

Histological Evaluation of Bone Regeneration Means Freeze Dried Bone Allograft (FDBA) in Post Exodontia Sockets

Evaluación Histológica de la Regeneración Ósea en Alvéolos
Post Exodoncia Mediante Uso de Freeze Dried Bone Allograft (FDBA)

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FUENTES, R.; OPORTO, V. G.; OLATE, S.; ROJAS, R. M.; BORIE, E. & ENGELKE, W. Histological evaluation of bone regeneration means freeze dried bone allograft (FDBA) in post exodontia sockets. *Int. J. Morphol.*, 29(1):286-292, 2011.

SUMMARY: Within oral rehabilitation alveolar ridge preservation following extraction is important. This research study shows a histological, histochemical and histomorphometrical evaluation in two cases of post extraction ridge-socket preservation performed with FDBA. In two patients dental extraction procedures were performed and sockets were immediately filled with FDBA. Six months later a biopsy of grafted area was obtained and rehabilitated through dental implant. Grafted bone samples were treated for histological and histochemical analysis. Bone tissue area was measured. Laboratory analysis of three samples showed inactive bone surfaces, neither osteoblasts nor osteoclasts were found, only osteocyte and osteogenous cells were observed. These findings do not mean that tissue is metabolically inactive, rather bone genesis develop from a tissue matrix with the potential to generate undifferentiated osteocytes, and a micro environment with proteins such as bone morphogenetic proteins (BPM). Inactive biomaterial particles were not observed. Samples showed 0% and 30% bone tissue respectively. Considering histological differences between this and other research studies, it is necessary to develop further investigation to increase knowledge of processes involved in bone regeneration as well as bone quality, considering the variability that could be seen in each patient.

KEY WORDS: Bone regeneration; Biomaterials; FDBA; Socket.

INTRODUCTION

It is possible to achieve bone regeneration and alveolar ridge increase by using autografts and biomaterials (Oporto *et al.*, 2008). Autogenous grafts (AG) have presented high rates of success (Chapasco *et al.*, 2008; Smolka *et al.*, 2006; Lizuka *et al.*, 2004; Cordaro *et al.*, 2002; Antoun *et al.*, 2001), with survival of dental implants very close to findings of nongrafted sites (Tonetti & Hammerle 2008). Despite AG being considered the gold pattern it always carries along an increased morbidity and limitation of the availability; in consequence development and use of biomaterials are a good alternative to be used in bone augmentation techniques (Nevins *et al.*, 2009; Kolerman *et al.*, 2008; Fagan *et al.*, 2008; Cenni *et al.*, 2009; Nevins *et al.*, 2007; Turonis *et al.*, 2006; Dallari *et al.*, 2006).

Clinical and histological research aimed at the study of bone augmentation still presents doubts in terms of

recognizing which biomaterial presents the best bone integration and best clinical performance. Histological studies in humans focus mainly on maxillary sinus lift (Choukroun *et al.*, 2006; Cammack *et al.*, 2005; Yukna & Vastardis, 2005; Kassolis & Reynolds, 2005; Lasella *et al.*, 2003; Markopoulou *et al.*, 2003), lateral or vertical increase of the alveolar ridge and bone regeneration by resection of residual cysts (Friedmann *et al.*, 2009; Galindo-Moreno *et al.*, 2008; Schwartz *et al.*, 2007; Suga *et al.*, 2006; Zerbo *et al.*, 2001).

Although some research points out that in post exodontia socket, bone regeneration improves with the application of biomaterials (Lasella *et al.*), there is an interest in certain research groups to study this area (Chiapasco *et al.*, 2006; Tonetti & Hammerle; Donos *et al.*, 2008), this in view of current results which are not necessarily a predictor

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of success. (Chiapasco *et al.*, 2008;. Cordaro *et al.*, 2008; Rochietta *et al.*, 2008; Antoun *et al.*). In view of the above it is interesting to identify histological results in post exodontia sockets treated with commercially available biomaterials.

The aim of this research is to realize a histological, histochemical and histomorphometric analysis of two patients where Freeze Dried Bone Allograft (FDBA) was applied in their post exodontia sockets.

MATERIAL AND METHOD

Patients and surgical procedures. Two male patients 35 and 40 years of age were treated by the Oral Implantology Division of the Universidad de La Frontera during 2008. Both patients required rehabilitation of pieces 1.1 and 2.1 with a unitary crown installed over osseous integrated implant. In both patients teeth had received endodontic treatment and extensive restoration making further dental treatment impossible. Surgical sites presented inadequate alveolar ridge, absence of inflammatory processes and/or active infection. Bone reconstruction through the use of biomaterials and subsequent rehabilitation through assisted implant prosthesis unit (AIP) was indicated.

With administration of local anesthesia (2% lidocaine with epinephrine 1:200.000), the first surgery consisted in the extraction of two pieces with high rotation drills in order to segment the radicular remnant and realize the exodontia in accordance with conventional techniques; subsequently FDBA cortical particle bone of 0.125 AND 0.850 mm was applied within the alveolar ridge (Community Tissue Service, a Department of Community Blood Center) with alveolar curette. Suturing was realized with 3-0 silk thread, simple suture without the use of membrane or any other type of protective element.

Six months later the second surgery was performed. Upon administration of local anesthesia (2% lidocaine with epinephrine 1:100.000) mucoperiosteal flap was realized starting from lineal incision; bone material was obtained in the first stage by using a 2 mm internal diameter trephine, perforation was made in the center of the alveolar ridge previously grafted with FDBA. The specimen was submerged in buffered formalin at 10% and subsequently processed in the Comparative Embryology Laboratory of the Faculty of Medicine of the Universidad de Chile, through routine histological techniques 72 hours after collection. In a second surgical stage the site prepared with trephine was extended with surgical drills achieving a site

for implant of 11 mm in length and 3.75 mm in diameter (internal hexagon) with a primary stability of 45 Ncm.

Histological and Histomorphometric study. Samples of bone tissue were decalcified and set to realize longitudinal 7µm cuts using microtome (Micron®). Subsequent staining with Hematoxylin-eosin and alcian blue (trichromic technique) (HEA), Masson (M), Von Kossa reaction (VK) and Picrosirius Junqueira method staining (PJ). Preparations were evaluated using optic microscope (Zeiss®) with magnification of 50X and 100X, using Axiovision® computer software to identify bone structure (quality and quantity) vascular structure and inflammatory infiltrate. The histological and histomorphometric study was carried out in all images obtained.

RESULTS

Figure 1 (Figs. 1A, 1B, 1C and 1D) show histological images in case 1. Figure 1A (HEA) presents one tissue with extracellular acidophilus matrix and osteocytes included along with dense fibrillar tissue. In the bone trabeculae neither osteoblasts nor osteoclasts were observed in the endosteum, however some osteogenic cells were observed. In Figure 1 B (M) blood vessels were observed, probably neoformed, as well as transition between dense fibrillar tissue and bone matrix. Figure 1 C (PJ) collagen type I was identified noted red in color, in view of the use of polarization microscope, in mosaic and with reduced longitude; collagen type III was not observed. Figure 1 D (VK) clearly indicates that the bone structure is not mineralized.

Figure 2 shows histological images for case 2. (Figs. 2A, 2B, 2C, 2D) Figure 2A (HEA) presents trabecular bone tissue with osteocytes in the matrix and few osteogenic cells in the endosteum. Neither osteoblasts nor osteoclasts were observed. With trichromic Masson stain, case 2 (Fig. 2B) exhibits two differences with case 1 (Fig. 1B). Trabeculae have mineralized, evidenced by the black color of the Von Kossa technique (Fig. 2 D), Haversian canals are in formation and connective tissue has changed from dense to lax in some segments.

Using Picrosirius Junqueira method stain (Fig. 2C) both cases present similar results observing collagen type I only. With the Von Kossa technique in Case 2 (Fig. 2D) trabeculae have mineralized and is evidenced by black color.

Histomorphometry of points carried out in the von Kossa method stain for evaluation of the bone tissue present

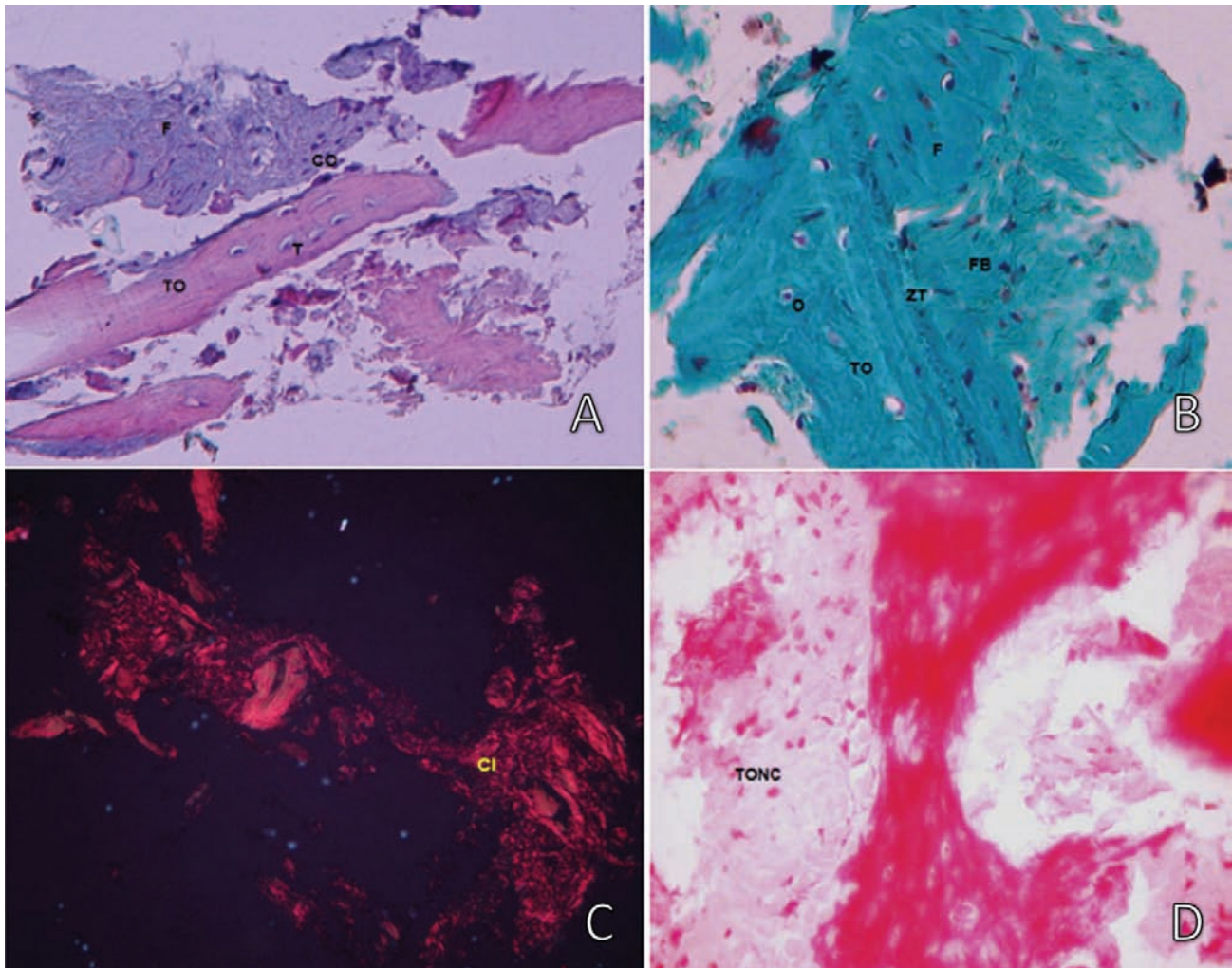


Fig. 1. Corresponds to case 1. A.- (HEA) Bone Tissue (BT), Plain osteogenic cells (OC), Bone Trabeculae (T). B.- (M) Transition area between dense fibrillar tissue and bone matrix (TA.), Osteocytes (O), Bone Tissue (BT), Fibroblasts (FB), Dense fibrillar tissue (F). C.- (PJ) Type I collagen fibers (CI). Fig 1D (VK) Noncalcified bone tissue (NCBT).

in the samples, determining a complete absence of mineralized tissue in case 1 and 30% mineralized tissue in case 2 (Figs. 1D and 2D).

DISCUSSION

The use of biomaterials has been evaluated in various regenerative techniques (Friedmann *et al.*; Feuille *et al.*, 2003; Herold *et al.*, 2002; Rosen & Reynolds, 2002; 2001; Danesh-Meyer *et al.*, 2001; Laurell *et al.*, 1998), with limited research on the use of FDDBA in post extraction alveolar ridges.

Neither osteoblasts nor osteoclasts were found in either sample, although osteocytes and osteogenic cells

were observed. However, the fact of the sample presenting inactive osteogenic cells and that neither osteoblasts or osteoclasts were observed, does not mean that in this state of quiescence (metabolically inactive), rather that the bone genesis begins from the matrix of the tissue where cells with the potential or forming undifferentiated osteocytes and a micro environment with proteins of the growth factor family transforming beta as a morphogenetic protein of the bone, with participation of growth cells and factors from the fibrillar matrix (similar to that observed in the bone formation process during embryonic development) and not from osteoblasts. These finding differ from what has been presented as ideal bone regeneration processes, where according to advancement of osteogenesis (in bone regeneration induced by biomaterials), the matrix of fibrillar tissue regresses; formation of bone type I is completed by

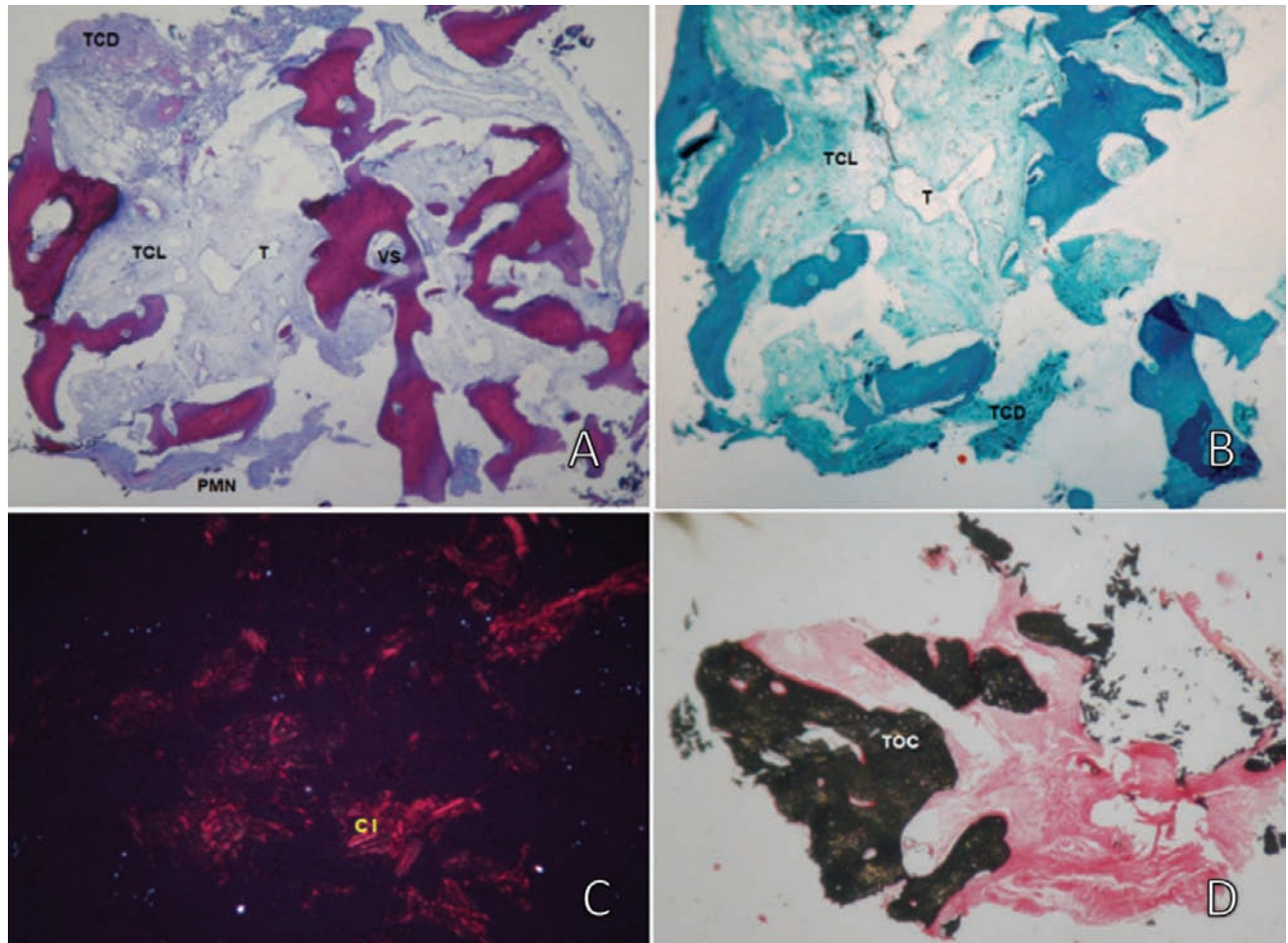


Fig. 2. Corresponds to case 2. A.- (HEA) Loose Connective Tissue (LCT), Dense Connective Tissue (DCT), Blood Vessels (BV), Polimorphonuclear Cells (PMC), Loose Matrix (LM). B.- (M) Bone Trabeculae (T), Loose Connective Tissue (LCT), Dense Connective Tissue (DCT). C.- (VK) Type I collagen fibers (CI). Fig. 1D (PJ) Calcified bone tissue (CBT).

the third or fourth week, and begins the phase of progressive substitution toward type II bone, where osteoclasts and osteoblasts appear (Anitua & Andia, 2000). At the same time, it also differs with the results presented by other research (Galindo-Moreno *et al.*; Suba *et al.*; Friedmann *et al.*; Gotz *et al.*, 2008; Lee *et al.*, 2008; Norton *et al.*, 2003, who have found that bone genesis produces starting with osteoblasts.

In case I non-mineralized bone trabeculae with osteocytes, dense fibrillar tissue and a transition between dense fibrillar tissue and bone matrix was observed. In case II, 30% mineralized bone was found, the latter in accordance with parameters published in other research developed with biomaterial and methodologies different than those used in this research (Artzi *et al.*, 2007); Norton *et al.*; Schwartz *et al.*; Galindo-Moreno *et al.*; Suba *et al.*; Friedmann *et al.*). Persistence of fibrillar tissue was observed in different amounts following six months after

the application of biomaterial, although no remaining intact biomaterial was observed in any of the biopsies. Furthermore, preservation of fibrous tissue areas for more than 4 weeks in a situation observed by other researchers using biomaterials (Lee *et al.*).

In view of results of this research, it is necessary to continue research associated to the relation between biomaterial and bone response in humans.

FUENTES, R.; OPORTO, V. G.; OLATE, S.; ROJAS, R. M.; BORIE, E. & ENGELKE, W. Evaluación histológica de regeneración ósea en alvéolos post exodoncia de humanos mediante uso de freeze dried bone allograft (FDBA). *Int. J. Morphol.*, 29(1):286-292, 2011.

RESUMEN: Dentro de la rehabilitación oral, es importante preservar el reborde alveolar post exodoncia. Se expone un análisis histológico, histoquímico e histomorfométrico de dos ca-

tos clínicos de terapias de regeneración ósea de alvéolos post extracción mediante FDBA. En dos pacientes se extrajeron piezas dentarias destruidas y se indujo regeneración ósea mediante FDBA. Seis meses después, se obtuvo una biopsia del injerto y mediante un implante de titanio fue rehabilitado. Para el análisis Histológico e Histomorfométrico, las muestras fueron tratadas con las técnicas Hematoxilina-Eosina, Azul de Alcian, Masson, Von Kossa y colorante Picrosirius de Junqueira. Se midió el área total de tejido, así como el área de tejido óseo. Las superficies de hueso de las muestras se observaron inactivas, no fueron encontrados osteoblastos ni osteoclastos, sólo osteocitos y células osteógenas, lo que no significa que el hueso esté en estado quiescente, sino más bien a que su génesis ocurre a partir de la matriz de tejido donde se encuentran células con potencialidad de formar osteocitos indiferenciados y un microambiente con proteínas de la familia de factor de crecimiento transformante beta. No fueron encontradas partículas de biomaterial inactivo. En las muestras se cuantificó 0% y 30% de hueso mineralizado. Dadas las diferencias histológicas encontradas con otros estudios, es necesario profundizar el conocimiento en los procesos involucrados en la regeneración ósea dependiendo del biomaterial utilizado, y la calidad ósea resultante en cada procedimiento en particular sin perder de vista la variabilidad que puede presentarse dependiendo de cada caso clínico.

PALABRAS CLAVE: Regeneración ósea; Biomateriales; FDBA; Alvéolo.

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Recibido : 17-08-2010

Aceptado: 19-11-2010