Original Article
Radiographical and clinical evaluation of critical size defects in rabbit calvaria filled with allograft and autograft: a pilot study

Gonzalo H Oporto V1, Ramón Fuentes2, Eduardo Borie2,3, Mariano del Sol4,6, Iara Augusta Orsi3, Wilfried Engelke5

1Dental School, Universidad de La Frontera, Temuco, Chile; 2CIMOFIR Research Centre, Dental School, Universidad de La Frontera, Temuco, Chile; 3Department of Dental Materials and Prosthodontics, Dental School of Ribeirão Preto, University of São Paulo, Ribeirão Preto, SP, Brazil; 4Department of Basic Sciences, Medicine School, Universidad de La Frontera, Temuco, Chile; 5Dentistry Centre, Department of Maxillofacial Surgery, University of Göttlingen, Göttlingen, Germany; 6Research Centre in Biomedical Sciences, Universidad Autónoma de Chile, Chile

Received May 16, 2014; Accepted June 23, 2014; Epub July 15, 2014; Published July 30, 2014

Abstract: Regeneration of resorbed edentulous sites can be induced by bone grafts from the subject himself and/or by the use of biomaterials. At present, there has been an extensive search for biomaterials that are evaluated by artificially creating one or more critical defects. The aim of this work was to clinically and radiographically analyze bone formation by the use of some biomaterials in artificially created defects in the parietal bone of rabbits. Six rabbits were used, creating defects of 8 mm in diameter in parietal bones. One defect was maintained with coagulum only, and in others, freeze-dried bone allograft (FDBA), autologous bone, and a combination of autologous bone with FDBA respectively, were added. Animals were sacrificed at 15-90 days with 2 weeks interval each, and calvaria were analyzed macroscopically, measuring by digital caliper the lack of filling at the surface of defects, identifying limits at anteroposterior and coronal view, realizing a digital photograph register of their external surfaces. This was subsequently evaluated radiographically by occlusal film radiography used to quantify its density through software. In conclusion, autologous bone showed the best behavior, clinically as well as radiographically. However, FDBA is a good option as an alternative to autologous bone as its behavior was slightly lower over time. The combination of autologous bone and FDBA in the same defect showed results considerably inferior to grafts used separately. Low radiopacity and clear limits were observed through time for the control coagulum filled defect.

Keywords: Bone regeneration, rabbits, autografting, radiography

Introduction

Osseointegrated implants are considered the ideal replacement for tooth loss [1]; however, the success of an implant is related to the bone resorption in edentulous sites [2]. It is very important to note that bone is a living tissue that constantly is being remodeled [3]. In addition, the rate of bone healing is slower than in other tissues; the use of biomaterials such as bone grafts that help the bone healing process is sometimes required [4].

Nowadays, there has been significant progress in techniques of bone grafting and bone substitute materials used to fill bone defects adjacent to dental implants [5, 6]. The current paradigm has changed from bioinert to bioactive materials with control of reaction and action in physiological environment [3]. In this context, allografts as FDBA (freeze-dried bone allograft) have been widely used in bone-healing processes and filling bone defects in the clinical field [7, 8].

The preparation of these allografts, such as FDBA, includes: removing most of tissue fluids by freeze-drying processes, vacuum packaging, and maintenance at room temperature to destroy osteogenic cells, keeping only a limited osteoinductive ability. The unavoidable bone protein denaturation has been considered an additional factor retarding revascularization of the graft. Also, the low water content inhibits
Calvaria defects with allografts and autografts

the action of microorganisms and enzymes that might degrade it [9]. Usually, the process to which they are subjected removes bone allograft immunity, without affecting the biochemical and structural bone properties. Despite this, the incorporation of the allograft is slow [7]. Otherwise, the risks of complications associated with harvesting autografts are avoidable because, according to a study by Lohmann et al. [10], the cost of autograft harvest for the user is similar to that of FDBA. Ideally, the role of allografts such as FDBA in bone reconstructive procedures is not just restricted to replacing the compromised bone area, but it must also stimulate the osteoconductive properties acting as scaffold for ideal bone healing. It is important to point out that these materials are required to comply with the demands of biocompatibility with null collateral effects, such as leukocyte infiltration and fibrosis [11]. Furthermore, despite the intense research and development associated with the use of non-autogenous biomaterials, they have still failed to reproduce the same features of the patient’s own bone, until now considered as the “gold standard” in these type of procedures [12], because they are immunologically inert, osteoinductive, and osteogenic [13]. Pansegrau et al. [14] showed better behavior for autografts than allografts as FDBA. Moreover, some authors recommend the use of allografts only when autografts are unavailable from the affected site [11, 15]. Therefore, the aim of this study was to compare, throughout a period of time, the macroscopic and radiographic behavior of the FDBA, autologous bone, and a mixture of both grafts in bone defects created in rabbit parietal bone.

**Material and methods**

Ethics Committee from Universidad de La Frontera previously approved the study.

Six rabbits (*Oryctolagus cuniculus*) were selected from the Biotherium of the Department of Basic Sciences, Faculty of Medicine, Universidad de La Frontera, Chile. To minimize the growth effect, only adult animals (7 months old, with a weight of $2.90 \pm 0.20$ kg) were used in this study. The care and management of the animals was realized according to ethical standards of the “Guide for Control for Research Animals” and the surgery took place in a sterile environment.

The rabbits were premedicated 1 hour prior to surgery with an oral dose in drops of amoxicillin 50 mg/kg. For sedation of the animals, ketamine 15 mg/kg i. m. supplemented with diazepam 1 mg/kg i. m. was used, to maintain levels of neuroleptanalgesia, with hypnorm 0.1 mg/kg i. m. at intervals of 30 minutes during the surgery. In addition, as a local anesthetic, 0.4 ml of lidocaine 2% was applied with a dose of 1:100,000 of epinephrine (Octocaine-100, Novocol Pharmaceutical, Ontario, Canada).

A median lineal incision was made from the frontal to the occipital region separating the skin laterally. The periosteum was elevated 5 cm in each animal and subsequently four bicortical defects were created, two in each parietal bone, fully bicortical, with an 8 mm internal diameter trephine bur. The block was removed according to the technique reported by Lu and Rabie [16]. As soon as it was extracted, the bone of the defects was particled in a bone mill with particle sizes between 350 and 500 μm to be subsequently used as autograft [17].

The defects created were randomly filled with (Figure 1): (1) Coagulum; (2) Particled autologous bone; (3) Human bone from bone tissue bank (FDBA, Community Tissue Services, Dayton, USA); (4) A mix of human bone of tissue

![Figure 1](image-url). Upper view of filled defects before suturing.
Calvaria defects with allografts and autografts

The wounds were closed with absorbable sutures type Vicryl® (Ethicon, Novartis Animal Health US, Inc., Greensboro, North Carolina, USA) 3-0 discontinued.

Following surgery, the rabbits were medicated for ten days with one daily oral dose of amoxicillin 50 mg/kg and were maintained with _ad libitum_ feeding in closed cages and with controlled temperature.

The animals were sacrificed with overdose of sodium pentobarbital i. m. (60 mg/kg in weight) once 2, 4, 6, 8, 10, and 12 weeks were met following surgery, the defect extracted immediately by slices with safety margin.

After removal of samples, the presence of resorption was assessed of each biomaterial used to repair the defects created. For the above, the lack of filling defects on the surface was measured with a digital caliper of 0-150 mm (Mitutoyo, Illinois, U.S.A.) identifying their limits in anteroposterior and coronal ways. Also, a record of digital photography of the external face of the calvaria was realized.

Subsequently, calvaria radiography was taken with an occlusal film in the radiology unit of the Universidad de La Frontera, with the aim of evaluating the evolution of bone healing throughout time. The radiographs were taken with an extraoral equipment (Planmeca Intra, Planmeca U.S.A. INC, Roselle, Illinois, U.S.A.) at a distance of 15 cm from the plate, perpendicular to the same (90°), with 63 kV, 8 mA and 0.64 seconds of exposure with a piece of 1 cm square plumb. The radiographs were developed in an automatic developer Durr Dental XR 24 (Bietigheim, Germany).

Finally, the occlusal radiographs were digitalized with a Nikon D-50 camera with a 90 mm Tamron lens to be analyzed later with APFill Ink Coverage Meter v.5.2 software, identifying the density of each zone of the film. The average value of gray scale used was 130 (acceptable values between 296 and 116, according to Efeoglu et al. [18]).

**Results**

**Macroscopic results**

Regarding macroscopic characteristics, identifying their limits in anteroposterior and coronal ways, the defect filled with FDBA presented the best consolidation throughout time because...
Calvaria defects with allografts and autografts

**Discussion**

Results obtained from the macroscopic measure of the defects lengths in rabbit calvaria, in the anteroposterior as well as crown aspects, indicated that autologous bone grafts and FDBA separately showed an improved macroscopic progress through time (Figure 2). After 60 days, clinical observation of a complete consolidation was noted up to 90 days (Figure 3); there upon, a value of measure zero was assigned. Previously, results agree well with information reported by some authors [19, 20], who indicated that the best graft also considered the “gold standard” are the autografts. This statement pertains to the non-immunogenic capacity and histocompatibility of autologous bone, in addition to providing the three elements to generate and maintain the bone: osteoconduction graft, growth factors for osteoinduction, and osteogenesis progenitor cells [21, 22]. It is noteworthy that its main disadvantage lies in the need of a donor site, increasing the morbidity of the procedure [4]. Nevertheless, FDBA has been reported by some authors as a successful biomaterial in bone regeneration of critical cranial defects in rabbits [23]. Nishida and Shimamura [24] reported that 61.5% allografts are efficient and that they fail in the union or do not join in approximately 11% of cases. Other authors mention that allografts such as FDBA can present a minor immunogenic reaction [25], in some cases, which could translate into graft failure, which did not occur during this study. It should be noted that deficiencies of FDBA are directly associated with the 25%-35% frequency of allograft failures as a result of a lack of union or fracture [26].

The combination of autologous bone grafts with FDBA only consolidated at 90 days could lead to the assumption that, when in a state of particles, the combination of both grafts slows the consolidation process.

Furthermore, defects that were only maintained with coagulum presented lesser macroscopic progress in reference to the centripetal bone formation, inasmuch that well-defined net limits of the defects used as control with length values close to those initially developed were
always observed throughout the time period. Additionally, coagulum-filled defects were the only ones that did not present firm consistency, rather they presented as fibrous or flaccid in accordance with some reports [27].

Radiographically, we were able to observe defects with autologous grafts presenting greater radiopacity in time (Figures 4 and 5). This finding agrees with some reports [12, 14], which mentioned that autologous bone presents greater bone regeneration when compared with allografts. Radiographic behavior of autologous bone could be related with a great amount of graft resorption during the first 30 days, which coincides with Clune et al. [28]. Subsequent to 30 days, an almost constant radiopacity can be observed, possibly due to bone matrix apposition and mineralization. Initial size of the particles of autologous graft could influence the rate of initial bone resorption along with osteoconduction potential, a situation positively indicated by Malinin et al. [29]. Grafts with FDBA material presented very low radiopacity during the first 45 days; subsequent to that time, grafts were observed to present greater density, achieving nearly 85% radiopacity. The previous information could be related to that stated by Tang et al. [7], who mentioned that consolidation of allograft is slow. Furthermore, in some studies, fibrous connective tissue has been found around the FDBA [23]. It is noteworthy that allografts may not have the same osteoinductive characteristics as autografts, depending on the method by which they are processed [22, 26]. FDBA behavior can be the result of immature bone modeling during the first 2 weeks, subsequently increasing bone volume, in accordance with results mentioned by Efeoglu et al. [18], who worked on bone defects with β-tricalcium phosphate and PRP (plasma rich in platelets). Pekkan et al. [30] indicate that radiopacity of bone biomaterial is reduced when used in the mandibular cortical bone. Something similar could occur with the calvaria, a bicortical bone, with its radiopacity affected in comparison to the autologous bone. Defects, in which grafts were a combination of autologous bone with FDBA, showed a lesser radiopacity than both grafts individually, achieving 50% radiopacity and maximum limit at 90 days; it is worthwhile noting that the combination of both grafts showed greater radiopacity compared with coagulum used as control. In the case of coagulum-filled defect, the least radiopacity was observed over time, which again is in accordance with the macroscopically located fibrosis.

As previously observed, results obtained during clinical evaluation and radiographic evaluation are relatively similar, presenting autologous bone as the first option in the use of grafts in bone defect, coinciding with some authors [14, 31]. Essentially, good behavior was appreciated for the combination of autologous bone with FDBA, although considerably lesser than for FDBA and autologous bone grafts separately. In reference to the differences between macroscopic and radiographic results for FDBA grafts, this could be related to the osteoinductive capacity of the periosteum in direct contact with the side surface of the defect, which would
encourage greater bone formation from external cortical to the interior of the defect, observing a macroscopically better behavior than in radiographs, the latter constituting only a bidimensional image where reduced density is observed. In the event that only osteoinduction properties of biomaterials are evaluated, making necessary the use of nonabsorbable membrane separating the periosteum from direct bone contact, in order to avoid its function. However, from a clinical point of view, it is advisable to maintain direct contact with the periosteum to favor bone regeneration of the defect through osteoinductive properties along with osteoconductive properties of biomaterials.

Furthermore, it can be ascertained that, in some cases, there are macroscopic or clinical observations that may not concur with radiographic findings, in which case a histological analysis is essential. Finally, of the most important steps in the development and validation of biomaterials is the completion of a rigorous experimental trial to determine and confirm biocompatibility. It should be noted that this is a pilot study; therefore, future research is necessary with a greater number of subjects to obtain statistically significant values with the opportunity to evaluate the degree of consensus in clinical and radiological exams in the application of different biomaterials as bone substitutes in bone defects as well as an evaluation of the effectiveness of each.

In conclusion, autologous bone showed the best behavior, clinically as well as radiographically; however, FDBA is a good option as an alternative to autologous bone since its behavior was only slightly inferior over time. The combination of autologous bone and FDBA in the same defect showed results considerably inferior to grafts used separately. In the control coagulum-filled defect, reduced radiopacity and clear limits were observed over time.

**Acknowledgements**

We acknowledge the financial support by DIU-FRO project (DI11-0069).

**Disclosure of conflict of interest**

None.

**Address correspondence to:** Dr. Gonzalo H Oporto V, Dental School, Universidad de La Frontera, Manuel Montt 112, Temuco, Chile. E-mail: gonzalo.oporto@ufrontera.cl

**References**


Calvaria defects with allografts and autografts


