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)


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 (Nevis 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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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 alcin 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 Axiosvision® 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 endosteam, however some osteogenetic 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 endosteam. 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...
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 FDBA 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...
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 (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 view of results of this research, it is necessary to continue research associated to the relation between biomaterial and bone response in humans.

Resumen: Dentro de la rehabilitación oral, es importante preservar el reborde alveolar post exodoncia. Se expone un análisis histológico, histoquímico e histomorfométrico de dos casos.
los 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 coloreante Picosirius 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 osteogénicas, lo que no significa que el hueso esté en estado quiescente, sino mas 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 plasma rico en factores de crecimiento (P.R. F.).

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

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