Histologic Evaluation of Human Intrabony Periodontal Defects Treated with Deproteinized Bovine Bone Mineral in Combination with Orthodontic Tooth Movement: A Case Series

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The aim of this case series was the histologic evaluation of guided tissue regeneration utilizing deproteinized bovine bone mineral (DBBM) when regenerative surgery was combined with (test) or without (control) early orthodontic tooth movement. Core biopsy samples were harvested from previously defected sites after 9 months. The histologic section showed integration of DBBM particles in newly formed bone in the apical and middle thirds of the defect, while in the coronal part, graft materials were mainly embedded in connective tissues in the control patient. DBBM particles showed partial resorption with more de novo bone formation in test samples. Int J Periodontics Restorative Dent 2020;40:321–330. doi: 10.11607/prd.4346

The treatment of teeth with pathologic tooth migration due to attachment loss represents a challenge for clinicians in periodontal therapy. Different orthodontic forces modify the healing of an intrabony periodontal defect. Some authors detected new connective tissue attachment formation within the osseous defects after thorough root surface debridement alone followed by orthodontic tooth movement (OTM) toward the defects. Other groups did not confirm this finding and reported long junctional epithelium interposition between the orthodontically eliminated osseous defect and the denuded root surface. A combined orthodontic and periodontal regenerative therapy may boost periodontal regeneration through reducing occlusal trauma and bone apposition on the tensile side. The process and potential benefit of bone remodeling induced by orthodontic forces is correlated with the existence of periodontal ligaments (PDLs). Therefore, a surgical procedure is necessary to regenerate lost periodontal tissues before tooth movement.

The protocol for treatment of wide and noncontained intrabony defects is the combination of enamel matrix derivatives and a graft, or a guided tissue regeneration (GTR) plus a graft. Both combinations employ a graft material to promote space maintenance, prevent membrane...
collapse, or use as a carrier for biologic agents. The behavior of the mostly documented xenogeneic materials is sometimes questionable in intrabony defects. It is known from human studies that deproteinized bovine bone mineral (DBBM) combined with GTR may enhance periodontal regeneration, nevertheless, particles located at the most coronal part of the previous defect can be embedded in connective tissues.

DBBM either with or without collagen content is considered a non- or minimally resorbing material in GTR and GBR settings. However, bone remodeling-induced resorption of xenogeneic graft particles is a histologically detected and proven phenomenon after different types of bone replacement interventions when DBBM is infused with recombinant human platelet-derived growth factor. Animal studies have shown that OTM is possible into an area that has been regenerated with DBBM, causing similar physiologic remodeling. There is neither histologic proof of DBBM resorption in humans nor sufficient information about the behavior and the clinical success of non-autogenous graft materials combined with OTM. Therefore, the present authors intend to histologically evaluate the behavior of DBBM graft materials in such a treatment approach. The aim of the present study is to highlight the impact of tooth movement modalities on the intrinsic healing pattern of unfavorable intrabony defects. The present case series represents preliminary data of an ongoing randomized controlled trial.

Materials and Methods

Three nonsmoking, generally healthy individuals without any concomitant medications were included in the study. They were referred for periodontal treatment at the Semmelweis University in Budapest. All individuals went through an initial cause-related periodontal therapy and oral hygiene instructions. For study inclusion, the subjects needed to display one vertical bony defect with a minimum intrabony component defect depth of 4 mm. The intrabony defect had to be wide (radiologic angulation > 25 degrees) and noncontained (no more than two bony walls), and furcation Class III lesions were excluded. To meet inclusion criteria, the study teeth must also have suffered pathologic migration (elongated, tilted tooth, etc.). Signed informed consents were collected during inclusion. The study protocol was approved by the Regional and Institutional Committee of Scientific and Research Ethics of the University (Nº 90/2015).

The following clinical parameters were recorded at baseline and at 9 months during a reentry procedure: probing pocket depth (PPD), gingival recession (GR), and clinical attachment level (CAL). Probing bone level was measured intraoperatively according to Tonetti et al. within the same time points in order to calculate the intrabony component of the defect. All clinical parameters were recorded at six surfaces of the study teeth using a manual periodontal probe (UNC 15, Stoma), which was performed by the same investigator masked to the study design. The surface of the study tooth representing the deepest PPD was selected and also served as the location for probing bone level. An acrylic stent on the occlusal surface of the study tooth was fabricated from composite material, and the probe was fixed to it in order to clearly localize the deepest defect position, which helped locate the same area for measurement during reentry.

Surgeries were accomplished under local or nerve-block anesthesia (articaine 4% with epinephrine 1:100,000). The coronally advanced flap technique for the treatment of vertical bony defects described by Zucchelli and De Sanctis was utilized. An intrasulcular incision was made around the teeth, and the papilla was dissected with the simplified or modified papilla preservation technique over the intrabony defect. The papillae mesial and distal to the defect were dissected with submarginal incisions on the buccal aspect. Therefore, the type of flap design was an extended envelope, and the buccal flap was a coronally advanced flap (split-full-split thickness), combined with a full-thickness repositioned oral flap. The regenerative strategy in all cases according to the unfavorable defect morphologies was the guided tissue regeneration technique, where the defect was covered with the resorbable native bilayer collagen membrane (Bio-Gide, Geistlich Pharma). In order to prevent the collapse of the membrane, the defects were filled with lightly packed DBBM particles (Bio-Oss, Geistlich Pharma). A double internal mattress of sutures was fabricated from composite material.

Materials and Methods

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was applied (5-0 nonabsorbable monofilament, B. Braun) over the defect. Circumdental sling sutures closed the neighboring interdental areas (Fig 1). Prior to the first surgery, a stent was made on the study tooth from an acrylic agent in order to standardize the correct placement for later harvesting of the microbiopsy sample, which has to be at the same site of the former intrabony defect after the potential tooth movement. This stent was snapped on the tooth, and the microtrephine was attached to it during surgery. The trephine had to look toward the bottom of the intrabony defect.

Individuals received antibiotic therapy (625 mg amoxycillin + clavulanic acid, thrice a day for 7 days) supplemented with painkillers. Local chemoprofilaxis (0.2% chlorhexidine) was directed twice a day for 2 weeks. Sutures were removed 14 days after surgery. Mechanical toothbrushing and interdental cleaning at the surgical site were forbidden for 3 weeks.

The patients were randomly allocated to three treatment modalities after the regenerative surgical procedures. One individual (C) did not have OTM and served as the control patient. Two subjects received multibond appliances 10 days after the surgery. A gentle and continuous tooth movement was initiated with a 0.014 round NiTi wire, with a 0.016 × 0.016 stainless steel overlay wire in order to fix the neighboring teeth. One tooth tilted toward the periodontal defect (T1) went through an uprighting movement with minimal distalization using a coil spring, which possibly exerted tensile force to the healing site (Figs 2, 3a, and 4), while the last patient’s tooth (T2) was moved toward the periodontal defect, applying pressure forces to the former defect (Figs 5 to 8). Appliance adjustments and professional oral prophylaxis occurred once a month.

Reentry procedure was scheduled at the 9-month endpoint visit, when the same clinical and intraoperative parameters were measured. Reopening the former defect site was performed under the same anesthesia, and a minimal invasive full-thickness flap was elevated interdentally. Due to ethical considerations, the teeth were not removed with a bony block for full periodontal regeneration assessment. Only a bony sample was harvested from the former defect site with a microtrephine (internal diameter 1.8 mm, Meisinger), which standardized with the help of the previously fabricated acrylic stent. Every attempt was made not to harm the potentially developed new periodontal attachment, and therefore the outer rim of the microtrephine was barely in contact with the root surface (Fig 3b). The depth of the drilling determining the length of the core is crucial, because it should not contain any bones from the still-intact apical periodontal structures. Therefore, the drilling depth was specified after calculating the bony fill of the defect (baseline – endpoint intrabony component). The bony cores kept in the trephine were sealed in a vial filled with 10% buffered formalin. Samples were further dehydrated in ascending ethanol series and embedded in an acrylic resin medium according to standard procedures. A 60-micron-thick ground section was prepared through the center of the trephine according to the
Donath and Breuner,\textsuperscript{27} stained with Sanderson's rapid bone stain (Dorn and Hart) and counterstained with acid fuchsin (Sigma-Aldrich).

The primary outcome variable was the histologic and histomorphometric bone analysis of the previously defected site, and the secondary outcome variable was the percentage of the intrabony fill.

**Results**

Neither any complications nor any adverse events were observed during the healing period. Orthodontic therapy of patient T1 eliminated the study tooth’s premature contacts and created freedom for centric movement during mandibular movements, therefore the appliance was removed before the reentry procedure. The study tooth of subject T2 was moved mesially toward the defect but did not reach the final planned position. A biopsy sample was harvested from the grafted area with the multiband appliance still in place. Baseline and endpoint measurements of the clinical and intraoperative parameters are listed in Table 1. The mean intrabony component reduction was remarkable (7.67 mm), which yielded an 85.47% intrabony fill of the original defect dimension (Figs 4 and 8).

Histology of the bony sample representing the control patient showed osteoconduction of DBBM; particles served as a scaffold for new bone formation mainly in the apical and middle thirds of the previous defect. The bone substitutes...
in the coronal third of the sample were embedded in connective tissue (Fig 9a). A higher magnification from the middle-apical part of the core discloses favorable framing and interlinking of the bone substitute materials by new bone (Fig 9b). Histomorphometry showed the presence of 37.2% graft particles with a combination of 22.4% de novo bone formation, while the remaining part was filled with soft tissue components (40.4%) measured in the whole core. While the distribution of the DBBM particles among the core seems to be consistently proportional, soft tissue elements tend to be more frequent in the coronal third.

Evaluating the biopsy sample of the T1 patient, which served as a tension site, showed partial resorption of the DBBM particles (23.7%) with more de novo bone formation (34%) compared to control, while soft tissue components remained similar (42.3%) according to histomorphometric examination (Fig 10a). There was an intimate contact of new bone with the graft particles observed in the enlarged pictures. Ongoing bone formation was present, characterized by the woven bone, osteoids, and osteoblasts. New bone bridged the space between the graft particles. The presence of blood vessels suggests the presence of revascularized vital tissues in the healed site (Figs 10b and 10c).

T2 patient’s tooth moved toward the periodontal defect, and therefore the histology’s core section was an area of a pressure site, which revealed 30.2% newly formed bone combined with an increased
8.2% of resorbed graft. Soft tissue elements filled the remaining 61.6% part of the section (Fig 11a). Under greater magnification, the authors observed cutting cones with osteoid formation inside the graft particles. There was new bone formation and secretion of osteoid by osteoblast present at 9 months postoperative (Figs 11b and 11c).

Discussion

The present literature suggests that throughout eliminating the inflammation developed after periodontitis, periodontal treatment can be successfully and safely combined with OTM. It would seem essential to recommend the optimal time point of initiating such a movement after surgical intervention. Conservative approaches suggest a period of 6 to 9 months of healing after surgery, while modern treatment methods initiate orthodontics immediately or at an early phase following 2 weeks of healing. One randomized controlled trial found a statistically significant difference with immediate application of OTM regarding clinical parameters, radiologic bone density, and bone fill. The same group demonstrated in an animal study that the immediate application of orthodontics showed an increase in the trabecular count and in the total surface area of newly formed bone compared to other groups, with or without a later-initiated tooth movement. A case series in which intrabony defects were treated with DBBM with collagen content demonstrated that by activating the orthodontic appliances 2 weeks after surgery, this early movement did not have negative effects on the augmentation material nor any detrimental effects on healing. At this period, the periodontal defects are past the most sensitive healing period, the blood clot phase, where most wound failures happen.

Table 1

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<th>Case</th>
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<th>Intraoperative parameters</th>
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<tr>
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PPD = probing pocket depth; CAL = clinical attachment level; C = control; T1 = test patient 1; T2 = test patient 2.
Fig 10  (a) Core section from test patient T1 ($\times$30 magnification). Graft particles are embedded in newly formed bone. Less bone substitute is located coronally, where the most tensile strength was utilized during uprighting of the tooth. (b) Development of blood vessels between the areas of newly formed bone prove the revascularization of the former defect ($\times$150 magnification). (c) The middle part of the core sample presents ongoing bone formation, where new bone is bridging the space between the DBBM particles ($\times$150 magnification).

Fig 11  (a) Histologic image from patient T2 shows a low number of graft pieces and an increased amount of intrabony space for soft tissue elements ($\times$40 magnification). (b) Observation of cutting cones with osteoid formation inside the graft particles might be due to ongoing bone remodeling ($\times$150 magnification). (c) Formation of woven bone together with the secretion of osteoid by osteoblast activity is visible ($\times$150 magnification).
At 2 weeks, the healing period is transitioning from the inflammatory to the regeneration phases and is less susceptible to be jeopardized by orthodontic forces compared to an immediate initiation of OTM. Nevertheless, cautious tooth movement with low orthodontic forces might be mandatory even in an early initiation of OTM, which was utilized in the present protocol.

The application of GTR in association with orthodontic treatment seems to improve periodontal conditions during teeth movement into or from a bony defect. There is available data that suggests tooth movement into regenerated areas can be performed without jeopardizing the formation nor the level of the new periodontal attaching apparatus. It is still controversial which bone substitute should be used, whether and how these materials are degraded, and if any side effects result from the material’s breakdown or encapsulation in connective tissue in the long-term. The data on whether PDLs are responsible for the morphologic changes that occur in a grafted area are still lacking. So far, only animal histologic evidence is available and suggests that OTM into a previously grafted area with DBBM can be performed safely. In that animal study, the bone substitute particles showed resorbing patterns on the pressure site, but there was no sign of graft remnants observed at the proximity of the PDL space. The site where the tooth was moved entirely through showed complete resorption of DBBM particles combined with natural bone replacement.

According to the results of the clinical parameters, the minimal subject size prevents determining relevant findings; therefore, statistical analysis of the parametric data is not reasonable at this stage. However, all patients showed beneficial improvement in the recorded parameters.

The histologic intention of this study was not to evaluate a full periodontal regeneration with the evidence of a new cementum with embedded PDLs, but rather to only analyze the behavior of the DBBM particles when they heal uneventfully or are expressed to a different kind of orthodontic forces. In order to fulfill this aim, it is acceptable to have a microbiopsy sample harvested using a trephine with the smallest diameter possible under clinical circumstances. The control patient’s sample, whose tooth healed splinted without any movement, showed nice healing and incorporation of the graft, but according to other human histology findings, DBBM particles can also be encapsulated at the coronal part of the defect and into the connective tissues. The authors lack any data about the long-term behavior and stability of these healed sites, but the question arises as to whether these areas might be more susceptible to a periodontal breakdown in case of an oral hygiene deterioration.

A former periodontal defect exposed to tensile forces of OTM (patient T1) after 9 months represents histologic data of a perfect de novo bone formation around bone substitute materials. Compared to the control sample’s histomorphometry, it seems to be a tendency for the amount of graft particles to decrease and for the amount of newly formed bone to increase. It is assumed that the tension caused by orthodontics induces bone remodeling and apposition, which interferes with the DBBM particles resulting in the change in amount of graft particles and newly formed bone. In the present case, tooth movement for T1 was an uprighting with minimal distalization. Due to the uprighting, the greatest tensile strength is located at the coronal aspect of the defect, where fewer graft remnants were observed in the histologic sample (Fig 10). These observations need further investigations, but this tendency could be beneficial in the long term, because a former defect healed with a larger amount of newly formed bone might be more stable and resistant to later periodontal bacterial attacks.

The core sample of patient T2 presented a further-decreased graft ratio while de novo bone formation was comparable with that of the T1 subject. While this site was under pressure by orthodontic force, the induced resorption capacity of the osteoclast affected the DBBM particles as well. Ongoing remodeling of graft material was evident by the presence of cutting cones. The greater amount of connective tissue might be due to the fact that the bony sample was harvested at the active phase of the OTM. It might be assumed that after removal of the appliance, ongoing bone formation with trabecularization would continue, which could possibly further increase the percentage of de novo
bone. However, this tendency should be regarded as an observation due to the minimal sample size.

As demonstrated by different authors, active remodeling of DBBM can be explained by the up-regulating effect of the recombinant human platelet-derived growth factor in bone metabolism, which created bone remodeling units around graft particles. The novelty of the present study is the histologic evidence of a similar DBBM resorption in humans caused by a different stimulus, OTM. The present results of pronounced graft resorption in patients T1 and T2 compared to C occurred presumably due to the effect of orthodontic-induced bone remodeling, which is strictly related to PDLs. Therefore, it seems that early initiated OTM does not compromise the periodontal wound healing phases nor the establishment of a possible new attachment; otherwise, the later-developing ligaments would not cause the remodeling effect. According to the human histologic findings on periodontal regeneration, bone formation around DBBM particles is linked to preferable new cementum and PDL formation. On the other hand, if a new periodontal attachment could not be verified, new bone development around DBBM was not reported. Therefore, the presented minimally invasive histologic harvesting and evaluation technique can be considered appropriate to fulfill the study aims demonstrating DBBM remodeling and to deduce simultaneous formation of a new attachment.

Conclusions

In the present case series, unfavorable periodontal defects were treated with the use of extended, coronally advanced flaps according to the guided bone regeneration techniques with the utilization of DBBM particles. The improvement of clinical parameters was desirable, and intrabony defect resolution was observed in all cases. Following OTM, increased active resorption of DBBM along with new bone formation was observed, which needs further investigation due to the low sample size of the present study. Nevertheless, DBBM resorption is a unique phenomenon and may be beneficial for patients treated with a combined periodontal orthodontic as it results in the long-term stability of grafted sites.

Acknowledgments

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References