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Processed Bovine Dentine and Bone Xenograft (BIO OSS) For Bone Regeneration and Repair-A Comparative Animal Study

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Abstract: Different forms of allogenous and autogenous bone grafts have been studied for their potential use as bone substitutes. Although autogenous bone grafts is a treatment alternative for bone regeneration and repair they have several disadvantages like limitation of graft volume available, donor site morbidity and is technique sensitive. Thus, use of other materials like processed bovine dentine and Bone Xenograft (Bio-Oss) presents an alternative and a relatively simple technique for bone regeneration and repair. The main aim of this study was to determine the role of Processed Bovine Dentine and Bone Xenograft (Bio-Oss) in bone regeneration and repair and to compare these two groups histlogically. A total of ten healthy adult Albino male rats, aged 4 weeks weighing between 150 – 200gms were used in the study. Three round surgical defects of approximately 1mm in diameter were drilled in both the right and left femur. Processed bovine dentine was placed in the right femur and Bio-Oss was placed in the left femur. Group I: - Processed Bovine Dentine.Group II: - Bone Xenograft (Bio-Oss). Bio-Oss graft displayed osteoconductive properties and the best bone formation with complete resorption and organization of the grafted materials. Based on histological findings, though Processed Bovine dentine displayed osteoconductive properties its effect on bone formation was slower compared to that of Bio-Oss. The results of the present study supports that the use of Processed Bovine Dentine seems to be an alternative bone substitute, although less effective than Bone Xenograft (Bio-Oss).
Keywords: Processed Bovine Dentine and Bone Xenograft (Bio-Oss).

INTRODUCTION

The human body might suffer from bone damage due to accidents or due to excessive removal of bone or due to aging. Regeneration or repair of bone is a natural process but takes its due course of time. Hence, autogenous bone grafts and other artificial bone materials have come as a new technique for bone regeneration and repair.

Diverse biomaterials have been used in dental surgery and, with continuous research and development as wel las academic advancements, a variety of new biomaterials have been commercialized. In oral and maxillofacial surgery, periodontal surgery, implant surgery, and diverse other fields, grafting biomaterials are used to repair hard and soft tissue defects, in conjunction with guided tissue regeneration and guided bone regeneration, and in esthetic and reconstructive plastic surgery [1].

Of the available bone graft materials, autogenous bone grafts, obtained from the host, are considered a gold standard as they possess desirable properties of bone formation such as osteogenesis, osteoinduction and osteoconduction [2]. Autogenous bone is an ideal material for the reconstruction of hard tissue defects, because it promotes osteogenesis, osteoinduction, osteoconduction, and rapid healing. However, the disadvantages of autogenous bone as a grafting material are that the harvest volume is limited, resorption is unavoidable, and a second defect is induced in the donor area. 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].

However, owing to concerns regarding the spread of infection and the high cost associated with allogeneic or xenogeneic bone, clinicians and patients may opt against these sources of grafting material. Synthetic bone, in contrast, is relatively inexpensive and involves no risk of disease transmission [1]. Owing to the limitations of autogenous grafts new artificial bone substitutes like Bovine dentine and Bone xenograft (Bio Oss) for have been used frequently.

An ideal bone substitute should possess the osteoinductive and osteoconductive properties. Osteoinduction is the process of stimulating osteogenesis. Osteoinduction is defined as transformation of non-osseous connective tissue cells into osteogenic and chondrogenic cells. The most commonly used materials are allogenic and autogenic bone grafts. Osteoconduction is characterized as bone growth by apposition from the surrounding bone. This process provides a physical matrix or scaffolding suitable for deposition of new bone. The most common osteoconductive bone graft materials are alloplasts and xenografts.

Dentine has been shown to have distinct advantage as it contains more than 20% organic matrix similar to that of bone. Demineralised dentine has shown to be a good osteoconductive material in repair and regeneration of bone [4]. Bio Oss which is similar to natural inorganic cancellous bone does not elicit inflammation providing osteoconductive scaffolding similar to those of autogenous bone [5].

The easy application of these materials in clinical practice and its possible beneficial outcome including bone repair and regeneration has yielded good results. These bone regeneration materials can be great adjuncts in Oral and Maxillofacial surgery practices after surgical extractions of teeth, implants and maxillofacial reconstructions.

The present study has been designed to compare between Bovine dentine and Bone xenograft (Bio Oss) in promoting a faster and better osteoconduction, with no possibility of chronic inflammation. This study focuses on comparison of processed Bovine dentine and Bone xenograft (Bio-Oss) as valuable adjuncts for bone regeneration and repair and thus promoting the better material among the two.

MATERIALS AND METHODS

Ten healthy adult Albino male rats aged about 4 weeks measuring 150-200gms were selected from the department of pharmacology and toxicology, St. John's Pharmacy College, Bangalore in which the materials to be compared for bone regeneration and repair were placed in the right and left femurs of these rats. The study protocol was approved by the ethical committees of the St. John's Pharmacy College, Bangalore and The Oxford Dental College, Hospital and research centre, Bangalore and was conducted as per the guidelines for research principles involving animals as set by the CPSEA, taking appropriate measures for minimizing pain and discomfort to animals. The study was carried out for a period of 4 weeks.

STUDY DESIGN

A total of ten healthy adult Albino male rats, aged 4 weeks weighing between 150 – 200gms were used in the study. Three round surgical defects of approximately 3mm in diameter were drilled in both the right and left femurs. Processed bovine dentine was placed in the right femur and Bio-Oss was placed in the left femur.

MATERIALS

Processing of Bovine dentine

Extracted young bovine teeth with open apices were washed with water, cleaned, and all soft tissues including the pulp and periodontal ligament were removed from the root. After the enamel had been removed with a high-speed diamond bur, the dentine was broken into small pieces (5–10 mm) using a mortar and pestle. Then the dentine was washed with distilled boiling water several times to eliminate the organic solvent, and then the fragments were air dried. Final stage involved grinding pieces of dentine into small particles obtained in powder form using a high-speed diamond bur.

Composition of Bio-Oss

Bio-Oss is a natural, non-antigenic, porous bone mineral matrix. It is produced by removal of all organic components from bovine bone. Due to its natural structure Bio-Oss is physically and chemically comparable to the mineralized matrix of human bone. It is available in the form of cancellous granules (spongiosa), cortical granules and in the form of blocks.

Properties/Action

The inorganic bone matrix of BIO-OSS has physical and chemical properties comparable to the mineralized matrix of human bone. The collagen facilitates handling of the graft particles and acts to hold the BIO-OSS at the desired place. The consistency of this material readily allows it to take the shape of the defect. The graft is slowly resorbed and replaced by new bone.

METHODS

The rats were anesthetized by using 10% Ketamine hydrochloride given intraperitoneally using an insulin syringe. The left and right hind limbs were shaved and prepared. A vertical incision on the ventral part of the hind limb was placed. The fascia and the muscles were dissected and the left and right femurs were exposed.

Three small round surgical defects measuring 3mm in diameter were drilled in the mid-shaft of the right and left femurs using a micro motor with copious amount of saline irrigation. Samples of sterile Processed Bovine dentine and Bone Xenograft (Bio-Oss) were placed into these surgical defects created in the right and left femurs respectively under aseptic conditions. Wounds were closed using interrupted silk sutures and the animals allowed recovering.

Post-Operative care

Once the rats recovered from anesthesia, they were housed in paddy husk covered flat bottom plastic cages under controlled environmental conditions for 4 weeks with free access to water and feed. _A course of antibiotic Injection Taxim 1mg in 3 divided doses for 5 days and analgesic Injection Inac 2.5mg in 3 divided doses for 3 days were given intramuscularly. After 4 weeks the rats were sacrificed by cervical dislocation. The femurs were dissected free and placed in formalin before decalcification and processing to haematoxylin and eosin stained paraffin sections.

HISTOLOGIC EVALUATION

The animals were sacrificed after 4 weeks of the surgery. The operated sites were located and dissected free and placed in formalin before decalcification and processed to haematoxylin and eosin stained paraffin sections. To assess the bone regeneration and repair the sections were then viewed under compound microscope.

RESULTS

Bio-Oss graft displayed osteoconductive properties and the best bone formation with complete resorption and organization of the grafted materials. Based on histological findings, though Processed Bovine dentine displayed osteoconductive properties its effect on bone formation was slower compared to that of Bio-Oss. (Table 1, Fig 1-6)

STATISTICAL METHODS

Descriptive statistical analysis has been carried out in the present study. Results on continuous measurements are presented on Mean ± SD (Min-Max) and results on categorical measurements are presented in Number (%). Significance is assessed at 5 % level of significance Chi-square/ Fisher Exact test has been used to find the significance of study parameters on categorical scale between two or more groups.

Table-1: Comparison of Outcome variables between two groups

Variables	Group I (N=10)		Group Ii (N=10)		P Value
	No	%	No	%	
Resorption Of The Implanted Material					
• Positive	9	90.0	10	100.0	1.000
• Negative	1	10.0	0	0.0	
Osteoblastic Activity					
• Positve	8	80.0	10	100.0	0.474
• Negative	2	20.0	0	0.0	
Osteoclastic Activity					
• Positve	0	0.0	4	40.0	0.087+
• Negative	10	100.0	6	60.0	
Newbone Regeneration					
• Positve	0	0.0	10	100.0	<0.001**
• Negative	10	100.00	0	0.0	

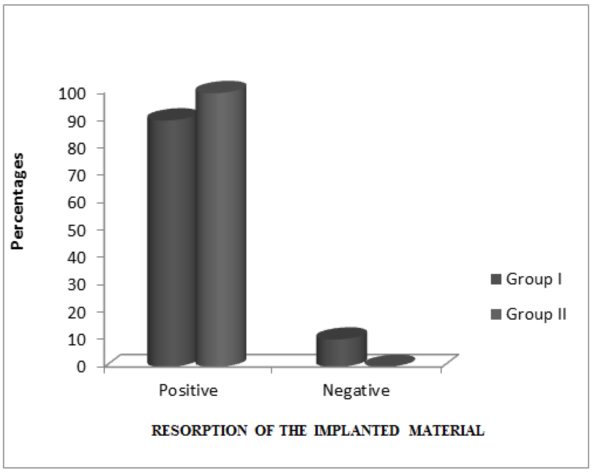


Fig-1

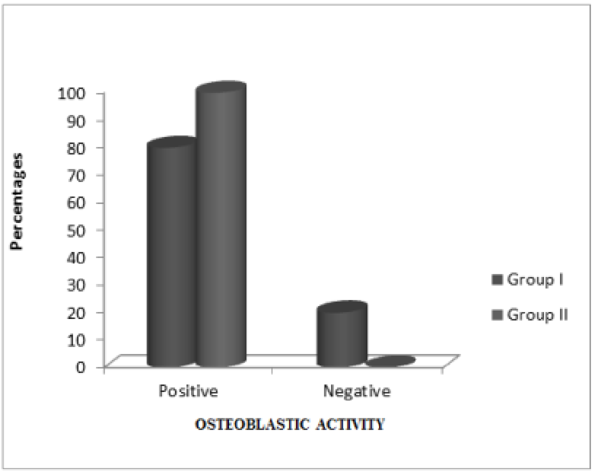


Fig-2

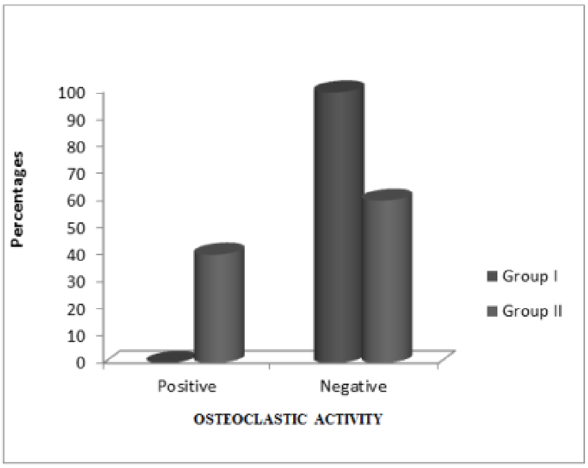


Fig-3

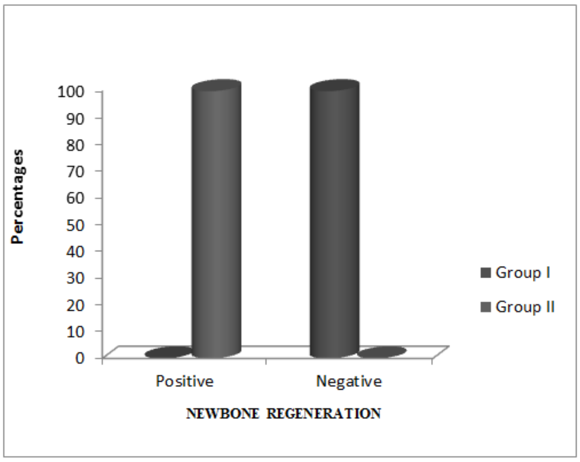
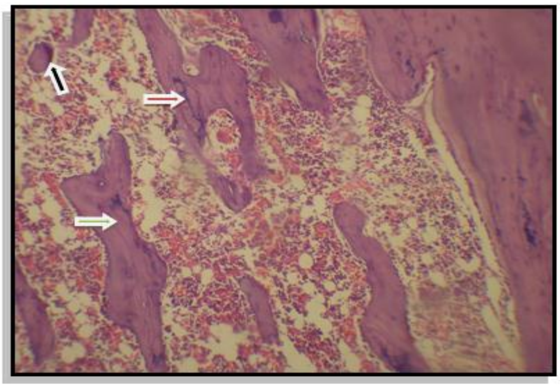


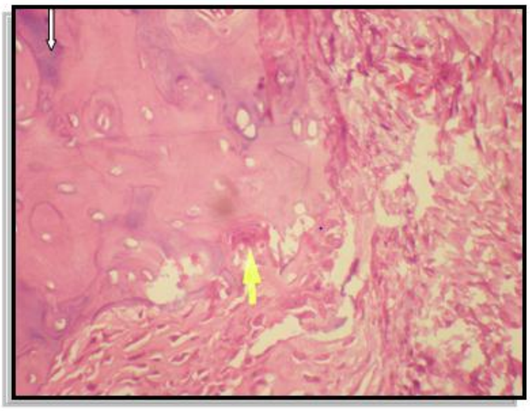
Fig-4



HISTOPATHOLOGICAL FINDINGS OF BIO-OSS

OSTEOBLASTS SEEN
RESORPTION OF GRAFT MATERIAL
ISLANDS OF NEW BONE FORMATION

Fig-5



HISTOPATHOLOGICAL FINDINGS OF PROCESSED BOVINE DENTINE SHOWING RESORPTION OF IMPLANTED GRAFT MATERIAL (White Arrow) AND OSTEOBLASTS (Yellow Arrow)

Fig-6

DISCUSSION

Excessive bone removal is a commonly encountered clinical problem in Oral and Maxillofacial Surgery and often results in long term functional defects and inability to replace the lost structure. Treatment of bone defects with bone grafts is mainly aimed to allow early correction of the defect and allow good bone regeneration and repair.

An ideal bone substitute should be biocompatible and gradually be replaced by new bone. Various materials like autogenous and allogenous bone grafts, plaster of paris, particulate dentine, xenografts and alloplastic materials have been used for bone regeneration and repair. The present study has been designed to compare two resorbable hydroxyapetite materials, Processed Bovine dentine and Bone xenograft (Bio Oss) which have proved to be biocompatible and have exhibited osteoconductive properties.

In a study by Keyvan Moharamzadeh *et al*.[6] for bone regeneration and repair showed that, the processed bovine dentine showed excellent biocompatibility in vitro, stimulated formation of new bone and was completely incorporated into new bone in vivo and hence processed bovine dentine has the potential to be used as a suitable substitute in bone regeneration and repair

In the present study Processed bovine dentine which was placed in defects created in the femur of the rats were non-irritative and histopathologic ally showed no signs of inflammation and with no foreign body giant cells thereby enhancing their ability for resorption of the material and new bone formation.

Marcelo Camelo, *et al*,[7] in a study to evaluate the clinical, radiographic, and histologic response to the use of Bio-Oss porous bone mineral for the treatment of periodontal osseous defects in humans showed substantial improvement in the clinical parameters of probing pocket depth, attachment level, and radiographic bone fill. Histologically, there was extensive new bone formation and an absence of inflammation.

Similarly, in the present study Bio-Oss which was placed in defects created in the femur of rats histopathologic ally showed islands of new bone formation with no signs of inflammation. Kim Su Gwan *et al*. [8], studied the combination of particulate dentine and plaster as bone substitute material in calvarial bone defect in rats and to compare it with Bone Xenograft (BIO-OSS). The defect was filled with different graft materials in each group. The study showed that the combination of particulate dentine and plaster is an alternative bone substitute although less effective than bio-oss.

The statistical results of the present study also supports the above study by illustrating that Processed Bovine dentine is less effective as an alternative bone substitute as compared to Bio-Oss. The resorption of the implanted graft material in the Bio-Oss group was to the extent of 100% while the Processed Bovine dentine group showed 90% of resorption with insignificant P value of 1.000 and both the groups showed good Osteoblastic activity, which was around 100% for the Bio-Oss group and 80% for the Processed Bovine dentine group with P value of 0.474 which is statistically insignificant for the comparative study. Bio-Oss group showed 40% of Osteoclastic activity whereas no Osteoclastic activity was present in the Processed Bovine Dentine group with a P value of 0.087, showing suggestive significance. 100% of new bone formation was present in the Bio-Oss group and no new bone formation in the Processed Bovine dentine group which is statistically strongly significant with a P value of <0.001.

In the present study the Bone Xenograft (Bio-Oss) graft displayed osteoconductive properties and the best bone formation with complete resorption and organization of the grafted materials. Based on histological findings, though Processed Bovine dentine displayed osteoconductive properties its effect on bone formation was slower than that of Bio-Oss.

CONCLUSION

As compared to the Processed Bovine Dentine group the group treated with the Bone Xenograft (Bio-Oss) showed significantly more positive new bone regeneration.

The resorption of the implanted graft material in the Bio-Oss group was to the extent of 100% while the Processed Bovine dentine group showed 90% of resorption with insignificant P value of 1.000 and both the groups showed good Osteoblastic activity, which was around 100% for the Bio-Oss group and 80% for the Processed Bovine dentine group with P value of 0.474 which was statistically insignificant for the comparative study. Bio-Oss group showed 40% of Osteoclastic activity whereas no Osteoclastic activity was present in the Processed Bovine Dentine group with a P value of 0.087, showing suggestive significance. 100% of new bone formation was present in the Bio-Oss group and no new bone formation in the Processed Bovine dentine group which is statistically strongly significant with a P value of <0.001.

The possible interpretation is that, the processed Bovine Dentine group showed less formation of Osteoblastic activity and absolutely no Osteoclastic activity which somehow inhibited the positive effects of processed bovine dentine as compared to Bio-Oss. More long term comparative studies of different durations are needed to develop these materials further as potential substitutes for bone.

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