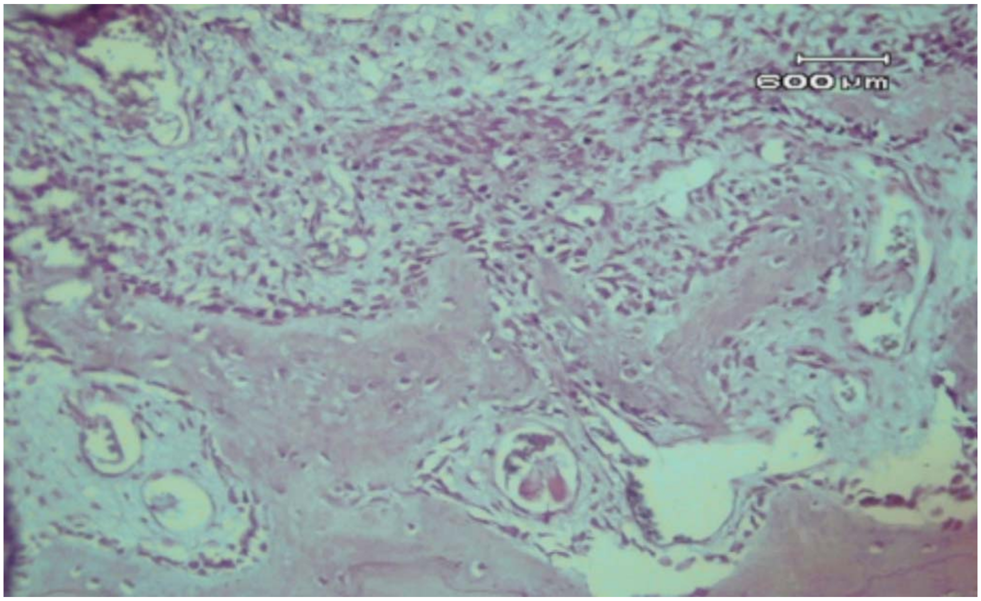

Figure 6. Fibroblast proliferation and depositional activity on the newly formed bone spicules and outlining areas of osteoid deposition. Distal side of the experimental tooth (H&E, ×400).

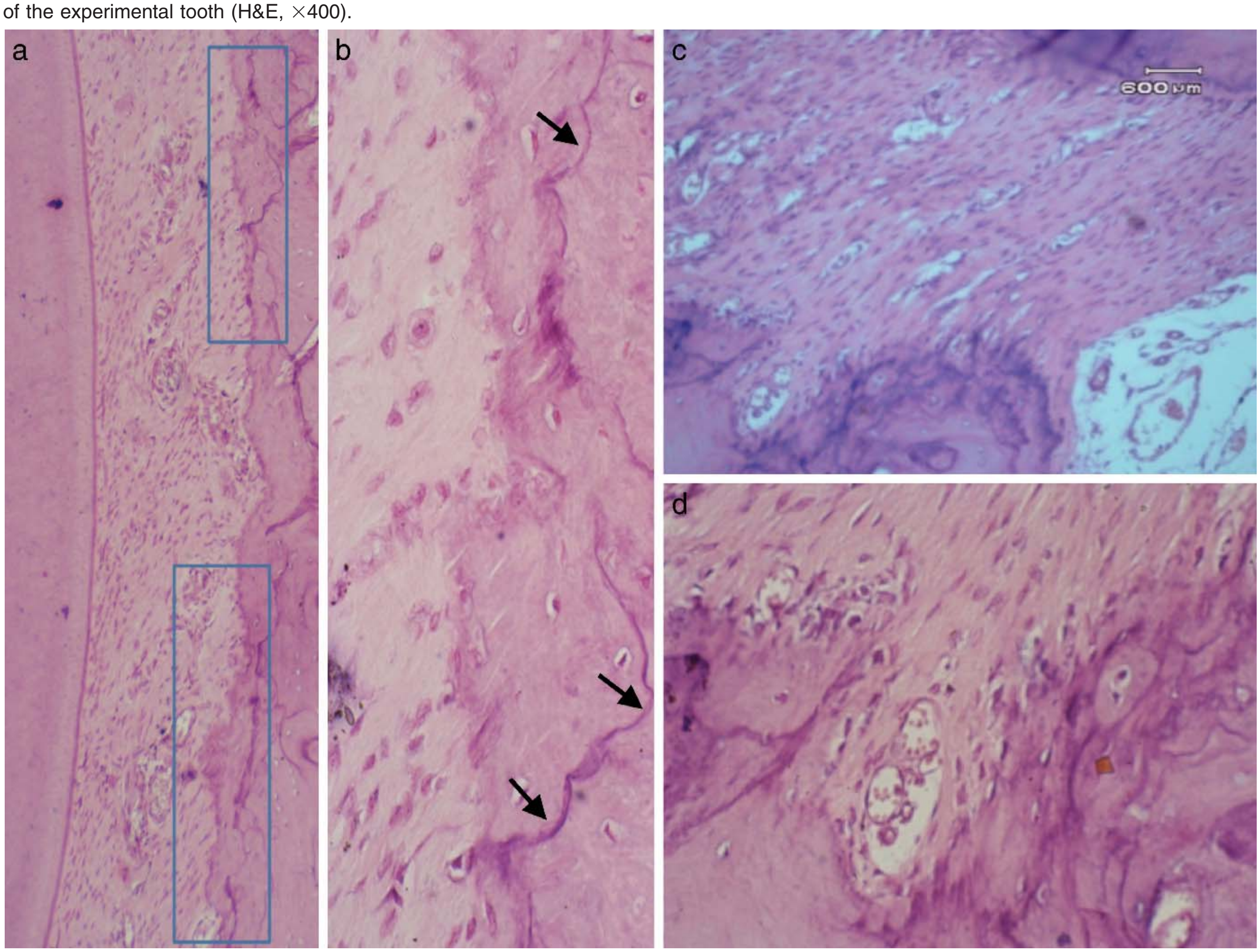

Figure 7. (a) A site of active remodeling in distal side of the experimental tooth showing new bone filling area of bone resorption (H&E, ×100). (b) Note the irregular and concave dark lines of previous resorption (arrows) and subsequent filling with new bone, also note the osteoblastic activity on the bone boundary (H&E, ×400). (c) Appositional activity at the alveolar crest of the interdental septum of the distal side of the experimental teeth (H&E, ×100). (d) Higher magnification of the remodeling lines and osteoblastic activity (H&E, ×400).

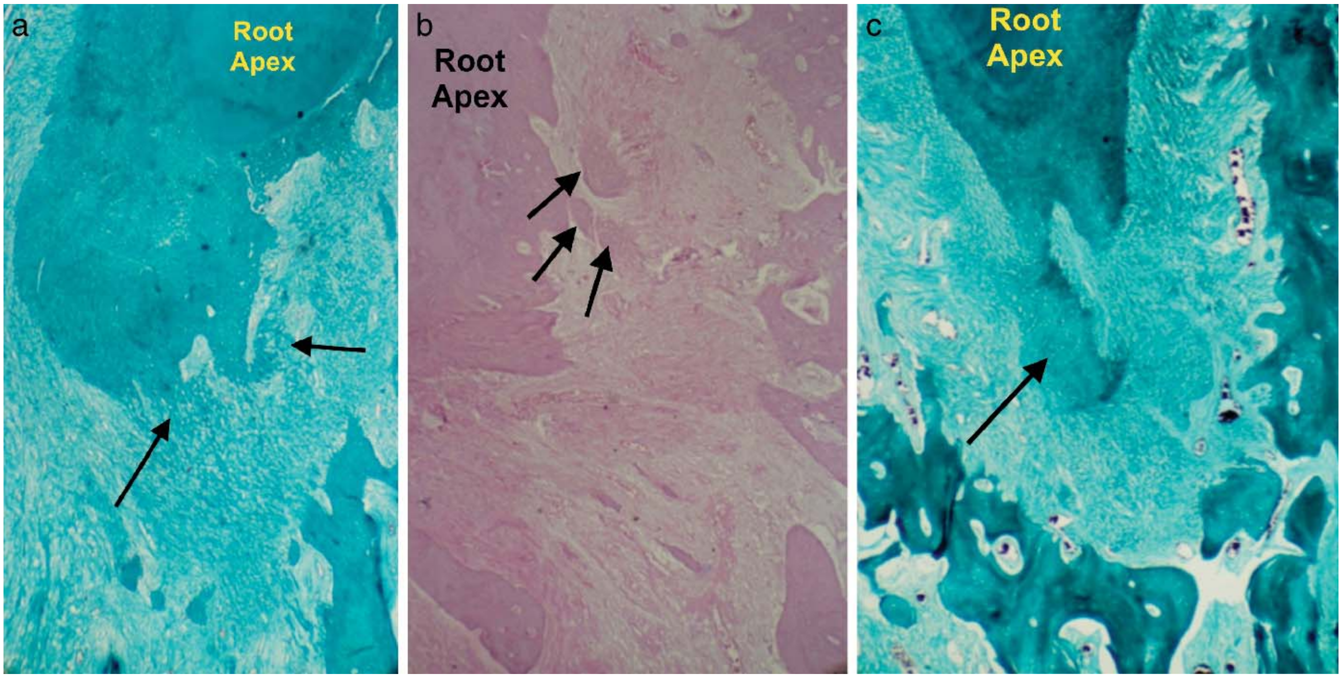

Figure 8. (a) The apical hypercementosis and its directivity to the distal side of the socket. Note the cellular proliferation adjacent to the apex (arrows) (trichrome stain, ×100). (b) Fusion between newly formed bone spicules (arrows) and the irregular hypercementosis on the root apex (H&E stain, ×100). (c) Actual fusion between the hypercementosed root apex and a large forming bone spicule (arrow) (trichrome, ×100).

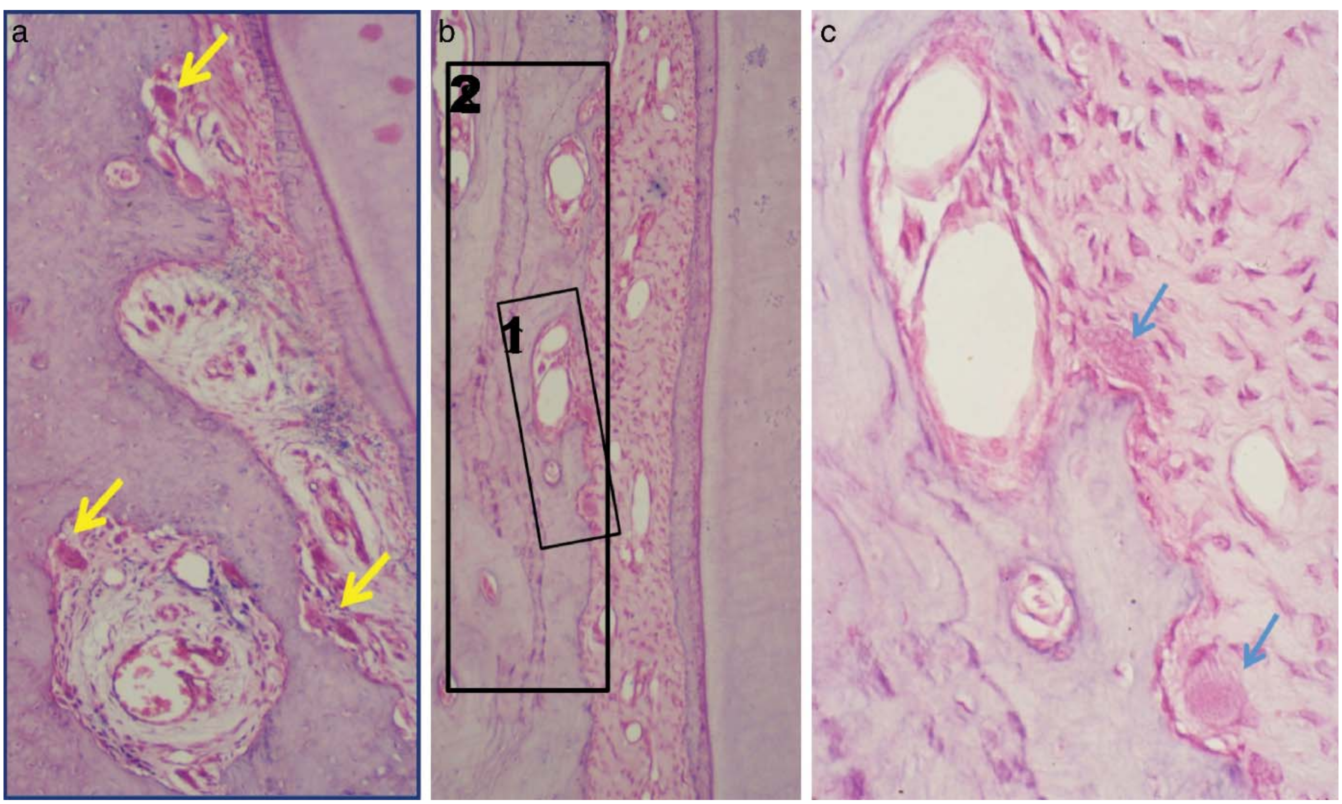

Figure 9. (a) Resorption bays and osteoclast activity (arrows) on both of the PDL and endosteal surfaces of the socket wall. Distal side of the control tooth (H&E, ×200). (b) Distal side of control tooth revealing bone remodeling, PDL compression, and narrowing (H&E, ×100). (c) Higher magnification of inset 1, showing resorption bays and osteoclasts (arrows) on the socket wall (H&E, ×400).

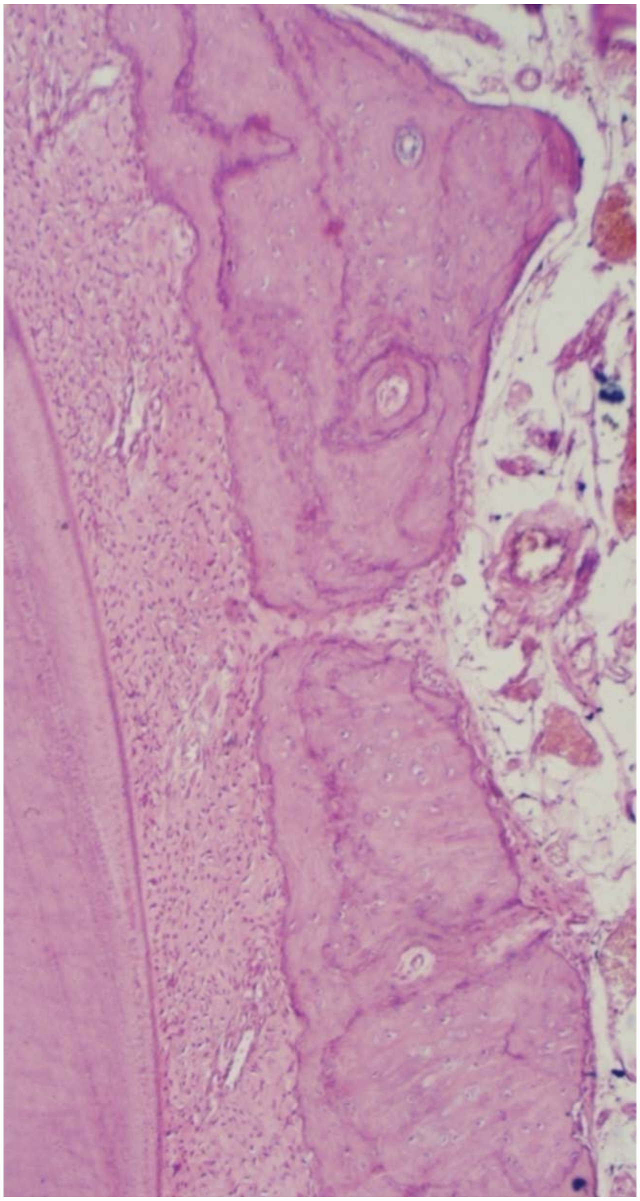

Figure 10. Pattern of bone remodeling on the mesial side of control teeth. Note the organization of the PDL fibers and cells (H&E, ×100).

DynaGraft II is known to contain BMPs and has been used and tested extensively for periodontal regeneration.[14–16] The clinical and histologic findings express strong support for the efficiency of using DynaGraft II to stabilize teeth after fast tipping movement followed by a short period of retention.

The histologic results confirm the clinical results. The histologic findings of the control side differed from those of the experimental teeth and reflected the clinical observation of the evident relapse, in which the control teeth had moved back to their original positions.

On the distal side of the experimental teeth there was a significant amount of bone formation filling the orthodontically widened periodontal space and restoring its original width in just 6 weeks. This provides evidence for the osteoinductive ability of DyanGraft II and for the possibility of using such material to prevent short-term relapse after orthodontic tooth movement. The findings also confirm the research theory of forming points of ankylosis or at least rearranging the periodontal fibers in a way to prevent postorthodontic relapse. There was histologic evidence for the start and progression of focal fusion between the newly formed cementum and the newly formed bone spicules at different locations. However, the progress to actual fusion between the tooth and the socket might need a longer period of observation and /or an increased dose of the injected DynaGraft II. The results of such approximation are still unknown, and further investigation is needed to determine whether it will proceed to real ankylosis or remodel to normal PDL.

The formation of new cementum observed in this study provides evidence for the efficiency of DynaGraft II in regenerating cementum in addition to its ability to regenerate bone. This indicates the possibility of using the same delivery technique of DynaGraft II to treat orthodontically induced root resorption. However, this was an incidental finding that requires further investigation in a more carefully designed study.

This was a pilot study, so the number of animals used was small and inadequate to perform statistics. Additional studies are required to confirm the results. A larger sample should be used, different doses of BMP should be tested, and histomorphometric analysis should be performed to evaluate the newly formed bone statistically. In addition, the long-term effect of such application should be evaluated to determine how the newly formed bone and cementum affect PDL.

CONCLUSION

- This study confirms a new method of postorthodontic retention in which a biologically safe osteoinductive material is used to retain the teeth via induction of points of approximation between the cementum and alveolar bone.
- This study also introduces a new and safe method of delivering the osteoinductive materials to the PDL. However, the long-term effect of such application is still unknown, and further testing is required using larger samples and more standardized and sophisticated techniques.

ACKNOWLEDGMENT

I would like to thank King Abdulaziz University for providing the necessary fund for this research.

REFERENCES

1. Edwards JG. A long-term prospective evaluation of the circumferential supracrestal fiberotomy in alleviating orthodontic relapse. *Am J Orthod Dentofacial Orthop*. 1988;93:380–387.
2. Boese LR. Fiberotomy and reproximation without lower retention, nine years in retrospect: part I. *Angle Orthod*. 1980;50:88–97.
3. Boese LR. Fiberotomy and reproximation without lower retention, nine years in retrospect: part II. *Angle Orthod*. 1980;50:169–178.
4. Sadowsky C, Sakols EI. Long-term assessment of orthodontic relapses. *Am J Orthod*. 1982;82:456–463.
5. Uhde M, Sadowsky C, Begole E. Long-term stability of dental relationships after orthodontic treatment. *Angle Orthod*. 1983;53:240–252.
6. Ras F, Korstjens CM, Kuitert RB, van Ginkel FC, Prahl-Andersen B. The stability of orthodontic treatment over the long term. *Ned Tijdschr Tandheelkd*. 1992;99:355–361.
7. Schutz-Fransson U, Bjerklin K, Kurol J. Mandibular incisor stability after bimaxillary orthodontic treatment with premolar extraction in the upper arch. *J Orofac Orthop*. 1998;59(1):47–58.
8. Melrose C, Millett DT. Toward a perspective on orthodontic retention? *Am J Orthod Dentofacial Orthop*. 1998;113:507–514.
9. Adachi H, Igarashi K, Mitani H, Shinoda H. Effects of topical administration of a bisphosphonate (risedronate) on orthodontic tooth movements in rats. *J Dent Res*. 1994;73:1478–1486.
10. Urist MR, Nfikulski AJ, Nakagawa M, Yen K. A bone matrix calcification-initiator noncollagenous protein. *Am J Physiol*. 1977;232:C115–C127.
11. Mori M, Isobe M, Yamazaki Y, Ishihara K, Nakabayashi N. Restoration of segmental bone defects in rabbit radius by biodegradable capsules containing recombinant human bone morphogenetic protein-2. *J Biomed Mater Res*. 2000;50:191–198.
12. Herford AS, Boyne PJ. Reconstruction of mandibular continuity defects with bone morphogenetic protein-2 (rhBMP-2). *J Oral Maxillofac Surg*. 2008;66:616–624.
13. Mellonig JT. Bone allografts in periodontal therapy. *Clin Orthop Relat Res*. 1996;324:116–125.
14. Bowers GM, Chadroff B, Carnevale R, Mellonig J, Corio R, Emerson J, Stevens M, Romberg E. Histologic evaluation of new attachment apparatus formation in humans. Part I. *J Periodontol*. 1989;60:664–674.
15. Bowers GM, Chadroff B, Carnevale R, Mellonig J, Corio R, Emerson J, Stevens M, Romberg E. Histologic evaluation of new attachment apparatus formation in humans. Part II. *J Periodontol*. 1989;60:675–682.
16. Bowers GM, Chadroff B, Carnevale R, Mellonig J, Corio R, Emerson J, Stevens M, Romberg E. Histologic evaluation of new attachment apparatus formation in humans. Part III. *J Periodontol*. 1989;60:683–693.
17. Citagenex. DynaGraft II, DBM in reverse phase medium putty and gel. Available at: http://www.citagenix.com/en/dynagraft_ortho.html. Accessed July 17, 2007.
18. Little RM, Riedel RA, Årtun J. An evaluation of changes in mandibular anterior alignment from 10–20 years post-retention. *Am J Orthod*. 1988;93:423–428.
19. Little RM. Stability and relapse of dental arch alignment: review article. *Br J Orthod*. 1990;17:235–241.
20. Pearce AI, Richards RG, Milz S, Schneider E, Pearce SG. Animal models for implant biomaterial research in bone: a review. *Eur Cell Mater*. 2007;2:1–10.