THE GUIDED BONE REGENERATION TECHNIQUE (GBR): A MORPHOLOGICAL AND IMMUNOHISTCOHEMICAL STUDY

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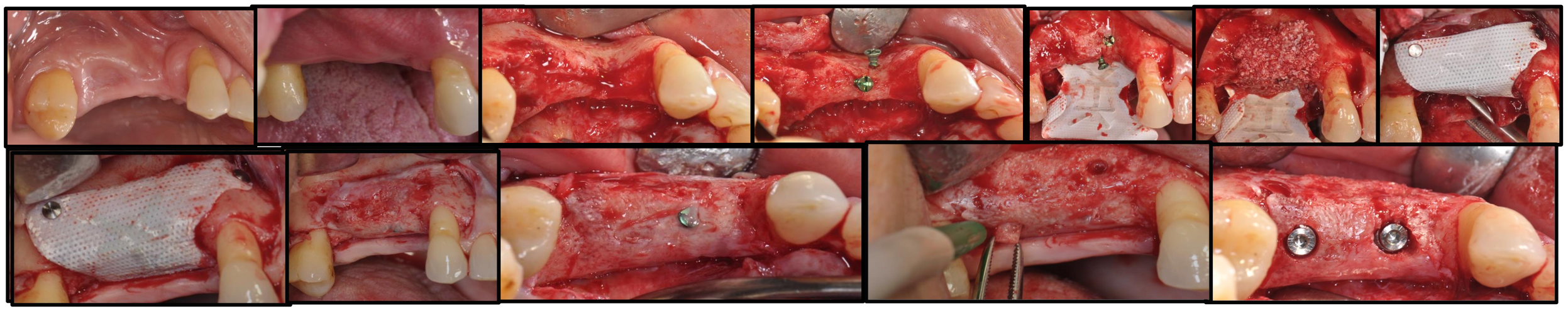
Background and Aim

The volumetric alterations of both maxillary and mandibular bone, resulting in limited implant-prosthetic rehabilitation, represents a critical consequence of tooth loss (1). Six months after tooth loss is generally observed both a horizontal (29 to 63%) and vertical (11 to 22%) reduction in bone volume (2). The Guided bone regeneration technique (GBR) has been proposed to correct these volumetric alterations. GBR technique has the advantage of promoting alveolar bone gain with predictable and stable results (3). The GBR involves the use of a mechanical barrier capable of isolating the surgical site from both epithelial cells and connective tissue to allow osteogenic cell proliferation and new bone formation (4). In this study, a combined horizontal and vertical GBR technique was performed. The graft was a mixture composed of 50% autologous bone (AB) and 50% allograft of bovine bone with termic deantigenation (ABBMT) or enzimatic deantigenation (ABBME). Finally, a titanium reinforced PTFE membrane stabilized with pinns and screws was used and the mucosal flap was closed free of tension with a PTFE suture. Starting from all these considerations, the aim of this study was to evaluate the quality of the regenerated bone in term of histology and immunohistochemical analysis.

Clinical Case



Materials and Methods

Based on the available clinical data, 7 patients were selected and underwent GBR technique. Eight months after surgery the membranes were removed and titanium implants were placed. The implant osteotomy was realized with a 3 mm diameter trephine burs, obtaining bone biopsies then investigated by morphological and immunohistochemical analysis. The study design was approved by the ethic commitee of Unicamillus.

Histological analysis.

Specimens were fixed in 4% formalin and paraffin embedded. Four-µm serial sections were used to perform morphological studies (hematoxylin and eosin staining and toluidine blue staining) . Hematoxylin and eosin staining sections were analyzed to evaluate the bone matrix in terms of thickness and number of trabeculae as well as for the presence of bone cells (osteoblasts and osteocytes). The structure of the bone was studied by analysis of toluidine blue staining.

Immunohistochemistry

Immunohistochemical analysis was performed to study the expression of molecules involved in bone metabolism such as BMP-2, BMP-7 and PTX3 in all collected samples. Antigen retrieval was performed on 4-µm-thick paraffin sections using EDTA citrate buffer pH 7.8 (PTX3) or citrate buffer pH 6.0 (BMP-2 and BMP-7) for 30 min at 95°C. The sections were then incubated for 1 h at room temperature with the primary antibodies (BMP-2 Mouse monoclonal clone 1A11 Dilution 1:500; Novus Biologicals, Littleton, CO, USA; BMP-7 Mouse monoclonal clone ab54904; Dilution 1:250, AbCam, Cambridge, UK; PTX3 rat monoclonal clone MNB1, Dilution 1:100, AbCam). Washes were performed with PBS/Tween20 pH 7.6. Reactions were revealed by the HRP-DAB detection kit (UCS Diagnostic, Rome, Italy). Immunohistochemical signals were analyzed by assigning a score from 0 to 3 according to the number of positive cells (Table 1) for each section.

Score	Positive cells (10x)
0	no
1	1<x>3 positive cells
2	4<x>6 positive cells
3	>7 positive cells

Table 1: immunohistochemistry scoring system.

Results

Histologic analysis.

Haematoxylin and eosin analysis showed new matrix formation in all analysed biopsies. Specifically, in 4/7 patients bone tissues were well formed with neither sign of degeneration nor presence of inflammatory infiltrate. In 2/7 patients bone matrix was well structured but the new bone was less than in previous cases. Lastly, low new bone matrix was observed in 1/7 patients. Similar data were obtained by studying the toluidine blue sections.

As concern the number of both osteoblasts and osteocytes, no difference was observed in the analysed biopsies. This confirm the presence of new bone matrix in all patients.

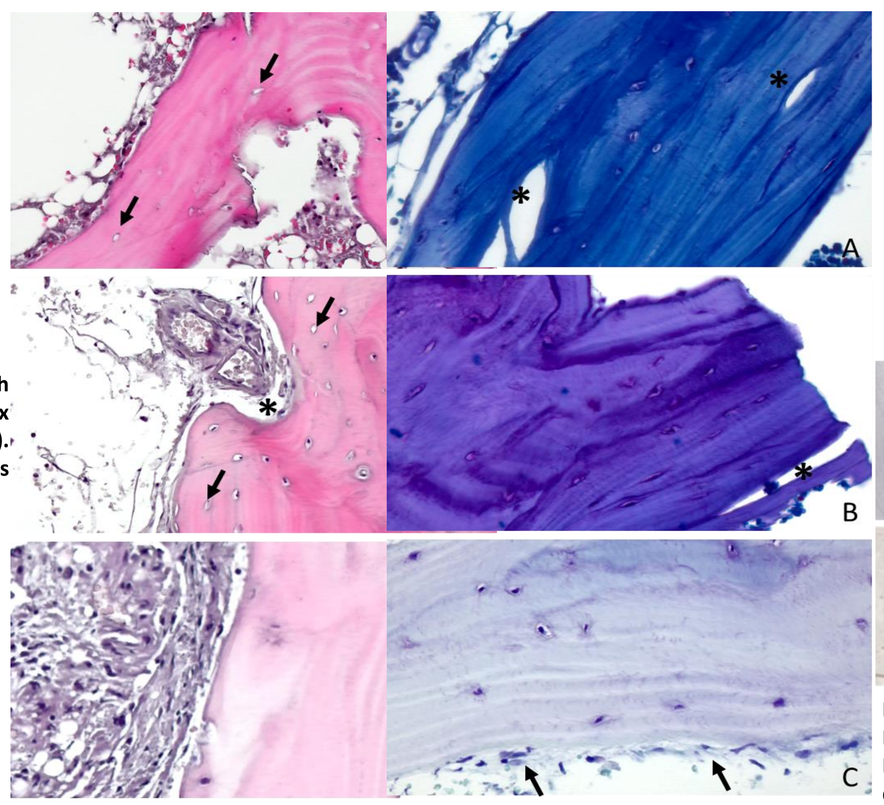


Figure 2 Morphological analysis A) Sample shows new bone matrix with some empty osteocyte lacunae (arrows). B) Sample shows new bone matrix with some osteoblasts (asterisk) and few empty osteocyte lacunae (arrows). C) Image displays well structured regenerated bone with several osteoblasts (arrows). Magnifications 60x for each image.

ABBMT	Anorganic bovine bone thermally demineralized
ABBME	Anorganic bovine bone enzymatically demineralized
AB	Autologous bone

TABLE 2: acronyms.

Immunohistochemistry

Immunohistochemical reactions showed an association between the expression of molecules capable of inducing bone matrix deposition and the morphological characteristics of bone specimens. In particular, the highest score values for BMP-2, BMP-7, and PTX3 were observed in patients with the higher bone quality in term of both trabecular number and bone thickness. All these molecules were mainly expressed by osteoblasts and osteocytes. It is important to note that BMP-2 and PTX3 represent the most powerful inducers of osteoblast differentiation and activity thus contributing to bone regeneration.

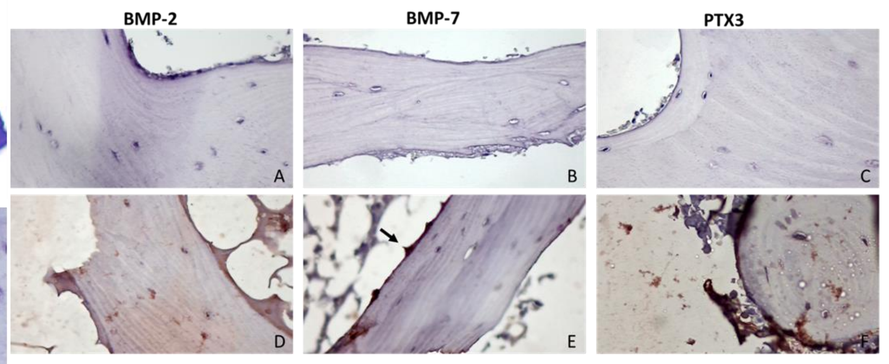


Figure 3 Immunohistochemical analysis. A-C) Regenerated bone with no/rare BMP-2, BMP-7 and PTX3 positive cells is characterized by some empty osteocyte lacunae. D-F) Regenerated bone shows focal expression of BMP-2, BMP-7 and PTX3. Bone tissue displays numerous osteoblasts (arrows). Magnifications 40x for each image.

SEX	AGE	PATHOLOGY and PHARMACY	SMOKE	POSITION	TYPES OF ATROPHY	%Osso-Bio	BIOMATERIAL	MEMBRANE	HEMATOXYLIN EOSIN LEVEL 1: trabecular alteration	HEMATOXYLIN EOSIN LEVEL 2: section 50 um from the previous one. Differences from the first level	TOLUIDINE BLUE LEVEL 1 bone alterations	TOLUIDINE BLUE LEVEL 2 section 50 um from the previous one. Differences from the first level	BMP-2 (score 0-3)	BMP-7 (score 0-3)	PTX3 (score 0-3)
F	69	breast cancer- Etirox e Lobivon	yes	24-25	mixed	50-50	ABBME	CYTOPLAST	Yes, by thickness and number. Signs of osteocytes degeneration.	No	Partial loss of firmness. Presence of trabecular rarefaction.	No	0	0	0
F	68	coarotid stenosis and hypertension	NO	26-27	mixed	50-50	ABBMT	CITOPLAST	No	No	No	No	1	0	1
F	54	thyroid nodules	NO	15-16	mixed	50-50	ABBMT	CYTOPLAST	No	No	No	No	1	1	0
F	58	Drug allergy	yes	15-16	mixed	50-50	ABBMT	CYTOPLAST	Moderate. Slight thinning of the thickness.	No	No	No	1	0	0
M	72	Ndr	yes	15-16	mixed	50-50	ABBMT	CYTOPLAST	Yes, by thickness and number. Signs of osteocytic degeneration.	No	No	No	1	1	0
F	63	Allergy to Aulin	NO	24-25-26	mixed	50-50	ABBMT	CYTOPLAST	No	No	Slight	No	0	0	1
F	55	Ndr	yes	35-36	mixed	50-50	ABBMT	CYTOPLAST	No	No	No	No	1	2	0

TABLE 3: protcol data.

Discussion

In this study we highlighted that in all histological cores (taken in the vertical regeneration component) and with both histological stains (hematoxylin-eosin and toluidine blue) there were newly formed bone matrix with absence of inflammatory cells, presence of vital osteoblasts and osteocytes and incremental lines which demonstrates neo-osteogenesis. The regenerative technique was effective for regeneration of new bone also in the vertical component. This is in agreement with previous literature data (1). The news of this study is about immunohistochemical research related to BMP-2, BMP-7 and PTX3 proteins. These proteins are always expressed by osteoblasts and osteocytes. Moreover, the BMP-2 and the PTX3 are the most powerful inducers of osteoblastic differentiation and activity. Their presence suggest the presence of an active and vital bone capable of driving the osseointegration process and thus supporting the implants. In all samples of the grafted cases autologous bone + thermally deantigenated bovine bone we highlighted the presence of these proteins, while in the only sample in where the biomaterial used is enzymatically deantigenated bovine bone, they are absent. the limit of our study is the number of cases examined.
In conclusion, the GBR technique with PTFE membranes and underlying graft of AB and ABBMT is able to vertically regenerate a new bone suitable to support implants.

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