A Comparative Evaluation of Decalcified Freeze Dried Bone Allograft, Hydroxyapatite and Their Combination in Osseous Defects of the Jaws

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Abstract

Objectives To evaluate decalcified freeze dried allograft or hydroxyapatite and a combination of both as bone autograft substitutes in the healing of osseous jaw defects.

Materials and Methods 24 patients participated in the study which involved the filling of osseous defects in the maxilla/mandible with decalcified freeze dried bone allograft (DFDBA) or hydroxyapatite (HA) or a combined graft composed of these two in equal proportions.

Results Bone formation occurred as early as 4 weeks in the DFDBA and combination groups and 12 weeks in the HA group which was verified by radiographs, Dentascans (DentaScan® Software Program, General Electric, USA) and bone scintigraphy.

Conclusion Both these materials can be used as bone graft substitutes in smaller defects although their suitability in large defects is yet to be studied.

Keywords Bone · Demineralised · Freeze dried · Hydroxyapatite · Bone · Grafts

Introduction

Bone is a dynamic living tissue that shows marked structural alteration in response to injury, changes of stress and vascular, endocrine, genetic and nutritional influences. It is one of the few human organs that can undergo regeneration rather than repair with formation of scar tissue. Bone is a specialized connective tissue that provides support and protection for the delicate and vital organs of the body and also allows for locomotion.

The development of bone graft material to replace bone remains a formidable challenge in maxillofacial surgery. Although autogenous bone is the best material, however, the advantage of an autograft is offset by the limited supply of such bone and morbidity associated with surgery to harvest the graft.

This article aims to study and compare the efficacy of two such bone substitutes in the healing of osseous defects of the jaws.

Materials and Methods

The present study comprised of 24 patients who attended the outpatient department of Oral and Maxillofacial Surgery, U.P. King George University of Dental Sciences, and Lucknow for jaw defect corrections between January and December 2005.

An informed consent of each patient was taken to participate in the study.

And the patients were randomized in three groups:

Group A—where osseous defects were filled with decalcified freeze dried bone matrix allograft (DFDBA).

Group B—where osseous defects were filled with porous HA granules.

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Fig. 1 Preoperative radiograph showing periapical pathology in 22 region

Group C—combination of DFDBA and HA granules in equal proportions was used. The bone graft–alloplast combination was packed tightly in the osseous defect.

The defects of bone were mostly as a result of cyst enucleation and other such benign lesions larger than 1 cm diameter (Fig. 1). Third molar sockets and locally aggressive lesions were excluded.

The allograft material was prepared by the following procedure:

1. *Procurement of healthy bone:* Healthy femur heads, from the Department of Orthopaedic Surgery, King George Medical University, Lucknow and kept in an autoclaved container containing distilled water and stored in the freezer.
2. *Defatting of bone:* Cortico-cancellous bone was then cut by sterilized mallet and chisel into small pieces. The graft material was then immersed in 100% ethyl alcohol for 1 hour to remove fat from the bone marrow.
3. *Decalcification of allograft material [1]:* The bone was then decalcified with 0.6 N HCl to remove the calcium. The graft was then washed in sodium phosphate buffer to remove the residual acid. The demineralised bone was re-frozen for 2 weeks.
4. *Freeze drying of bone:* The cortico-cancellous bone was then pulverized in an autoclaved kitchen mixer especially kept for this purpose, in the presence of liquid nitrogen at a temperature of -196°C and sieved to almost powder form.
5. *Sterilization of allograft material:* Prepared allograft material was stored in an air tight glass container and

Fig. 2 Enucleation of cyst followed by obliteration of the defect with the graft (DFDBA and HA)

sterilized by the γ -radiation of 2.5 Mrad at Central Institute for Medicinal and Aromatic Plants, Lucknow and stored at $2-3^{\circ}\text{C}$ until the allograft was used.

Hydroxyapatite with average pore size 0.8 mm available in ready to use in pre-sterilized (Gamma irradiated) granules/block form used in the study was HAG 3 manufactured by Surgiwear Limited India.

The bone graft/alloplast/combination was packed tightly but not too tightly in the osseous defect. Closure of wound was achieved by interrupted sutures using 3-0 black silk (Fig. 2).

A pressure pack for half an hour was given to achieve haemostasis. Patients were instructed to continue antibiotic, analgesics, vitamins for 5 days. Sutures were removed on the fifth post-operative day.

Clinical evaluation was done for post-operative pain (Visual Analogue Scale), post operative swelling, signs of infection (persistent post-operative swelling, pus discharge, pain) and signs of implant rejection (discharge, extrusion, tissue dehiscence).

In all the post-operative cases intraoral periapical X-rays were taken at immediate post-operative day 1, 4, 12, and 24 week intervals for radiographic evaluation. A measuring grid of 1×1 mm size squares on a transparent sheet was used for measurement of osseous defect by placing the grid over the radiograph. The alveolar bone loss/gain was measured by counting the number of squares overlapping the defect at different follow ups. Squares which were half filled with bone were not counted.

Percentage was calculated using following formula:

$$\frac{\text{Sq. overlapping initial defect} - \text{Sq. overlapping the defects at different follow ups}}{\text{Sq. overlapping initial defect}} \times 100$$

for, evidence of calcification (evident by increased radio-opacity), bone formation (evident by formation of trabecular pattern) and bridging of gap by normal bone was calculated by using the above formula.

Three phase skeletal scintigraphy scan was done under the gamma camera after intravenous infusion of ^{99m}Tc MDP 4 weeks postoperatively.

Dentascan assessment was done between 4 and 6 weeks postoperatively to assess new bone formation. Dentascan is a specialized type of computed tomography study which is performed on a conventional CT scanner used for imaging all parts of the body. It uses thin 1 mm axial CT images of the maxilla and mandible to reformat a series of multiple cross-sectional and panoramic images of the jaw [2, 3].

None of the patients agreed for histological examination during post operative follow up, hence, it was not included in the study.

Statistical Analysis

The following have been used in the present study to analyse the data obtained.

1. Mean
2. Standard deviation
3. Student t test
4. Analysis of variance
5. *Chi square* (χ^2) ($P < 0.05$ least significance, $P < 0.01$ significant, $P < 0.001$ highly significant.)

Results

Analysis of the three groups did not reveal any statistically significant differences at any of the follow up periods except for swelling and radiological evidence at four weeks [4].

Swelling was seen only in group C at fourth week follow up. Also, there was moderate pain and pus discharge with extrusion of the graft and tissue dehiscence. This difference was statistically significant as compared to group A and B.

No signs of infection (persistent post operative swelling, pain or pus discharge) were seen in any patient of group A and B. In group C, however, all these three signs of infection were seen in 2 (25%) patients at fourth week.

No signs of implant rejection (discharge, extrusion, tissue dehiscence) were seen in any patient of group A and B. In group C, however, all these three signs of implant rejection were seen in 2 (25%) patients at fourth week. The graft material was surgically removed at fourth week.

Radiological evidence of calcification, bone formation and bridging of the gap with new bone as evident by formation of irregular trabeculae of bone and appearance of radio-opaque areas in the defect was evident from fourth

week onwards. It was seen in 62.5% cases of group A, in no case of group B and 50% cases of group C; by this time two grafts were rejected in group C. The difference between group A and B was statistically significant ($P < 0.01$) at this point of time.

At 12 week follow up 62.5% cases each of group A and B showed radiological evidence of calcification, bone formation and bridging of the gap while 66.7% cases of group C showed radiological evidence of calcification, bone formation and bridging of the gap. At 24 weeks follow up interval 75% cases of group A and B and 100% of group C showed radiological evidence of bone formation (Fig. 3).

The Dentascan and Bone Scintigraphic assessments although performed in only a few representative cases after 4 weeks of graft placement supported and confirmed the radiographic findings (Figs. 4, 5).

Fig. 3 24 weeks postoperative radiograph showing healed osseous defect with residual hydroxyapatite granules

Fig. 4 Dentascan done 4 weeks postoperatively showing no evidence of bone formation and radioopaque HA granules

Fig. 5 Scintigraphy done at 4 weeks after surgery showing increased uptake of ^{99m}Tc at surgical site representing new bone formation at grafted site

Discussion

The allograft, DFDBA and the alloplastic material, HA were tried in this study due to their osteoinductive and osteoconductive properties respectively and their availability in sufficient quantities as and when needed. There have been numerous encouraging results with both these bone substitutes.

Since both these materials were found to be useful, it was thought to combine them together and to assess their collective effectivity in bone formation and to see whether these collectively offered any additional advantage as opposed to their singular use.

The DFDBA was used because the very processes involved in its preparation i.e. decalcification exposes on its surface, the bone morphogenetic proteins (BMPs) which are osteoinductive [5–11] that is, they induce differentiation of mesenchymal cells into cartilage and bone [12]. BMPs are natural proteins which play important roles during embryogenesis and mediate in specific aspects of skeletal growth and development during later adult life [13]. BMPs have been extracted from bone, dentin, and osteosarcoma tissue [12].

Thus, in group A, the bone formation ensued immediately after filling DFDBA because of the BMPs which were exposed during the decalcification process.

Also, the freeze drying at -196°C destroys the antigenicity of the DFDBA [14–17].

Porous HA is an abundantly available non toxic material [18] that is bioactive and allows new bone to be formed directly on its surface without any intervening layer of fibrous tissue [19].

Synthetic HA possesses a similar composition to HA crystals present in bone, enamel and dentin and exhibits

osteoconduction by acting as a scaffold for new bone to grow through the implant material as long as there is enough vital host bone surrounding it [20].

HA, when implanted does not evoke an inflammatory or foreign body response [9, 21, 22] and has a good tissue tolerance [23].

Thus, in group B, the initiation of bone formation is different i.e. by osteoconduction alone as the HA granules used merely act as a trellis for blood vessels and migration of osteoblasts from surrounding healthy bone, a process called “creeping substitution”, whereby the HA is slowly resorbed and replaced by bone.

HA when used with DFDBA as opposed to their single use also showed good results as a synergistic effect of osteoinduction and osteoconduction was observed [24–26].

The cause of failure in 2 patients of group C who showed signs of infection along with extrusion of the grafts and wound dehiscence in the fourth week was therefore thought to be possible because of the presence of a sub-clinical infection.

The response in group C was thus intermediate between groups A and B in terms of timing of bone formation, calcification and bridging of gap.

The study attempted to use CT (Dentascanner) as a monitor of bone remodeling and is in accordance with many reports on the use of CT for subtle bone changes in the jaws. There was however, no evidence of bone formation at 4 weeks in the few CT scans that were taken.

The Scintigraphic assessment with ^{99m}Tc -MDP was done in four patients between 2 and 4 months post-operatively. The perfusion, blood pool and delayed images were obtained in all patients. The delayed images revealed increased tracer uptake indicating a well perfused graft with no evidence of its necrosis. The sites showed increased osteoblastic activity as a sign of reparative process [27].

Conclusions

DFDBA when used for the obliteration of osseous defects bypasses the phase of obligatory resorption and shows early evidence of new bone formation. HA when used for obliteration of osseous defects shows (a) delayed first evidence of bone formation as compared to the decalcified freeze dried bone matrix allograft and (b) it undergoes resorption but takes a long period to resorb completely and be replaced by bone. The radiograph assessment score over time frame indicates early bone formation with DFDBA and the combination of DFDBA and HA than HA alone. Dentascanner offers three-dimensional visualization of the jaw bones. In cases where we did Dentascanner, no evidence of bone formation was seen although in one case appearance

of mild amount of trabeculae formation was seen, but a statistical conclusion could not be drawn because of the small sample size and high cost of the investigation. Bone Scintigraphy assessment results showed increased osteoblastic activity and viability of the graft, however, both Dentascan and Bone Scintigraphy are very expensive investigative tools and not affordable by most patients who seek treatment at our institution. More recent and newer investigative tools were not used due to their unavailability at my institution.

A prospective randomized controlled trial with a large sample size and over a long period, is however, warranted to validate these observations more conclusively.

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