Evaluate the use of fresh Autogenous cement and dentine as bone graft to repair bone defects in dogs: experimental study

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Abstract

Objective: This study aimed to evaluate the role of fresh autogenous cement and dentine as bone graft to repair bone defects in dogs.

Study design: 3 adult dogs were used as subjects, 2 bony defect were created on the buccal surface of the mandible in each dog, one of them was grafted with fresh autogenous cement and dentine (test group) and the other was served as control, Samples were then biopsied in 4, 8, and 12 weeks.

Results: After 4 weeks there was new cell lining attached to bone graft pieces in test group, after 8 weeks bone formation surrounding the bone graft pieces was observed. On the other hand less bone formative activity was evident in control group. After 12 weeks we observed that most bone graft pieces underwent resorption, and the volume of new bone increased and became interconnected with the surrounding bone, while only fibrosis and small area of new formed bone could be observed in the control group.

Conclusions: The use of fresh autologous cement and dentine as bone graft led to active bone formation with excellent quality.

Introduction

Autogenous bone is an ideal material for the reconstruction of hard tissue defects, because it promotes osteogenesis, osteoinduction, osteoconduction, and rapid healing, but it does induce immune rejection. However, the disadvantages of autogenous bone as a graftingmaterial are that the harvest volume is limited, resorption is unavoidable, and a second defect is induced in the donor area[1,2]. To overcome these limitations, allogeneic bone, xenogeneic bone, and synthetic bone have been used in clinical practice; nevertheless, efforts have continued to develop more ideal bone grafting materials.[3,4] Kim et al reported the use of particulate dentin as an implant material to avoid the high cost of HA 5-10. However, the use of particulate dentin presents a problem for the retention of graft materials as well as the preparation of graft through many stages and this increases time and cost. This study aimed to evaluate the role of fresh Autogenous cement and dentine as bone graft to repair bone defects in dogs.

Material and Methods

1. **Experimental Animal:** 3 dogs (aged 12 months; weight 12 to 15 Kg) were used as subjects in this study. The research protocol was approved by the ethics committee for animal research, Al Andalus University for medical science. 2 bony defect were created on the buccal surface of the mandible in each dog, one of them was grafted with fresh autogenous cement and dentine mixed with tetracycline presteron (test group) and the other was served as control, Samples were then biopsied in 4, 8, and 12 weeks.
2. **Graft Material:** Autogenouscement and dentine graft material produced from teeth extracted from the experimental animals and each dog received graft originating from itself. The attached soft tissues were removed from the teeth then the surface of roots was chopped with diamond bur with copious irregation of salin, the pieces were collected and mixed with tetracycline presteron paste and used to graft the holes in test group.
3. **Animal Experiment:** General anesthesia was induced by using (ketamine, 5 mg/kg, and xylazine, 2 mg/kg IM) Infiltration anesthesia was done in the site of surgery for anesthesia and hemostasis with lidocaine (2% lidocaine HCl-epinephrine, 1.8 ml). Full-thickness mucosal flaps were raised and the first, second,and third mandibular premolar were extracted to prepare the graft, two defects with a diameter and depth of 5 mm and 4 mm were created over the buccal surface, one of them was grafted with autologous cement and dentine mixed with tetracycline presteron paste® (Nippon Shika Yakuhin Dental Pharm). The flap was pulled to position and sutured with absorbable suture after operation was completed antibiotic therapy (Ampicillin sodium; 25mg/ kg intramuscularly daily) was administrated for 5 days.

Histological procedure

Samples were then biopsied in 4, 8, and 12 weeks under general anesthesia bone samples were obtained from around the defect sites, fixed in 10% neutral formalin for 72 hours, and decalcified in nitric acid for 4 hours. The bone samples were then cut into 3-mmthick sections, followed by washing in running water. Each bone sample was then treated using an auto-processing machine (Hypercenter XP, Shandon, U.K.). After paraffin embedding, each section was cut into 4 to 5m slices and was stained with hematoxylin-eosin and Goldnertrichrome. Evaluations of the stained sections were then performed under an optical microscope and were photographed.

Results

After 4 weeks: There was new cell lining attached to bone graft pieces, large quantity of capillaries and Newly deposited osteoid formations were observed in the test group indicating bone-forming activity while only fibrosis could be observed in the control group in addition to some capillaries and inflammatory cell accumulation.

After 8 weeks: In test group; bone formation surrounding the bone graft pieces was observed, the newly formed bone included many osteocytes and there were regularly lined with many osteoblasts and there was no evidence of inflammation or foreign-body reaction. On the other hand less bone formative activity was evident with some newly deposited osteoid formations in the control group.

After 12 weeks: We observed that most bone graft pieces underwent resorption, and the volume of new bone increased and became interconnected with the surrounding bone, thus forming a more stable structure in test group. While only fibrosis and small area of new formed bone could be observed in the control group.

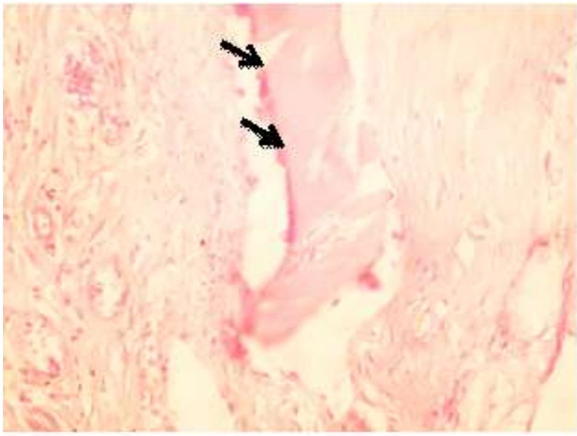

Fig. 1: Histologic analyses of 4week biopsy sample in test group. The new cell lining and attachment to bone graft piece (arrows). (H&E staining, X 40)

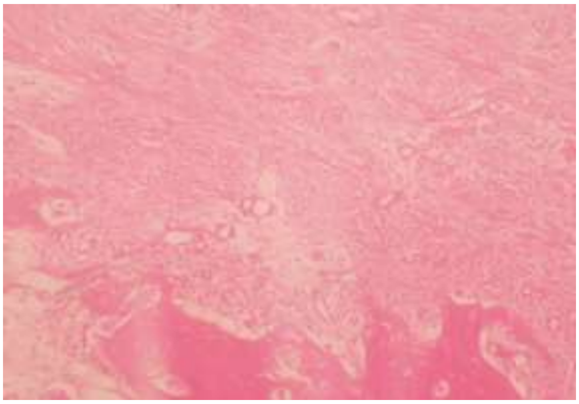

Fig. 2: Histologic analyses of 4week biopsy sample in control group: fibrous connective tissue contains capillaries and inflammatory cell accumulation. (H&E staining, X 40)

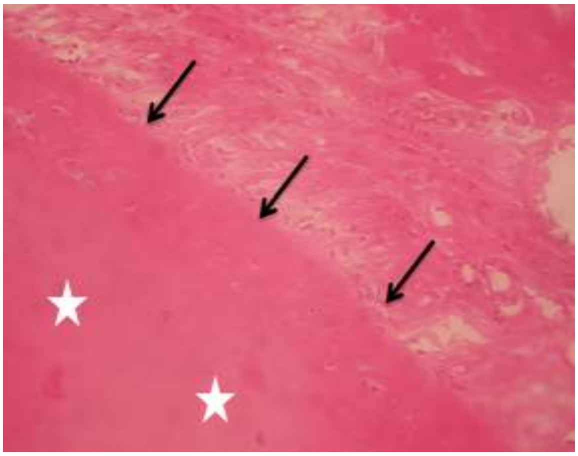

Fig. 3: Histologic analyses of 8 week biopsy sample in test group. New bone is actively formed around bone graftpiece. Asterisks and arrows indicate bone graft piece and new bone formation around the tooth granules, respectively. Hematoloxylin and eosin staining (×100)

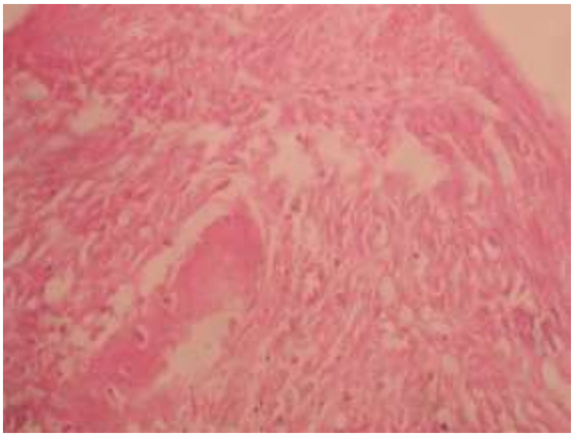

Fig. 4: Histologic analyses of 8week biopsy sample in control group. Limited bone formation Hematoloxylin and eosin staining (×100)

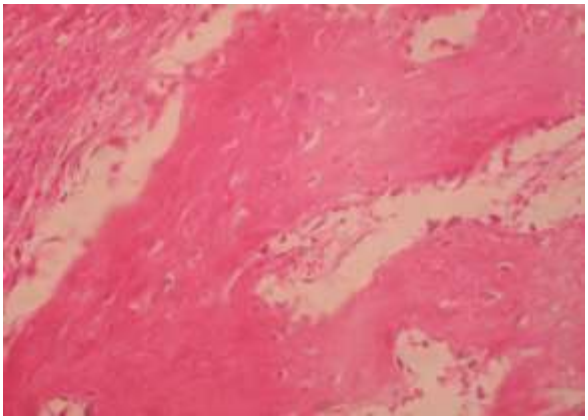

Fig. 5: Histologic analyses of 12week biopsy sample in test group: newly formed bone with osteocytes Hematoloxylin and eosin staining (×100)

Discussion

To aid in the stimulation of osteogenesis and the reconstruction of bone defects in patients with injuries, disease, and congenital malformation in dentistry, orthopedics, and neurosurgery, various types of bone grafts and biomaterials have been developed.[6] Ideally, the graft material is required to have the ability to facilitate osteogenesis, stability when implanted with the bone graft, low risk of infection, ready availability, low antigenicity, and a high level of reliability.[11] Research on graft materials has been steadily increasing since HA materials were first developed. Hydroxyapatite is the primary inorganic natural component of bone and is extremely biocompatible and bonds readily to adjacent hard and soft tissues.[12] However, the high cost of HA and its complicated application during surgery are drawbacks associated with its use. Although HA is biocompatible, one major challenge is that HA can create significant problems through rejection and the inability of the graft material to develop a stable fusion with the surrounding bone owing to its fluid nature.[10] The chemical composition of teeth is very similar to that of bone. In the enamel, the total inorganic content is 95%, the organic content is 0.6%, and water makes up 4%. However, in the dentin, the inorganic content is 70%-75%, the organic content is 20%, and water makes up 10%; and in alveolar bone, the inorganic, organic, and water contents are 65%, 25%, and 10%, respectively.[13] Approximately 90% of the organic material present in the dentin consists of collagen fibers, primarily type I collagen, and these fibers play an important role in calcification. The remaining organic components consist of non-collagenous proteins, carbohydrate, lipid, citrate, lactate, etc.[13] Young-Kyun Kim et al developed a novel bone grafting material that incorporates autogenous teeth (AutoBT), and provided the basis for its clinical application. AutoBT contains organic and inorganic mineral components and is prepared from autogenous grafting material, thus eliminating the risk of an immune reaction that may lead to rejection. AutoBT was used at the time of implant placement, simultaneously with osteoinduction surgery, and excellent bony healing by osteoinduction and osteoconduction was confirmed.[3,5-11] In the present study, the defects were grafted with fresh autologous cement and dentine graft which is prepared during the operation, Our histologic examination of the grafted area revealed that the grafting material was gradually resorbed and replaced with new bone, and the new bone formed a direct union with the remaining graft. The healing process, promoted by osteoconduction and osteoinduction, was observed in all samples, and abundant lamellar bone was observed, confirming that bony remodeling was achieved rapidly. Three months after surgery, the autologous cement and dentine had induced active new bone formation by osteoinduction and was gradually being resorbed. Kim, et al reported bone healing capacity of demineralized dentin matrix materials in a mini-pig cranium defect[14]. At 4 weeks, the inside of the bur hole showed fibrosis, and there was no sign of bone formation in the control group. On the other hand, bone formation surrounding the tooth powder granule was observed at 4 weeks in the experimental group wherein the bur hole was filled with tooth powder. Nampo et al.[15] compared the bone formation ability of autogenous tooth bone graft material and autogenous bone harvested from the long bone of rats in a mandibular defect area and reported that autogenous tooth bone graft material showed better bone formation compared to long bone. Studies based on autogenous tooth bone graft material as experimental and HA as control material grafted to a mandibular bony defect of mini-pigs reported that the experimental group showed better initial bone formation and histometric evaluations showed that the experimental group had better quantitative results.[16] The limitation of our study include a relatively small number of samples. Therefore, our data set may not be representative of all outcomes, and supplemental studies are required.

Conclusions

The use of fresh autologous cement and dentine as bone graft led to active bone formation with excellent quality.

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