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Osteopromotion of Biphasic Calcium Phosphate granules in critical size defects after osteonecrosis induced by focal heating insults

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Abstract

Heat-induced osteonecrosis represents a simple, rapid, and inexpensive method for reproducing the effects of bone disease. In the present study, we employed this technique to induce osteonecrosis in femoral defects in rabbits and assessed the efficacy of treatment using Biphasic Calcium Phosphate (BCP) granules (MBCP+^{TM}, Biomatlante SA). After 3 weeks, the osteopromotion effects of BCP granules could be statistically proven ($P < 0.05$) through image analysis of newly formed bone in osteonecrosed sites containing BCP granules when compared to empty control sites. Increasing mature and woven bone presence was observed after 6 and 12 weeks, forming new trabeculae in necrosed site. Significant statistical differences were evidenced at each time between empty necrosed and filled necrosed defects in terms of new bone volume.

1. Introduction

The development of relevant models for reproducing the effects of bone diseases has been of great interest. In this regard, osteonecrosis has been artificially induced using several techniques, including induction by cryogenics or heating [1], surgical ischemia [2], or active moieties [3].

In order to reduce costs and simplify implementation, we have utilized a thermocouple to induce local tissue necrosis in rabbit femoral epiphyses. Since Biphasic Calcium Phosphate (BCP) granules are known to be osteoconductive [4] and/or osteoinductive [5], we have evaluated the osteoinductive effect of MBCP+^{TM} micro-macroporous substitutes using this heat-induced model of rabbit osteonecrosis.

This study has been conducted as part of the European project on Gene Activated Matrices for Bone and Cartilage Regeneration in Arthritis (GAMBA; NMP 2009-2.3-1), which is focused on osteoarthritis gene therapy. Therefore, after assessing the therapeutic osteogenic potential of these BCP granules, the ultimate objective of this work will be to combine MBCP+^{TM} granule therapy with specifically designed gene vectors [6].

2. Materials and methods

Critical size defects (5–6 mm in diameter and 8 mm in length) were induced in femoral epiphyses of 18 New Zealand rabbits (Table 1). A thermocouple, which was controlled by a temperature probe, was heated to 80 °C. Rotation of the thermocouple was performed in order to improve homogeneity of the thermal treatment, which lasted for 45 seconds. Following implantation, the animals were confined for 3, 6, and 12 weeks.

MBCP+^{TM} granules were provided by Biomatlante SA (Vigneux-de-Bretagne, France), and had a composition of 20% hydroxyapatite (HA) and 80% beta-tricalcium phosphate (β-TCP). The osteonecrosed critical sized defects were filled with 2 cc of MBCP+^{TM}.

The epiphyses were processed for microtomography (μCT) using the MicroCT Skyscan 1072 system. Three-dimensional (3D) qualitative and quantitative image analyses of the bone ingrowth were performed. After μCT acquisition, the implants were embedded in methyl methacrylate resin (GMMA), sectioned, polished, coated with gold palladium, and examined by

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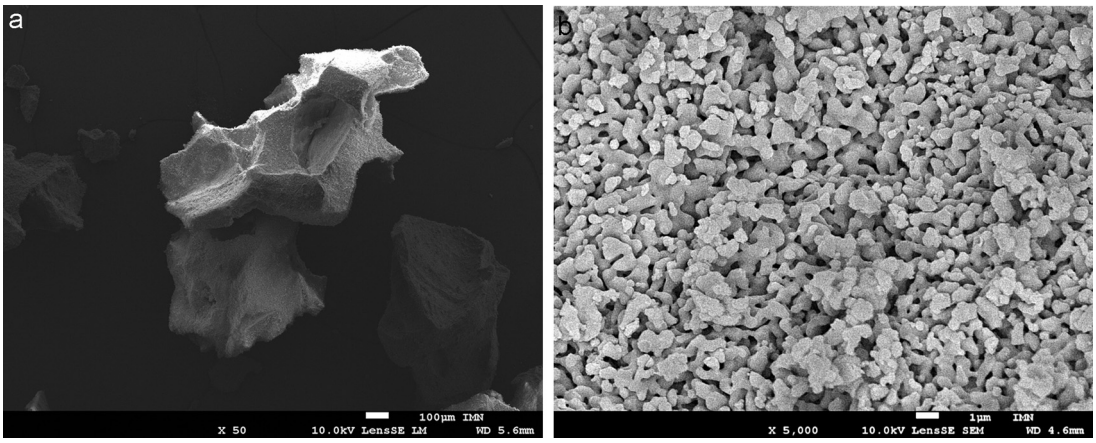

Fig. 1. Microstructure and porosity of MBCP+^{TM} granules, visualization by SEM. Macrostructure with macropores (a) and microstructure with micropores (b).

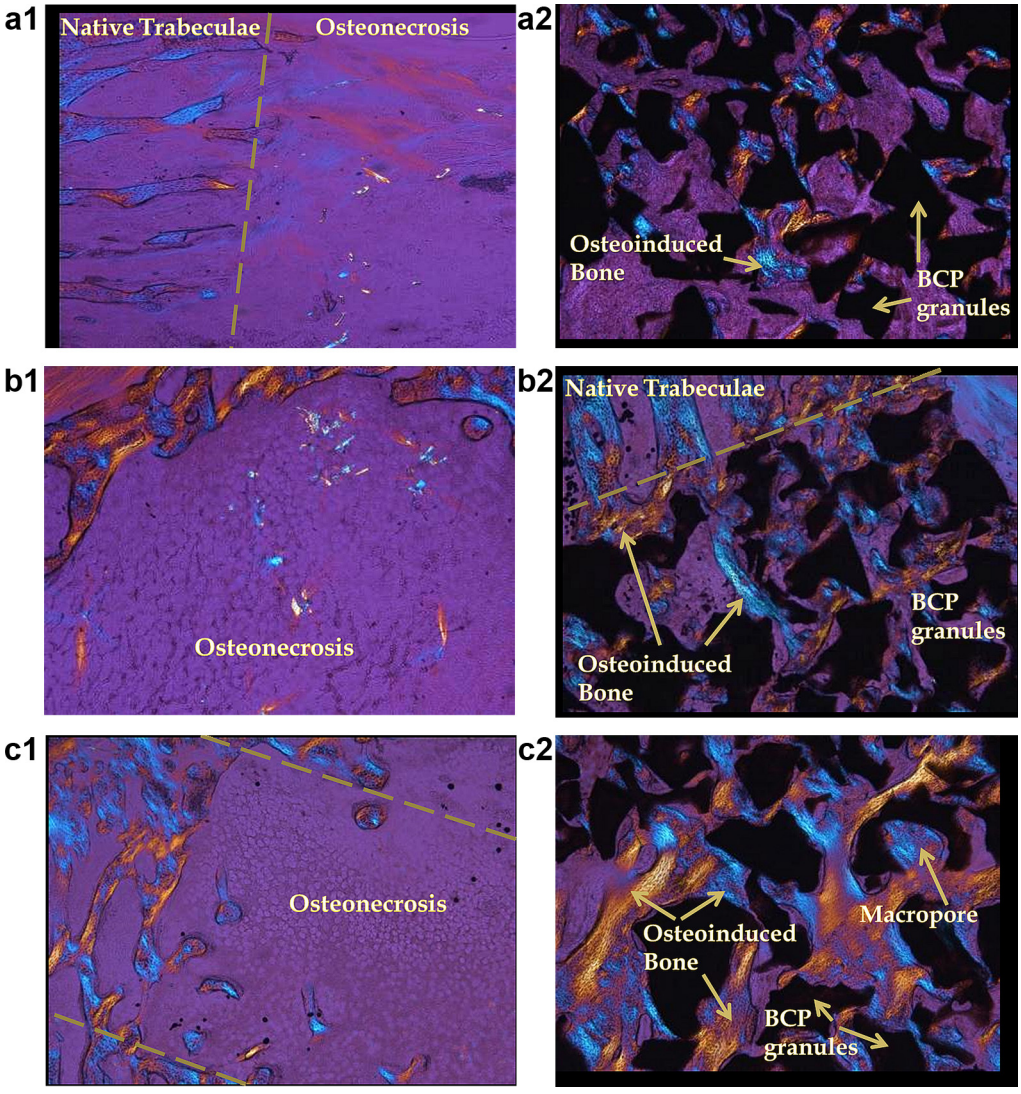

Fig. 2. Polarized light microscopy of histological sections. Newly formed bone is blue and brown, CaP biomaterials are black, and cellular environment is purple. Control osteonecrosis defect at 3 weeks (a1), 6 weeks (b1), and 12 weeks (c1). MBCP+^{TM} in osteonecrosed defects at 3 weeks (a2), 6 weeks (b2), and 12 weeks (c2).

Table 1
In vivo implantation study plan.

Conditions	Number of defects by time: 3/6/12 weeks	Critical size defect diameter/length (mm)	Type of epiphysis
Osteonecrosis control	6/6/6	5/8	Femoral
Osteonecrosis and MBCP+^{TM}	6/6/6	6/8	Femoral

scanning electron microscopy (SEM) using the backscattered electron (BSE) mode (LEO1450VP). Two-dimensional (2D) image analyses were performed using Quantimet Leica Q500 software. Processing of the 3D images from μCT was performed using CT-AN software (SkyScan, Belgium). Statistical analyses, including analysis of variance (ANOVA) and Bonferroni's post-hoc test, were performed on the quantitative 2D and 3D imaging data sets using Graphpad software (Graphpad Inc., USA).

Sections were prepared with a diamond saw microtome and observed via polarized light microscopy. Thin sections (7 μm) were stained according to Movat's pentachrome method and observed by light microscopy.

3. Results

BCP scaffolds were observed by SEM, which revealed homogeneous macroporosity (100–500 μm) (Fig. 1a) and microporosity (< 5 μm) (Fig. 1b). Also, crystallographic grain size, determined by examining the clearly visible grain boundaries, was found to be highly homogeneous (approximately 1 μm; Fig. 1b).

Using polarized light microscopy, we characterized bone ingrowth in histological sections obtained from the MBCP+^{TM} granule-filled defects. Three weeks after treatment, we could observe osteopromoted bone in direct contact with the biomaterial granules (Fig. 2a). However, this newly formed bone was not yet completely woven. Images obtained at 6 weeks revealed a greater amount of new bone and indicated initiation of trabeculae formation (Fig. 2b). At 12 weeks, the newly formed bone was woven, and BCP granules could be found embedded in the surrounding mature bone (Fig. 2c).

Histological sections, which were stained by Movat's method, clearly demonstrated an increase in bone formation from 3 to 12 weeks (Fig. 3). In addition, there was an absence of fibrous encapsulation and inflammation, which is consistent with previous findings using MBCP+^{TM} scaffolds [7].

Images were quantitated, and based on ANOVA analysis, statistically significant differences in bone formation were observed between MBCP+^{TM}-filled defects and osteonecrosis controls from 3 to 12 weeks. Indeed, new bone formation in osteonecrosed sites was greatly enhanced following treatment with MBCP+^{TM} (Fig. 4). These results were confirmed by 2D area analyses of SEM images (Fig. 5) and 3D volume analyses of μCT data (Fig. 6).

Finally, the BCP scaffold resorption rate from 3 to 12 weeks was calculated using 3D volume analysis of the μCT results.

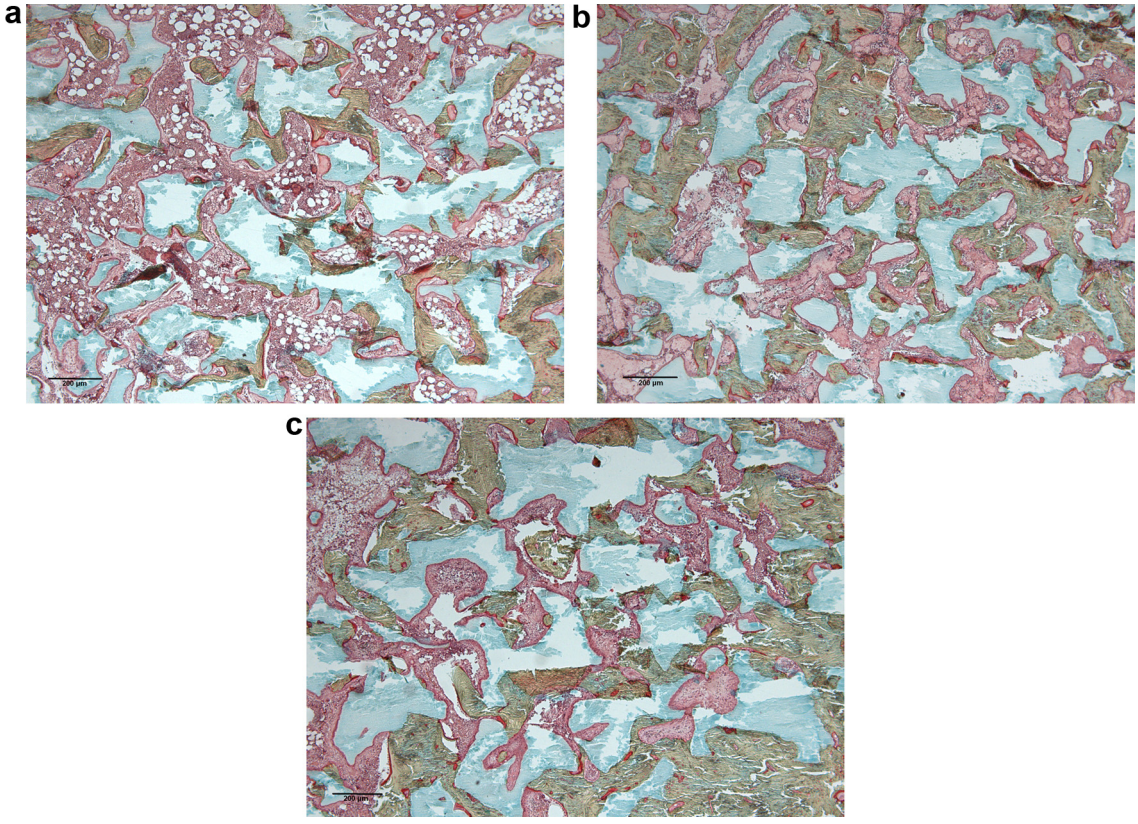

Fig. 3. Movat stained histological sections from MBCP+^{TM}-filled defects after 3 weeks (a), 6 weeks (b), and 12 weeks (c). Newly formed bone is dark green, MBCP+^{TM} residues are white and light green, and bone marrow is light and dark pink.

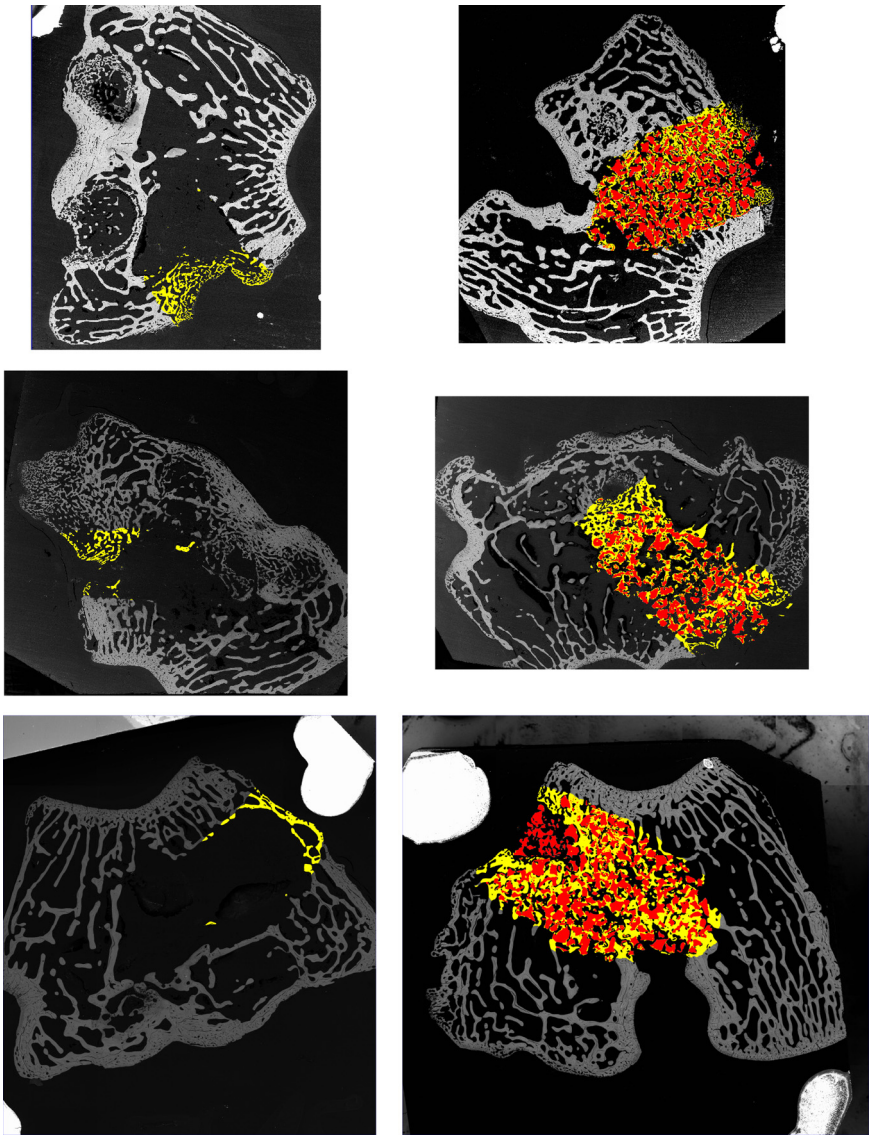

Fig. 4. 2D SEM image analyses of empty defects (left column) and defects filled with MBCP+^{TM} (right column) after 3 weeks (top row), 6 weeks (middle row), and 12 weeks (bottom row).

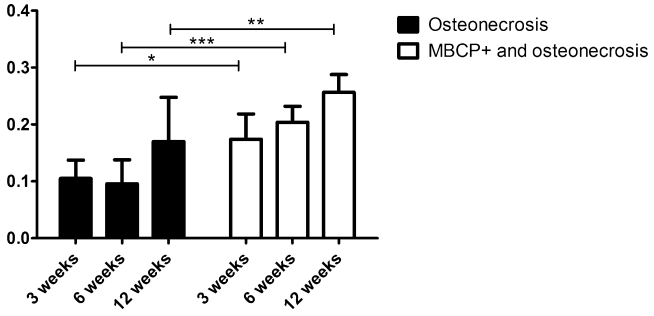

Fig. 5. Ratio of new bone to defect area from 2D SEM quantitative image analysis. ANOVA with Bonferroni post-hoc tests. *: $P<0.05$; **: $P<0.005$; ***: $P<0.001$.

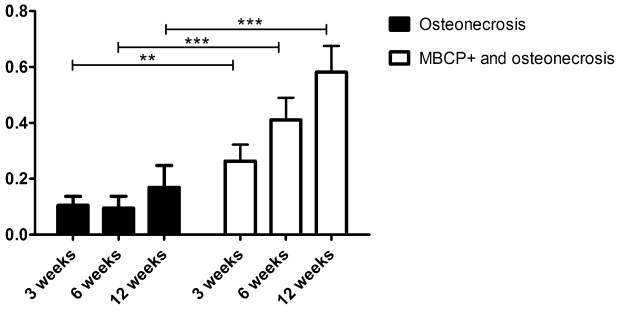

Fig. 6. Ratio of new bone to defect area from 3D μCT quantitative image analysis. ANOVA with Bonferroni post-hoc tests. *: $P<0.05$; **: $P<0.005$; ***: $P<0.001$.

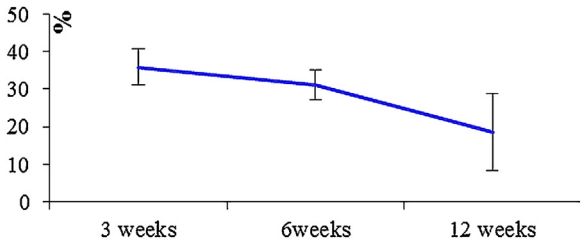

Fig. 7. Resorption rate of MBCP+^{TM} granules measured through 3D μCT. Scaffold volume is displayed as percentage of total defect volume.

We observed that the proportion of granules relative to the total defect volume decreased continuously from 40% at 3 weeks to 20% at 12 weeks (Fig. 7). This result is consistent with our previous image analysis. Taken together, our findings indicate that there is a steady increase in newly osteopromoted bone along with concomitant biomaterial resorption occurring in MBCP+^{TM}-filled defects [4].

4. Discussion – Conclusion

A principal goal in the development of preclinical models for the study of bone pathologies has been to identify simple, effective, and inexpensive methods. In this regard, our study has highlighted the efficacy of a thermocouple-based model for inducing osteonecrosis through heat. Although this technique was effective, it was not perfect because it did not completely inhibit bone remodeling due to intrinsic limitations of the animal model. In addition, we have statistically proven the osteopromotion potential of MBCP+^{TM} (ratio HA/TCP 20/80) after 3 weeks of treatment. Moreover, MBCP+^{TM} promoted bone formation could be observed in direct contact with BCP granules, highlighting the osteopromotion ability and osteoconductivity of these calcium phosphate (CaP)-based biomaterials. Notably, a lack of osteopromotion has often been associated with the use of CaP, which does not have the specific micro-macroporous structure that MBCP+^{TM} granules display [8]. These important porosity/interface properties cooperate with the chemically biomimetic composition of BCP (i.e., crystallographic phases of HA and β-TCP) to participate in the dissolution–precipitation process [9]. In future studies, it will be critical to evaluate the osteopromotion and therapeutic potential of these BCP granules in combination with emerging gene-based therapies using the same animal model of osteonecrosis. Ultimately, this research could contribute to effective treatment strategies for osteoarthritis and other bone degenerative pathologies in humans.

Disclosure of interest

T.M., F.M., and P.B. are directly involved in the Biomatlante company, which provided the scaffolds.

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