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Osteopromotive property of allogenic demineralized dentin matrix: a pilot study

Introduction

Alveolar bone deficiency poses a significant problem in the treatment of traumatic injuries, periodontal diseases, tumor resections, and in many cosmetic/reconstructive surgeries. The primary reasons for bone deficiency in dentistry are periodontal disease and tooth extraction. Periodontal disease involves infection and degradation of the periodontium, which if not treated leads to losing the bone support and most likely loss the teeth.[1] Tooth extractions may also cause bone deficiency. Periodontal ligaments distribute the mastication forces on the surrounding bone and as a result keep the bone healthy.[2] However, periodontal ligaments are extracted along with tooth, resulting in bone resorption.

This has led to numerous studies on different techniques and materials for bone regeneration over the years. Previous studies[3-7] have shown that autogenous demineralized dentin matrix (DDM) is a powerful bone regenerative material. Nevertheless, it is impractical as it requires sacrificing a healthy tooth. Furthermore, this approach is not feasible in edentulous patients. It is therefore best to utilize teeth that are extracted from other individuals during orthodontic treatments and wisdom teeth extractions, which are otherwise routinely discarded, in order to produce a bone regenerative material as an allograft. The pilot experiment outlined here tested the central hypothesis that *allogenic DDM increases bone regeneration*.

Materials and methods

In this pilot study, 6 female 120 d old New Zealand white rabbits were used. All the animal procedures were approved by and carried out in accordance with Shahed University Animal Care and Use Committee guidelines.

Rabbits were anesthetized with an intramuscular injection of a mixture of ketamine and xylazine (35 and 5 mg/kg, respectively). The left mandibular central incisor teeth were then extracted after local anesthesia using a lidocaine/epinephrine mixture (1:80,000, 8 mg/kg). All the periodontal ligaments were removed from the teeth and dental pulp was removed using the retrograde technique. Teeth were demineralized using the method of Urist et al.,[8] with some modifications. The teeth were demineralized in 0.6 mol/L hydrochloric acid for a week, followed by multiple washing periods in normal saline and 70% ethanol until their pH reached 7.5. Allogenic DDM specimens were cut into small pieces (about 2 mm^3) and pieces from all 6 rabbits were homogenized and stored in room temperature until use.

Two weeks postextraction, rabbits were anesthetized for a second time using the same anesthesia. A full thickness incision was made on the midline of the forehead. Two circular index grooves were made using an 8 mm trephine drill and a surgical micro-motor to a depth of 0.5 mm on the joint between the two parietal bones. These index grooves were filled with gutta-percha and guided the authors to the exact position of the defects during sample collection. Two bi-cortical defects were made in the center of index grooves using a 6 mm trephine drill. The floor of both defects was covered by resorbable collagen membrane (Paroguide, Acteon, France). One defect in each rabbit was filled with allogenic DDM (experimental defect), while the other one was left empty (control defect). Both left and right defects were covered by collagen membrane. The periosteum and the skin were sutured separately using Vicryl 4,0 (Ethicon Inc., Texas).

Rabbits were euthanized by IV barbiturate overdose at 15, 30, 45, 60, 75, and 90 d postsurgery (1 rabbit at each time point). Bicortical bone samples were collected using a 6 mm trephine drill. Histology samples

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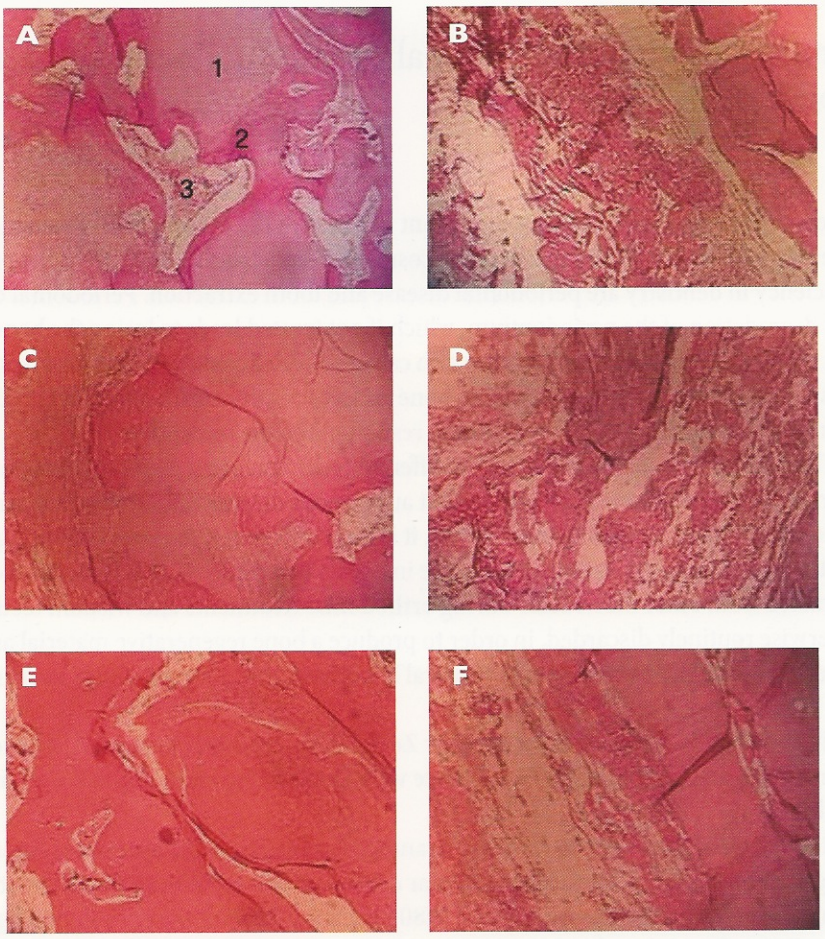

FIGURE 1: Histologic view of the experimental and control samples: **A**, experimental sample 30 d postsurgery: 1 denotes allogenic demineralized dentin matrix, 2 denotes newly formed bone surrounding the graft pieces, 3 denotes the bone marrow surrounding the newly formed bone; **B**, control sample 30 d postsurgery; **C**, experimental sample 60 d postsurgery; **D**, control sample 60 d postsurgery; **E**, experimental sample 90 d postsurgery; **F**, control sample 90 d postsurgery

were prepared using hematoxylin and eosin staining. Histologic and histomorphometric analyses were performed on two nonconsecutive slides from each sample. The average amount of newly formed bone was calculated for experimental and control groups irrespective of timing of euthanasia, and this was compared using paired sample t-test, where $p<0.05$ was considered statistically significant.

Results

HISTOLOGY RESULTS In the experimental defects at 15 d postgrafting, the area between the graft pieces was filled with fibrotic connective tissue with no inflammatory cell infiltration. In addition, a minute amount of newly formed bone was observed around the defect. At 30 d postgrafting, graft pieces had undergone remodeling and newly formed bone was observed bordering the pieces. Fibrotic tissue and bone marrow filled the area between the graft pieces (fig. 1A). At 45 d postgrafting, the remodeling process extended toward the center of the graft pieces. Multiple bone marrow spaces were observed. At 60 d postgrafting, woven bone had remodeled to lamellar bone and bone marrow spaces were observed in the mature bony tissue (fig. 1C). At 75 d postgrafting, the entirety of the graft pieces had remodeled to newly formed bone, and bone marrow spaces were observed in and around the bone trabeculae. At 90 d postgrafting, complete closure of the defect with mature bone was observed and no remnants of graft pieces were visualized (fig. 1E). No signs of an inflammatory reaction or infectious process were observed at the tissue level in any of the samples.

In the control defects, at 15 d postgrafting, the sample was filled with fibrotic tissue and no bone formation was observed. At 30 d postgrafting, minimal woven bone surrounded the defect while the

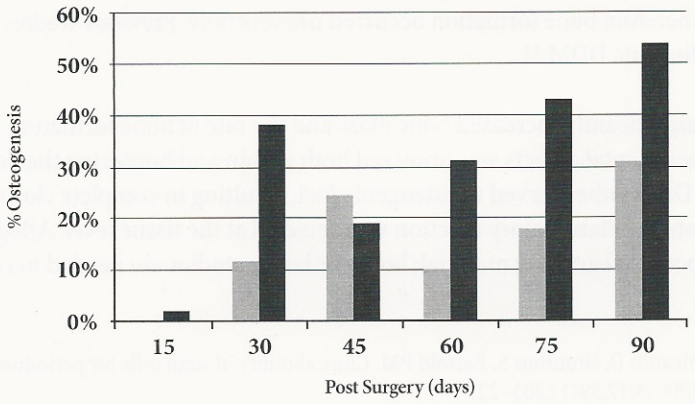

FIGURE 2: Histomorphometric analyses of the samples depicting the amount of bone regeneration in the samples. Light bars represent control samples and dark bars represent experimental samples.

remainder was still filled with fibrotic and necrotic tissue (fig. 1B). At 45 and 60 d postgrafting, the bone surrounding the defect had become more mature, however, the center of the defect remained composed of connective tissue (fig. 1D). At 75 d postgrafting, the amount of bone around the defect had increased as compared with the 60 d interval, and bone marrow spaces were observed in areas of the lamellar bone. At 90 d postgrafting, increased bone formation was observed, however, the center remained filled with fibrotic tissue (fig. 1F). The bone around the defect was completely mature and bone marrow spaces were observed in the bony tissue. No signs of inflammation or infection were observed in the control samples.

HISTOMORPHOMETRIC RESULTS The areas of newly formed bone were measured in two nonconsecutive slides of each sample. In the experimental group, newly formed bone was measured at 2%, 38%, 19%, 31%, 43%, and 54% after 15, 30, 45, 60, 75, and 90 d, respectively. In the control group, newly formed bone was measured at 0%, 11.5%, 24.5%, 10%, 18%, and 31% after 15, 30, 45, 60, 75, and 90 d, respectively (fig. 2). The overall average of the newly formed bone was measured at 31% and 16% for experimental and control groups, respectively. The average amount of bone regeneration was significantly higher in the experimental group when compared with the control group using paired t-test (p=0.03).

Discussion

Autogenous bone graft is considered the gold standard in periodontal treatment.[9] However, autologous grafting is associated with complications, such as the need for a second surgical site to obtain the graft and also donor site morbidity.[10] Moreover, several studies have reported varying amounts of graft resorption, which might lead to the need for a second augmentation procedure.[11-13] As a result, several studies have been performed to develop new grafting material with comparable osteogenic potential.

There are 3 different properties of graft material that increase the bone mass and bone formation rate:[14-17] osteoconduction: materials that provide a scaffold and give support to angiogenesis and calcification; osteogenesis: materials that contain either growth factors which promote cell proliferation and differentiation or mature osteoblast cells; and osteoinduction: materials that contain morphogen substances which trigger differentiation of undifferentiated mesenchymal cells into chondroblasts or osteoblasts.[15,17]

Histological analyses in this pilot study showed osteoclast cells surrounding the allogenic DDM pieces, thereby leading to resorption of the pieces and remodeling into woven bone. In addition, a line of osteoblast cells was observed adjacent to the pieces which may suggest osteoinductive property of allogenic DDM. This finding is in congruence with previous studies. Inoue et al.[18] implanted dentin into subcutaneous connective tissue, periodontal ligaments, femoral muscles, and rectus abdominis muscles of Wistar rats and reported that dentin induced chondrogenesis, which is the first step in endochondral bone formation. To confirm its osteoinductive potential, a graft should have the ability to induce bone formation in tissues other than bone.[19] Ike and Urist[20] implanted human demineralized dentin particle capsules into the femoral muscles of nude mice and observed induction of bone and cartilage formation. It has been suggested that bone morphogenic protein is responsible for the osteoinductive property of DDM.[18,21,22,23,24]

Our observation also showed that allogenic DDM cubes served as foci for bone formation and therefore increased the number of bone formation centers. As a result, in the experimental samples bone formation was observed around and within the center of the defects. Conversely, in the control group, bone formation was observed solely around the defect, whereas the center of the defects was filled with connective tissue even after 90 d. This phenomenon can be explained by the osteoconductive property of DDM, which has previously been reported.[3-7]

The major concern regarding allografts is their antigenicity and potential to trigger an immune response.

The results of this study showed that implantation of allogenic DDM did not cause inflammatory cell infiltration and therefore bone formation occurred uneventfully. Previous studies have also reported lack of antigenicity of allogenic DDM.[25]

Conclusion

Allogenic DDM significantly increased bone mass and the rate of bone formation. Additionally, bone formation in experimental defects was observed both within and bordering the defect. In the experimental group, allogenic DDM cubes served as osteogenic foci, resulting in complete closure of the defects with bone. No infection or inflammatory reaction was detected at the tissue level. Allogenic DDM may be considered as a potential grafting material; however larger studies are needed to confirm the findings of the present study.

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