



## Assessment of Hydrophilia of Biomaterials Blocks from Different Origins Used for Bone Augmentation

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Received: July, 12, 2019; Published: October 09, 2019

DOI: 10.31080/ASDS.2019.03.0664

### Abstract

The aim of this study is to compare topographically the hydrophilic potential of two different types of biomaterials through argentic impregnation. The experiment used five blocks of integral bovine bone (n = 5) consisting of an organic portion and a mineral portion (Bio-Oss Block, Geistlich Pharma, Welhausen, Switzerland); five blocks of bovine bone (n = 5), consisting of a mineral portion (Lumina Block, Criteria, São Paulo, Brazil); five blocks of equine bone (n = 5) (Bio-Gen, BIOTECK, Arcangelo, Italy). A model tomography I-Cat was used. The images were created in DICOM with the following acquisition protocol: MAX FoV of 6 cm with 40-seconds exposure of 0.2 mm voxel (MAX High Resolution), tridimensional image, with 4.2 mm of depth and 103.67 of diameter compared in terms of weight and density in the initial and final conditions. The non-parametric tests of Wilcoxon Signed Rank and Wilcoxon Mann-Whitney were used in the analysis of results

**Keywords:** Hydrophilia; Biomaterials; Bone Augmentation

### Introduction

[1] The long-term success of implants depends on the bone volume available for the implantation. Several techniques of preparation have been suggested for the osteointegrated implants. Reconstruction techniques are related to the volume of bone loss, measured in terms of thickness, height or both [2]. Horizontal bone augmentation consists of any procedure aiming to increase the alveolar ridge thickness in order to receive the implant with adequate diameter, usually larger than 3.5 mm.

The autogenous bone graft is the most commonly used and is considered the gold standard due to its biological advantages and osteogenic potential. However, it also presents longer convalescence time, increased morbidity and susceptibility to infections on the donor site, and progressive and continuous resorption. This has motivated the search for bone replacements, such as allogeneous, exogenous and alloplastic grafts. These materials range from par-

ticulate alloplastic to autogenous blocks of intra buccal areas, presenting different mechanical and biological properties [3]. In addition to that, the variety of available products and procedures represents a problem for the surgeon/implantology's, who has the responsibility of recommending the best surgical technique with the lowest risk of complication and morbidity.

The exogenous graft has been shown a promising alternative for reconstruction procedures of bone defects [4-5]. The particulate deproteinized mineral bovine graft has been widely used and studied for tissue regeneration guided with membranes and maxillary sinus lift procedures due to its osteoconductive properties and biocompatibility [6]. When this type of material is used as a block for the horizontal bone augmentation, its biological properties are added to the reduced surgical time, reduced risk of contamination, less trauma to the donor site, and reduced costs [7-8].

Given the advantages of this material as a block and its promising results, the aim of this literature review is to assess and discuss the studies carried with this biomaterial for horizontal bone augmentation.

### Literature review

The use of particulate inorganic materials of bovine origin for maxillary sinus lift and guided tissue regeneration has been well documented and widely used [6]; however, despite some promising results [4-5], little is known about the potentiality of this material when used as block graft.

In a case report with human subjects, new bone was formed in contact with the residual particles of the replacement when the only horizontal graft technique was used without membranes, serving as a osteoconductive support [9-10]. The osteoconductive property relates to the ability to function as a support for the migration, adhesion and proliferation of osteoprogenitor cells and for their differentiation into osteoblasts for the production of extracellular matrix [11]. When incorporated to the bone tissue, the inorganic bovine bone is maintained as inactive filling material and is reabsorbed when the tissue is extensively remodeled [12].

In addition to that, this material has other advantages such as its easy intra-operative handling, its availability in large amounts, and affordable cost [5].

The inorganic bovine bone block configuration is, obviously, less osteoconductive when applied to lateral [8] or vertical reconstruction [13].

In an *in vivo* study, these blocks showed no dimensional changes but were mainly filled with connective tissue and only a small amount (26%) of new bone formation was observed on the graft base [8-13].

Studies with dogs using inorganic bovine bone blocks for the vertical bone augmentation showed an increase of bone formation in groups that used membrane barriers in comparison to those that lacked the use of membranes. However, histology showed little new bone formation occurring only between the fixing screw and the base of the receptor bone; the outermost part of the graft was filled with connective tissue [14].

In another case report using mineral bovine bone block (Bio-oss spongiosa block, Geistlich Biomaterials, Wilusan, Switzerland) and coating with collagen membrane (Bio-Guide, Geistlich Biomaterials), a period of six months prior to the implant placement was given for healing. During the implant placement, the elimination of bone defect and the integration of the graft to the bone level of the surrounding teeth was clinically observed, with an excellent integration to the receptor region. A period of six months was also given before the second stage surgery and prosthetic rehabilitation. One year after the implant's placement, the marginal bone loss was smaller than 1 mm; no additional changes were observed within two years of function [5].

In a report of 12 cases with 15 receptor regions using bovine mineral bone (Bio-oss spongiosa block, Geistlich Biomaterials, Wolhusen, Switzerland) and coating with collagen membrane (Bio-Guide, Geistlich Biomaterials), the subjects were given a period of 9 to 10 months prior to the implants placement and another period of four months before the second stage surgery. Their results show that the combination of this biomaterial with the collagen membrane is an effective treatment option for horizontal bone augmentation prior to the implants placement, with reduced patient morbidity [15]. The use of absorbable membranes avoids a new surgical exposure for the membranes removal and reduces the risk of dehiscence and contamination, that could impair the graft [4].

Particulate deproteinized bovine bone either isolated or in association with autogenous bone and/or membranes has shown low rates of resorption.

Hydroxyapatite is integrated to the new bone, with a close contact of the lamellar bone and the particles surfaces [16]. A similar behavior is observed in the block configuration [17].

### Methodology

Group A consisted of five bovine bone blocks (Bio-Oss Block, Geistlich Pharma, Wolhusen, Switzerland, registered on ANVISA). The blocks were numbered, cataloged, photographed, weighted and submitted to an initial cone beam computed tomography (T1).

Group C consisted of five bovine bone blocks, on a non-ceramic shape (Lumina Bloco Critéria, São Paulo, Brazil, registered on ANVISA). The blocks were numbered, cataloged photographed, weight-

ed and submitted to an initial cone beam computed tomography (T1) in order to gather all the individual records. Group B consisted of five equine bone blocks (Bio-Gen, BIOTECK, Arcugnano, Italy). The blocks were numbered, cataloged, photographed, weighted and submitted to an initial cone beam computed tomography (T1).

To assess the materials hydrophilia, a solution of 0.5g of powdered Silver Nitrate (AgNO<sub>3</sub>, formed by the nitrate anion (NO<sub>3</sub><sup>-</sup>) and Silver cation (Ag<sup>+</sup>)) and 10 ml of distilled water to promote Argentic impregnation, as suggested by Barravieri in 2012. The solution was poured into a sterile acrylic Petri dish with 11.62 cm of diameter and weighting 13.06 g. With a pipette, 6 ml of the Argentic solution was added to the Petri dish. The set weighted 19.07 g and the liquid reached 3.5 mm of height. The blocks were not submerged.

All materials were weighted on an analytical balance (Ohaus Comp., Adventurer, USA) and weights were registered in grams.

The blocks were then placed individually on the Petri dishes and left for 120 seconds, when they were weighted and photographed. Finally, they were submitted to a final cone beam computed tomography (T2).

**Results**

The analysis was carried out separately for the initial and final conditions, considering weight and density. Table 1 lists average, median, standard deviation, minimum and maximum weight for each of the items. The averages and medians of all three products on the final readings are higher both for weight and density.

Product and Condition	Minimum	Average	Median	Standard Deviation	Maximum
Bioss Block Final	4.50	4.98	4.90	0.50	5.50
Bioss Block Initial	1.30	1.80	1.80	0.34	2.20
Bioteck Block Final	0.60	0.88	0.90	0.24	1.20
Bioteck Block Initial	0.40	0.48	0.50	0.04	0.50
Lumina Bloco Final	0.60	1.36	1.60	0.51	1.90
Lumina Bloco Initial	0.40	1.02	1.10	0.37	1.30

**Table 1:** Descriptive measures of the variable weight on the initial and final conditions.

The highest average weight and density are found for Bioss Block on the final condition (average weight of 4.98 and average density of 742.59) and the lowest are found for Bioteck Block on the initial condition (average weight of 0.48 and average density of - 633.15).

As for weight variability, Lumina Bloco showed the largest standard deviation on the final condition (0.51), Bioteck showed the lowest dispersion on the initial condition (with standard deviation of 0.04). Regarding density, Bioteck Block shows the largest standard deviation on the final condition (462.95), and Bioteck on the initial condition had the lowest standard deviation (12.33).

Product and Condition	Minimum	Average	Median	Standard Deviation	Maximum
Bioss Block Final	709.54	742.59	733.02	32.19	790.35
Bioss Block Initial	-490.09	-416.43	-421.52	76.26	-300.75
Bioteck Block Final	-560.96	-3.96	249.77	462.95	433.18
Bioteck Block Initial	-651.50	-633.15	-629.89	12.33	-618.27
Lumina Block Final	-550.58	-72.65	6.86	305.65	218.05
Lumina Block Initial	-573.79	-234.07	-129.56	254.52	35.11

**Table 2:** Descriptive measures of the variable density on the initial and final conditions.

**Discussion**

Among the techniques available for the augmentation of alveolar ridge thickness, autogenous bone blocks, with or without the use of membranes, result in larger gains in terms of crest width and smaller complication rates in comparison to particulate materials [3-18].

The survival rates of implants placed in regions that underwent horizontal reconstruction are high [2,3,5,15]. Autogenous bone block grafts are the most common choice for the reconstruction of deficient ridges for future rehabilitation with osteointegrated implants [19].

Its osteogenic, osteoconductive and osteoinductive properties promote a rapid integration of the grafted material; however, the bone is considerably reabsorbed, the amount of donor area is limited and the risk of complication is higher [20]. The ideal behavior would be the resorption and complete remodeling for the new bone. The rate of resorption, however, depends on the material and can impact its use, mainly on areas with aesthetic appeal.

This relatively high rate of resorption of autogenous bone grafts has motivated the search for more stable bone replacements. To this date, none of the commercially available biomaterials combine all of the features defined as ideal [22].

The ability of autogenous grafts to keep their initial volume varies. Studies have shown conflicting results regarding factors such as: type and site of reconstruction; the use of total prosthesis over the reconstructed areas; donor site [23]. Some reports show a horizontal bone graft resorption varying from 10 to 50% [24-25].

The success of vertical bone augmentation can also be based on the rate of success/survival of the implants placed on the site since it ultimately reflects the treatment result, which includes masticatory function, patient health condition and aesthetic [26]. The placement of implants seems to inhibit the residual bone and the grafted bone resorption [24].

Xenogenics biomaterials have a tridimensional structure similar to that of the bone. They should be free of all protein residues to avoid immune reactions [20]. However, some studies have identified the presence of bioactive factors such as TGF $\beta$  and BMP-2 on some samples [10].

The presence of these proteins may explain why this material is deemed more effective clinically than tridimensional replacements. Although a chronic immune response is not normally triggered by these factors, the number of procedures has increased and, thus, antigenicity has become more critical [10].

The concern with the use of this type of material was related to spongiform encephalopathy (mad cow disease) contagion, but thermal treatment above 300°C or with alkali followed by neutralization removes virtually all possible contamination with prions [27].

Inorganic bovine bone is a natural deproteinized bone with a high degree of biocompatibility [28]. This replacement undergoes a slow remodeling process and is eventually incorporated into the native bone [29], justifying the maintenance of the graft volume, thereby promoting better stability of the interproximal height of bone until the natural remodeling process begin with the loading of the implants [5].

Graft coating with membranes, whether absorbable or not, aims to isolate the repaired area (graft) to avoid migration and proliferation of undesired cells (in this case, connective tissue cells), allowing the population of the graft with bone cells [30]. Literature lacks studies comparing bone formation *in vivo* of exogenous graft blocks associated with membranes for horizontal bone augmentation. Nonetheless, when this type of graft was studied for vertical bone augmentation, they showed osteoconductive properties and a level of organization equivalent to that of the autogenous graft, leading to the conclusion that it can be used as support. It also showed that the combination with collagen membranes yielded no significant difference [17].

The studies presented here show promising results; however, they used different intervals between the graft placement and the implant placement, as well as different post-operative control times with small samples. Therefore, further studies should be carried on the role of absorbable membranes associated with the graft, the potential for bone formation on the graft, and the long-term behavior of implants implanted on these areas.

## Conclusion

According to the methodology used here, it is possible to conclude that, regardless of its animal origin, exogenous blocks were presented, to a lesser or greater extent, hydrophilic potential, showing variation not detected on the submitted statistics, able to be used as an osteoconductive.

When the products were submitted to pairwise match, significance was observed between Bioss Block and Lumina Block. This means that these products are statistically different, regarding both weight and density.

The same analysis was carried for the match between Biotech and Lumina, that showed no significant differences regarding weight and density.

In the match between Bioss and Biotech, results were similar to those of the first match: averages of weight of the Bioss product are significantly larger than those of the Biotech product, and the same is observed for density.

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**Volume 3 Issue 11 November 2019**

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