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Comparative study of bone microarchitactural structure after porous bioglass and Strontium doped bioactive glass bone graft in Wistar rat model

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Abstract

Osteoporosis is an important systemic skeletal disease, characterized by reduction in bone mass and disruption of the microarchitectural structure of bone tissue, resulting in loss of mechanical strength and increased risk of fracture. Wistar rats were divided into four groups: the group (I) was used as negative control (T), after ovariectomy, groups II, III, IV and V were used respectively as positive control (OVX), implanted tissue with Strontium doped bioactive glass (BG-Sr) (OVX-BG-Sr) implanted tissue with Porous bioactive glass (OVX-PG) . The histomorphometric analysis demonstrated that BV/TV, N. Ob was significantly higher in PG treated rats groups than those of BG-Sr groups. However, the (Oc.S/ BS) parameter was significantly decreased in PG groups when compared with that of BG-Sr treated rats. On the other hand, the MS/BS had not significantly decreased in the PG treated rats groups when compared with that of BG-Sr rat groups. After 60 days of implantation, the microarchitecture in PG bone were more ameliorated then that of BG-Sr bone. Both of PG and BG-sr groups were more amilorated than that of OVX groups. Therefore, our findings suggested that both PG and BG-Sr might have promising applications in osteoporotic diseases.

Keywords: Histomorphometry, Bioglass, Strontium, Bone tissue regeneration.

Introduction

Many therapeutic advances in the management of osteoporosis were studied first in diverse animal models and then entered clinical practice.[1] numerous treatments were available for reducing bone loss but each has limitations. For example, selective estrogen receptor moderators[2] and synthetic parathyroid hormone[3] can be found useful for osteoporosis prevention and treatment. Although recent evidence has confirmed their benefit in terms of fracture reduction, adverse outcomes such as increased risks for breast cancer have been identified.[4] Cement biomaterials have demonstrated considerable efficacy in bone reconstructive surgery.[5] Strontium are well known anti-resorptive agents largely used in clinical treatments for osteoporosis.[6] In the present study, two different biomaterials have been developed in order to biologically reinforce osteoporotic bone by increasing the bone fraction and improving bone micro-architecture. In previous study, Strontium doped bioactive glass and porous bioactive glass have been elaborated.[7, 8] The kinetics of chemical reactivity and bioactivity at the surface of biomaterials were studied.[8] Strontium was introduced as trace element at different contents in the glass matrix, according to its concentration in the bone

matrix.[9, 10] Investigations were conducted on the surface of biomaterials by using in vitro assay after immersion in SBF (simulated body fluid) and 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay cell test to highlight the effects of the strontium doped bioactive glass.[11] *In vivo* studies in relevant animal models are necessary to explore the effect of two implantable biomaterials on femoral condyle bone structure by performing histomorphometric analysis. Knowledge of bone microarchitecture is a clue for understanding osteoporosis pathophysiology and improving its diagnosis and treatment; the response of microarchitecture parameters to treatment should allow assessment of the real efficacy of the osteoporosis therapy.

Material and Methods

Animals and experimental design

Female Wistar rats (16–19 weeks of age), were used in this study. The rats were fed on a pellet diet (Sicco, Sfax, Tunisia) and water ad libitum. All the animals were kept under climate-controlled conditions (25 °C; 55% humidity; 12 h of light alternating with 12 h of darkness). All rats were randomly divided into four groups, the first group (I) used as control (T). Groups II, III and IV were used as ovariectomised rats (OVX), implanted with BG-Sr implant and implanted with PG. Anaesthesia was induced with xylazine (7 to 10 mg/kg (i.P) ROMPUN® 2%) and ketamine (70 to 100 mg/kg (i.m) imalgene®) depending on the body weight. A drilled hole, 3-mm diameter and 4-mm deep, was created on the lateral aspect of the femoral condyle using a refrigerated drill to avoid necrosis. Both PG and BG-Sr were sterilized by γ-irradiationfrom a 60 Co Source gamma irradiation at a dose of 25 Gy (Theratron external beam teletherapy, Equinox, Ottawa, ON, Canada) implant. The drill-hole was filled with 10 mg of biomaterials. The filling was done carefully in a retrograde fashion to ensure both minimal inclusion of air bubbles and direct implant – bone contact. The rats were obtained from the central pharmacy, Tunisia, and bred in the central animal house. The handling of the animals was approved by the Tunisian ethical committee for the care and use of laboratory animals.

Histomorphometric evaluation

The implanted femoral condyles were harvested, fixed in Burdack (formalin) and refrigerated. Specimens were dehydrated, using alcohol titrated solutions from 70 to 100% EtOH. Then, the specimens were infiltrated by methylmethacrylate that was allowed to polymerize, before placing it in a mixture of methylmethacrylate and glycolmethacrylate, without prior decalcification. Using a sliding microtome (Reichert- Jung), sections of 6 to 7 µm thick were debited by cutting along a transverse plane. Moreover, the sections were stained with modified Goldner trichrome, toluidine Blue. Bone/Tissue Volume (BV/TV, expressed in %), Osteoid/Bone Surface (OV/BV expressed in %), Bone/Tissue Volume (BV/TV, expressed in %), Osteoid/Bone Surface (OV/BV expressed in %), Osteoclast /Bone Surface (Oc.S/BS, expressed in %), Osteoblast/Bone Surface (Ob.S/BS, expressed in %), Osteoblast Number (N.Ob expressed in mm^{-2}) and Mineralizing Surface (MS/BS) were measured by a point count method using a 25-point integrating filter.[12]

Statistical analysis

The statistical analysis of the data was calculated using Student's t-test.

Results and Discussion

After 60-day of the surgery, the quantitative analysis demonstrated that BV/TV, N. Ob were significantly higher in PG treated rats groups than those of BG-Sr groups (Fig. 1A, 2A. 1B, 2B). BV/TV presented respectively, 46 and 43% in PG treated rats and BG-Sr groups. N. Ob parameter presented respectively, 39 and 35 mm-2 in PG treated rats and BG-Sr groups. However, Oc.S/BS% was significantly lower in PG treated rats group than that of BG-Sr groups and presented respectively, 0.87 and 1.2 % (Fig. 1C, 2C). On the other hand, the MS/BS had not significantly decreased in the PG treated rats groups when compared with that of BG-Sr groups and presented respectively, 1.1 and 1.2% (Fig. 1D, 2D). In fact, there were no signs of excessive mineralization or other detrimental alterations of the mineralized bone matrix. That augmentation parameter was not an indicator of a mineralization defect, because all values were evidently within the physiological range[13], but rather indicated osteoblasts activation. Moreover, those new bones had a trabecular architecture that incorporated the glass particles within the bone structure. In fact, Sr substitution had been proven to promote mesenchymal cell differentiation and osteoblast proliferation as well as bone formation.[14] Therefore, the effect of 0.1Sr W% therapy did not permanently inhibit the recovered physiological rises in bone forming activity, shortly following the cessation of antiresorptive treatment.[15] Furthermore, it was unsurprising that the experimental dose of 0.1% Sr similar to that found in natural bone stimulated a rapid matrix synthesis. On the other hand, the PG bone graft seems to enhance new bone

tissue ingrowth and reinforce the trabecular structures which may control the excessive resorption activity of osteoclasts. Herein, the measured histomorphometric parameters show that PG has an important capacity to influence both mineralization and cell activity, which is demonstrated to be successful and prompt bone healing. Herein PG presents an efficacious therapy for the bone rarefaction caused by the hormonal insufficiency. Four weeks after surgery, PG was shown to induce intensive remodeling with the highest affinity to the bone receiving BG biomaterial. The experimental data confirmed that both PG and BG-Sr materials were able to locally re-balance significantly the osteoclastic and osteoblastic activities in this induced form of osteoporosis. In this study, the PG was suitable for bone repair and regeneration because after implantation in bone defects, it was rapidly integrated into the bone structure, and then it was transformed into new bone thanks to the activity of osteoclasts and osteoblasts. The PG biomaterial filled the bone defect and permitted subsequent osteointegration. When applied to surgical femoral defects, PG generated a novel histoarchitectural order in the newly-formed bone within 4 weeks, and the spongious trabecular architecture was restored. Moreover, the decrease in bone volume fraction that is classically described between normal and osteoporotic bones[16] was not significantly modified after 30day of implantation and did not compensate efficaciously the bone microarchitectural disorder. By the end of the experiment, a very important similarity was observed between the treated femurs and the healthy ones, demonstrating a spectacular efficiency of a both PG and BG-Sr biomaterials in the considered animal.

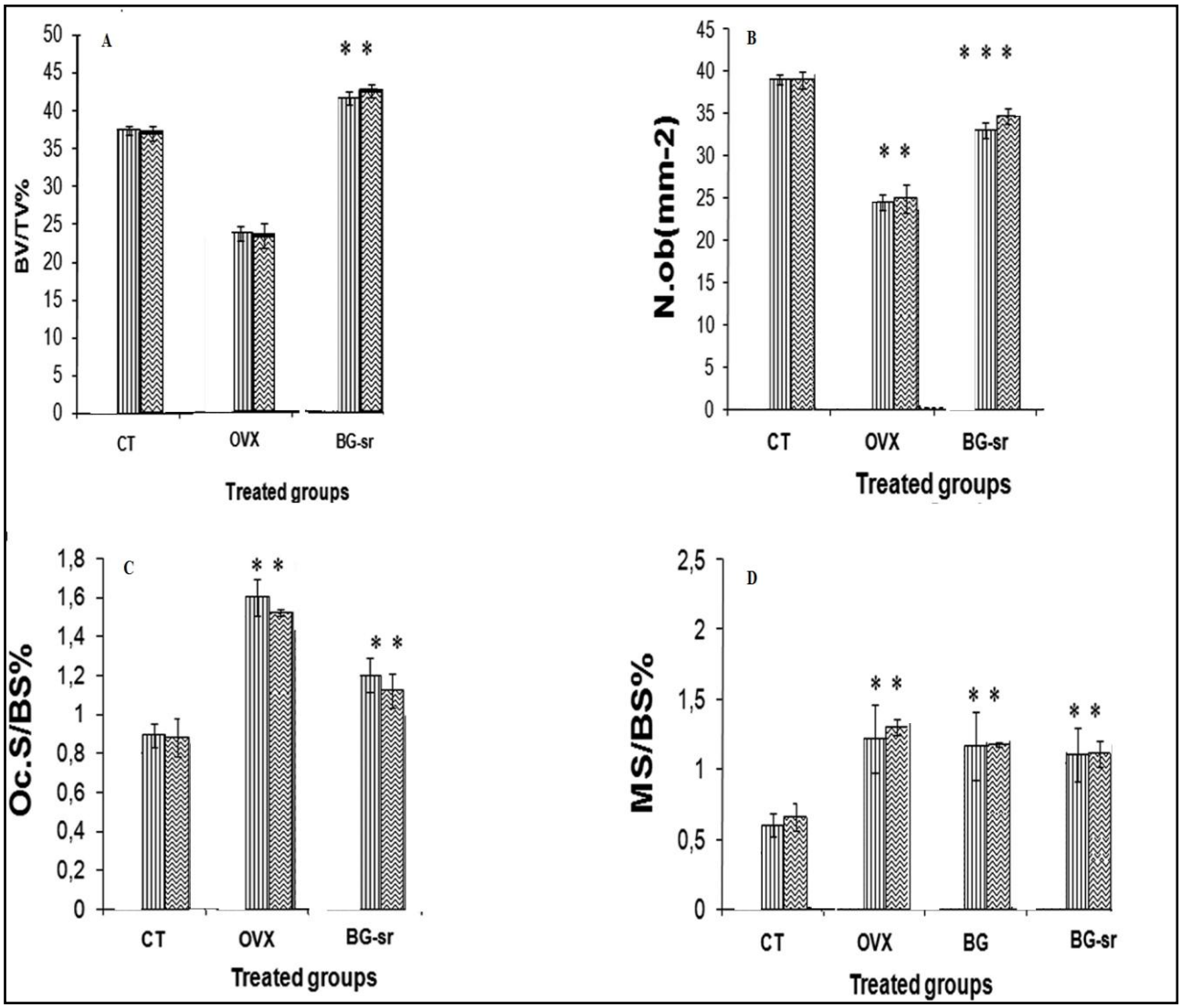

Figure 1: Determination of (BV/TV) (A), (N.Ob) (B), (Oc.S/BS) (C), (MS/BS) (D) after 30 and 60 days in control femoral condyle Wistar rats (CT), ovariectomised (OVX) and filled with strontium doped bioactive glass (BG-Sr) * significantly higher activity in the indicated group than CT. + significantly higher activity in the indicated group than (OVX) treated rats.

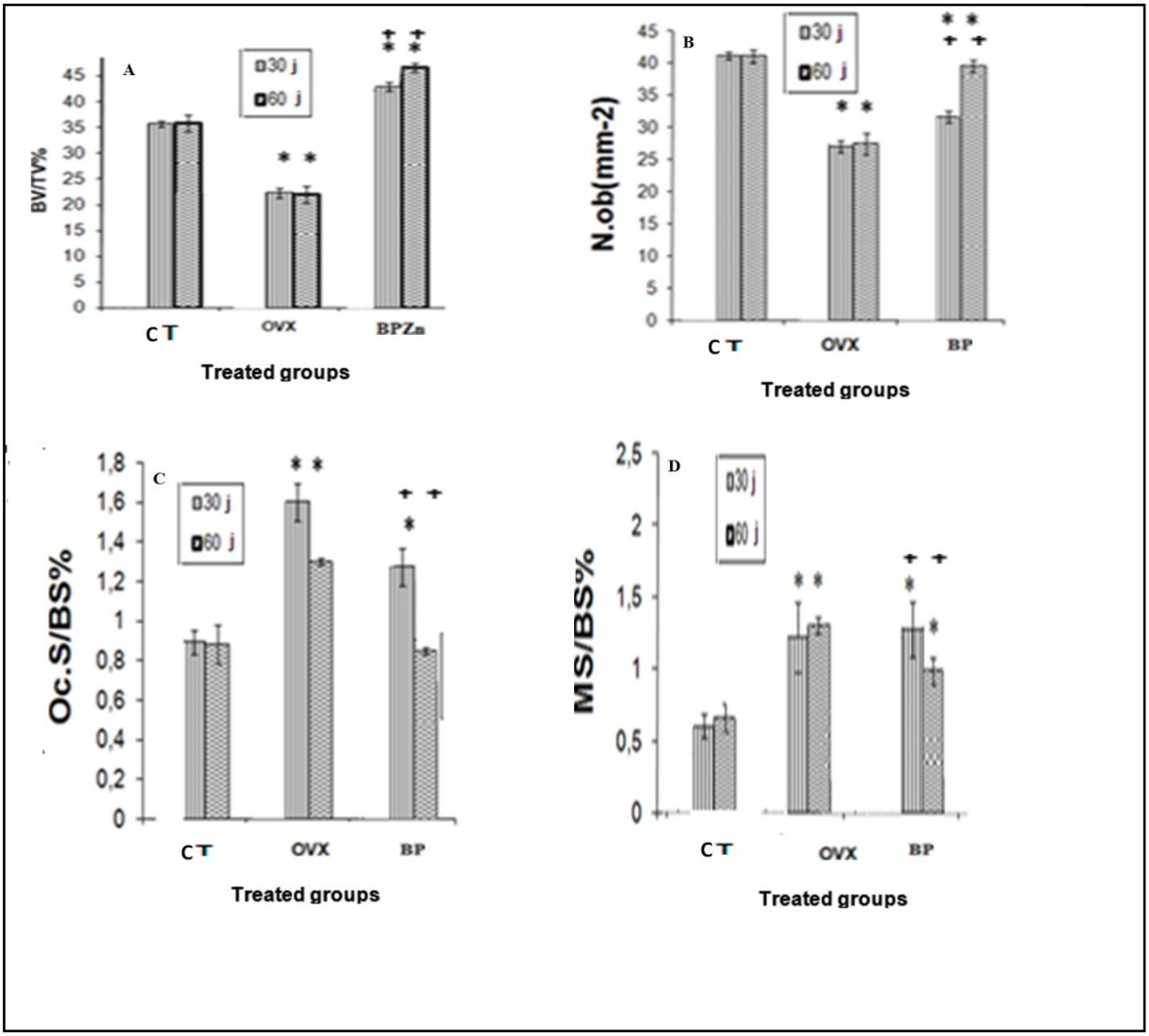

Figure 2: Determination of (BV/TV) (A), (N.Ob) (B), (Oc.S/BS) (C), (MS/BS) (D) after 30 and 60 days in control femoral condyle Wistar rats (CT), ovariectomised (OVX) and filled with porous bioactive glass (PG) * significantly higher activity in the indicated group than CT and OVX groups. + Significantly higher activity in the indicated group than (OVX) treated rats.

Conclusion

The overall results indicate a positive ceiling effect of Strontium doped bioactive glass and porous bioactive glass on bone microarchitecture. This study reveals that the all biomaterials were shown to ameliorate bonding to the bone. Furthermore, the study validates that bone mineral undergoes a maturation process. Moreover, osteoproductive properties are evidenced for both biomaterials analyzed in this study. The histomorphometric measures of the implanted bone status, in ovariectomized rats hope that PG and BG-Sr represents a promising biomaterial for bone regeneration in patients suffering from osteoporosis. This approach could be considered in the future for preventing osteoporotic fractures that are preferentially localized in the femoral condyle.

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