Characterization of Bone Substitute β-TCP Block for Maxillofacial Reconstruction

Caracterización del Bloque β-TCP de Sustitución Ósea para la reconstrucción Maxilofacial

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SUMMARY: The aim of this study was to characterize a commercial beta tricalcium phosphate (β-TCP) block allograft for use in maxillofacial reconstruction, evaluating its homogeneity, porosity and mineralization. Two commercial 5 x 5 x10 mm chronOS Vivify β-TCP blocks were used, which were evaluated by a STEM SU-3500 variable pressure scanning electron microscope (SEM-STEM) (Hitachi, Japan). For the semi-quantitative microanalysis of elements, the QUANTAX 100 energy dispersive x-ray spectrometer detector (EDX) (Bruker, Germany) was used. The homogeneity of the structural morphology, macropore and micropore size and component homogeneity were evaluated. The microscopic analysis showed micropores of 164.92 mm (± 35.032 mm) in diameter in the outer area and micropores in the inner area of 54.44 mm (± 17.676 mm). The formation of porosities and irregularities present in the block was heterogeneous between the outer and inner surfaces. The mineral content of the blocks presented homogeneity with the presence of carbon (2.02 %), oxygen (44.33 %), phosphate (16.62 %) and calcium (37.87 %). The β-TCP block can be used in bone reconstruction but the presence of reduced macropore and micropore sizes could limit efficiency in the substitution and bone regeneration phase.

KEY WORDS: b-TCP; Scaffold; Craniofacial reconstruction; Bone graft.

INTRODUCTION

The craniomaxillofacial defects associated with tumors, trauma and their sequelae are frequent and present a variety of conditions; bone reconstruction is needed and must respond to esthetics and function, which determines a technically demanding process (Sándor *et al.*, 2012; Fariña *et al.*, 2016). The four essential properties in bone reconstruction - osteoconduction, osteoinduction, osteogenesis and osteopromotion (Ripamonti *et al.*, 2001) - are related to autogenous grafts, the gold standard in reconstruction. They bring together the best properties; however, the morbidity of the donor site is their main disadvantage (Pogrel *et al.*, 1997).

Allografts and xenografts are subjected to various dehydration, sterilization and lyophilization treatments to reduce antigenicity reactions (Molly *et al.*, 2008). But these lack osteogenic and osteopromoter properties, their osteinductive property has not been confirmed and they only present osteoconductive capacity (Busenlechner *et al.*, 2008); their main disadvantage is related to the possibility of causing immunogenicity reactions and the transfer of diseases from the donor species (Eid *et al.*, 2001).

Alloplastic grafts are totally synthetic substitutes, do not transmit diseases and have osteoconductive properties, such as hydroxyapatite, bioactive glass, calcium carbonate or tricalcium phosphate (LeGeros, 2002). These substitutes have become an accepted alternative in the reconstruction of small defects due to their ease of use and versatility. Their development and characterization continue to be relevant (Martinez *et al.*, 2015), so that biocompatibility and time spent in the host are two decisive factors in their choice (Jensen *et al.*, 2009; Araújo *et al.*, 2011). In addition, their resorption is done through a process of synthesis, combining

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the distribution of grain size, pore size and their porosity (Knabe *et al*., 2000; Daculsi *et al*., 2003; Cheng *et al*., 2013).

The aim of this study is to characterize a solid commercial β-TCP block for use in maxillofacial bone reconstruction using scanning electron microscopy.

MATERIAL AND METHOD

The study was conducted at the Center for Excellence in Morphological and Surgical Studies at the Universidad de La Frontera; two 5 mm x 5 mm x 10 mm (250 mm³) blocks were selected, made with chronOS Vivify β-TCP (Synthes, Switzerland) for the study.

The products were removed from their packaging under sterile conditions to be placed on the analysis surface of a STEM SU-3500 variable pressure scanning electron microscope (SEM-STEM) (Hitachi, Japan), with a range of magnification variables and the detectors Everhart-Thornley secondary electron (SE) detector, backscattered electron (BSE) detector, compositional, topographic and 3D mode and a variable pressure ultradetector (UVD) STEM holder for transmission.

For the semi-quantitative microanalysis of elements, the QUANTAX 100 energy dispersive x-ray spectrometer detector (EDX) (Bruker, Germany) was used. The acquisition parameters of the images went to 15 kv, magnifications of 100x, 250X, 500x, 1000X and 4000x, capture speed "slow", vacuum time 2.23 seconds.

The study initially analyzed the external image of the block on all its sides to obtain data on mineral present, porosities of the block and ridges; later, maintaining the conditions of sterility, the block was sectioned in the middle part to divide it into two; the section was taken with a sterile saw blade installed in a low-speed motor (20,000 rpm). Then, the block was again installed on the image surface to identify the conditions observed inside the block, using the same type of analysis. The results were placed in a table designed on Microsoft Excel (Microsoft Corporation, USA), performing a descriptive analysis of the data.

RESULTS

The outer measurements revealed irregularities in the shape of the pores that were similar in the analyzed blocks; the macro pore diameters in these areas were on average 164.92 mm (± 35.032 mm) with a range from 110 mm to 235 mm (Fig .1).

When the measurements of the inner area of the blocks were taken, differences were noted in the porosities compared to the outer layer, which was standard in the three analyzed blocks. An average diameter of the micro porosities was observed of 54.44 mm (± 17.676 mm) with a range from 30 mm to 86 mm (Fig. 2). The formation of porosities and irregularities present in the block was heterogeneous between the outer and inner surfaces.

In terms of the mineral content of the blocks by semi-quantitative analysis, a standard condition of mineral concentrations was confirmed on the outer and inner surfaces of the blocks, demonstrating the homogeneity of this analysis with the presence of carbon (2.02 %), oxygen (44.33 %), phosphate (16.62 %) and calcium (37.87 %) (Fig. 3 and Table I).
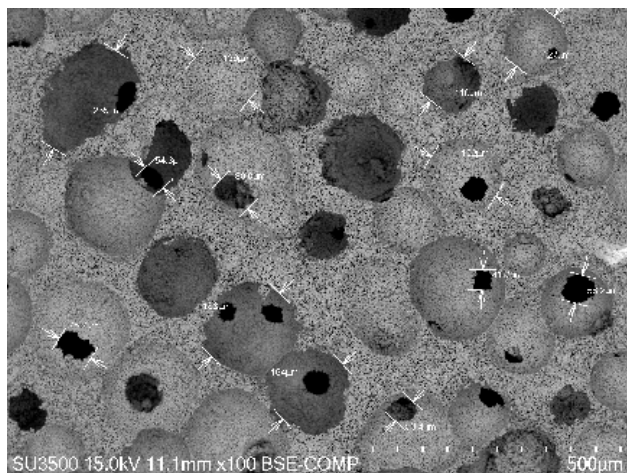

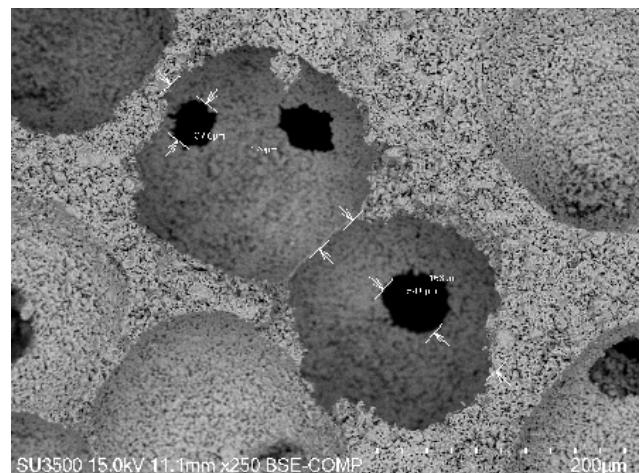Fig. 1. View of the size of macro pores (100x)

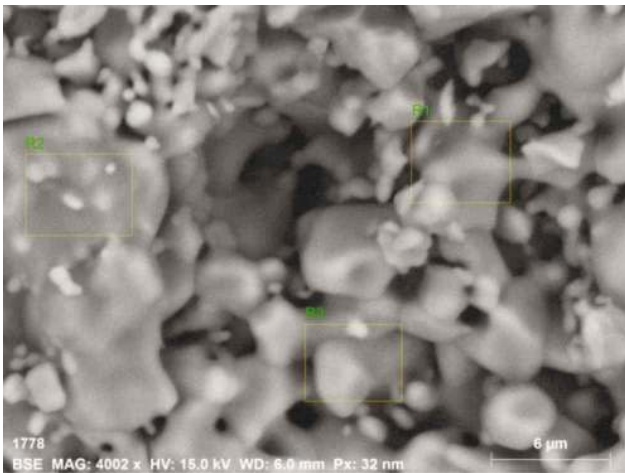Fig. 2. View of the size of micro pores (250x)

Fig. 3. Analysis area for the detection of mineral content of the blocks.

Table I. Mineral content of the blocks by semi-quantitative analysis.

Spectrum	C	O	P	Ca
R1	1.77	42.96	16.40	38.11
R2	2.13	45.31	17.02	37.71
R3	2.15	44.72	16.42	37.77
Mean	2.02	44.33	16.62	37.87
sigma	0.21	1.22	0.35	0.22
Sigma mean	0.12	0.71	0.20	0.13

DISCUSSION

The synthetic substitutes or alloplastic grafts based on hydroxyapatite (Ha), ceramic calcium phosphate and b-tricalcium phosphate are widely used as an alternative to autologous bone grafts in maxillofacial reconstruction (Spagnoli *et al*., 2001; Hoexter, 2002; Eppley *et al*., 2005). Several studies have shown these materials to be highly biocompatible, and they can also maintain the volume based on the capacity to regulate the resorption rate (Valentini & Abensur, 1997; Schlee *et al*., 2014). The use of b-TCP has been applied as a scaffold in the reconstruction of craniomaxillofacial defects together with stem cells and morphogenetic protein (BMP) (Alister *et al*., 2016; Uribe *et al*., 2017).

New studies have focused on the development of scaffolds based on graft elements like b-TCP (Billström *et al*., 2013; Liu *et al*., 2014), where the porosity that enables cell colonization, biomechanical resistance and resorption is integrated in times that allow for the adequate replacement by newly formed bone tissue.

In our analysis of the microstructure of pores in the block, an average size of 164 mm was observed for macropores and an average of 54.4 mm for micropores; and when these results were compared with other developed matrices, we found significant differences, considering pore averages of 200 to 400 mm and interconnecting channels and micropores from 100 to 200 mm (Wang *et al*., 2006; Tanaka *et al*., 2008).

It has been noted that ideal sizes would be over 300 mm in order to favor and increase bone tissue and capillary formation (Ohgushi *et al*., 1990; Karageorgiou & Kaplan, 2005). However, having an ideal porous structure, as this increases the size of the support material to repair larger defects, the number of air bubbles contained within the block increases. Therefore, it becomes more complicated for the bone marrow stem cells (BMSC) to enter the block and for the in vitro culture medium to perfuse into the central areas of the porous support (Vunjak-Novakovic *et al*., 1998; Holy *et al*., 2000; Wendt *et al*., 2003).

If we analyze that the size of the mesenchymal cells varies according to their cell phase, they can have an average diameter of 19 to 26 mm (Ge *et al*., 2014; Majore *et al*., 2009; Marklein *et al*., 2016); our study shows macropores with theoretically suitable sizes (below the average indicated in the international literature), but with very small micropores and communication channels, in some cases similar to the size of cells, which could limit the incorporation of a cellular layer.

On the other hand, angiogenesis elements can also be limited with this pore structure. The sizes vary according to vascular type, such as approximately 5 mm capillaries, the microvessels that present between 10 and 50 mm and the arterioles that present from 40 to 300 mm (Chan & Leong, 2008; Traore & George, 2017); one of the challenges in the blocks and matrices for reconstruction is the limitation of the distance for oxygen diffusion (approximately from 100 to 200 mm), the passage of nutrients and subsequent elimination of residues from the cell colonies (Lovett *et al*., 2009; Sakaguchi *et al*., 2013), so that the block analyzed in this study could have limitations in the substitution phase.

One positive aspect in this study is the homogeneity of mineral concentration, similar to those observed in other investigations (Daculsi *et al*., 1990; Miao & Sun, 2010; Sheikh *et al*., 2015). The relation of mineral composition and their relation to porosity involves the resorption speed, which is mediated by demineralization in the first stage and then resorption by macrophages and osteoclasts.

The scaffold must be highly biocompatible, biodegradable, bioactive, and during healing it must promote

osteoconduction, osteoinduction and osteogenesis. In addition, the scaffold must be osteoincorporative and osseointegrative to be accepted as part of the newly formed bone (Oryan *et al.*, 2014; Moshiri *et al.*, 2016). An ideal matrix block must imitate the native bone environment, macro- and microstructure, and must have a porous structure to allow the cells to enter the matrix to proliferate and be differentiated. This osteoconductive property is based to a large extent on the architecture of the block as well as on the materials used to make the desired matrix (Oryan *et al.*). The most important limitation during the healing of large bone defects is the lack of osteoinductivity of the matrices (Chen *et al.*, 2010; Oryan *et al.*; Seo *et al.*, 2014).

With the results of this study, it may be concluded that the b-TCP block can be used in bone reconstruction, but the presence of reduced micropore sizes could limit efficiency in the substitution and bone regeneration phase.

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RESUMEN: El objetivo de este estudio fue caracterizar un aloinjerto de bloqueo de fosfato tricálcico (β-TCP) comercial para su uso en la reconstrucción maxilofacial, evaluando su homogeneidad, porosidad y mineralización. Se utilizaron dos bloques comerciales de 5 x 5 x 10 mm de vivify β-TCP de chronOS, que se evaluaron mediante un microscopio electrónico de barrido de presión variable STEM SU-3500 (SEM-STEM) (Hitachi, Japón). Para el microanálisis de elementos semicuantitativo, se utilizó el detector de espectrómetro de rayos X de dispersión de energía QUANTAX 100 (EDX) (Bruker, Alemania). Se evaluó la homogeneidad de la morfología estructural, el tamaño del macroporo y microporo y la homogeneidad de los componentes. El análisis microscópico mostró microporos de 164,92 mm (± 35,032 mm) de diámetro en el área externa y microporos en el área interna de 54,44 mm (± 17,676 mm). La formación de porosidades e irregularidades presentes en el bloque fue heterogénea entre las superficies externas e internas. El contenido mineral de los bloques presentó homogeneidad con la presencia de carbono (2,02 %), oxígeno (44,33 %), fosfato (16,62 %) y calcio (37,87 %). El bloque β-TCP se puede utilizar en la reconstrucción ósea, pero la presencia de macroporos y tamaños de microporos reducidos podría limitar la eficacia en la fase de sustitución y regeneración ósea.

PALABRAS CLAVE: b-TCP; Andamio; Reconstrucción craneofacial; Injerto óseo.

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