# Morphometric and content assessment of the bone repair in rats treated with Guar Gum and X-ray irradiation

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## Aims

Bone tissue has high regenerative capacity, and in general, is able to recover from a damage without the need of major interventions. It involves inflammatory mediators and growth factors, as well as modulating protein from osteoclasts and osteoblasts. However, there are cases in which bone loss occurs, which, depending on the extension, could compromise bone repair. Thus, the use of bone graft is an alternative to provide matrix, allowing osteoblasts migration to form the organic matrix and, consequently, bone tissue repair. Materials that assist in the development of a healthy bone matrix have been studied to be used as graft, such as the polysaccharide derived from Guar Gum (PGG), which is a type of proteoglycan. Proteoglycans are connected with glycosaminoglycans (GAGs) that seems to affect the differentiation of progenitor mesenchymal cells into osteoblasts. It was observed that the osteoblasts treatment with GAG suppressed the ability of these cells to support the osteoclast differentiation, as well as their bone reabsorption activity. Natural polysaccharides with GAG structure have an effect on the inflammatory response. A PGG which reproduces the glycosaminoglycan structure of the extracellular matrix of articular cartilage and has protective properties of the cartilaginous tissue in the experimentally induced osteoarthritis has been developed and patented by UFC (02/09/2010 – nº PI1003401-3). The study of the effect of PGG application may represent the development of a new matrix to accelerate and optimize the repair of bone damages. The treatment used for bone neoplasia, in many cases, involves radiotherapy as an additional method. Despite of the high rates of success of this therapy, it must be highlighted the deleterious effects of radiation on the bone repair process. Studies have found that radiation induces an increase in osteoclastic activity, promoting substantial changes in morphology and bone strength and delayed bone repair. Considering that the search for materials that help in the bone regeneration process is very valuable, the aim of this study was to evaluate the effect of local administration of a polysaccharide derived from Guar Gum (PGG) solution on the repair process of bone defects in tibiae of irradiated rats.

## Method

Forty rats (*Rattus norvegicus*, Wistar Albinus), adults, males were divided into 4 groups based on treatment and on the use of irradiation (n=10): Control (Group 1), PGG administration (Group 2), Irradiation nontreated (Group 3), Irradiation + PGG administration (Group 4). Bone defects were produced on the right tibiae, with a round carbide drill #8 at low speed and PGG solution was administrated in these defects. Three days before the bone defect execution, the

animals on groups 3 and 4 were submitted to a single dose of 15 Gy of X-radiation. Twenty-one days after irradiation, all rats were sacrificed and their tibiae were removed.

The tibiae were scanned into a cylindrical plastic tube filled with water by Skyscan 1174 microCT system (Bruker, Belgium) using an aluminum filter of 0.5 mm, X-ray voltage of 50 kV, X-ray current of 800 µA, 0.5° rotation step, averaging (frames) of 2, 180° scanning and 6,4 µm of pixel size in a way that the defect was centralized in the scanned bone region. The acquired images were reconstructed by NRecon software (Bruker) and analyzed by CTAn software (Bruker). The volume was standardized on 400 axial images which fitted the repair region in the same amount in the upper and lower direction from the center of the repair (Figure 1A). The ROI was standardized in 2.3 mm of width and 0.8 mm of height based on the size of the bone defect preparation (Figure 1B) ensuring that all repair region was into the ROI. So, a rectangular VOI including the region of repair was created. Then, the morphometric parameters were analyzed. The analysis of the samples' mineral content was done by two-dimensional mapping using X-ray microfluorescence (µXRF). The results of morphometric parameters were statistically analyzed using ANOVA test and *post hoc* Tukey test**.** The level of significance was set at 5%.

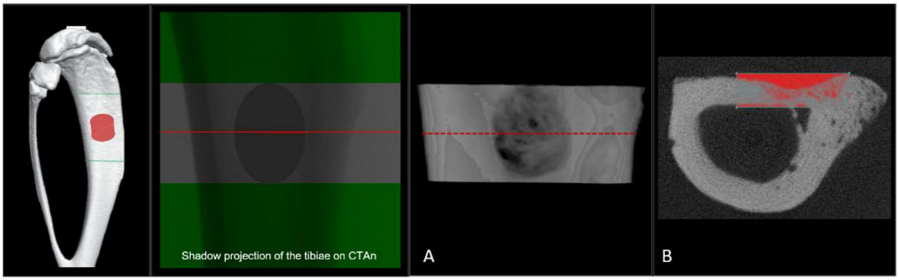


Figure 1: Shadow projection of the tibiae on CTAn. A. Volume composed by 400 axial images. B. Region of Interest.

## Results

The PGG group presented better morphometric parameters regarding the bone repair process (Figure 2).

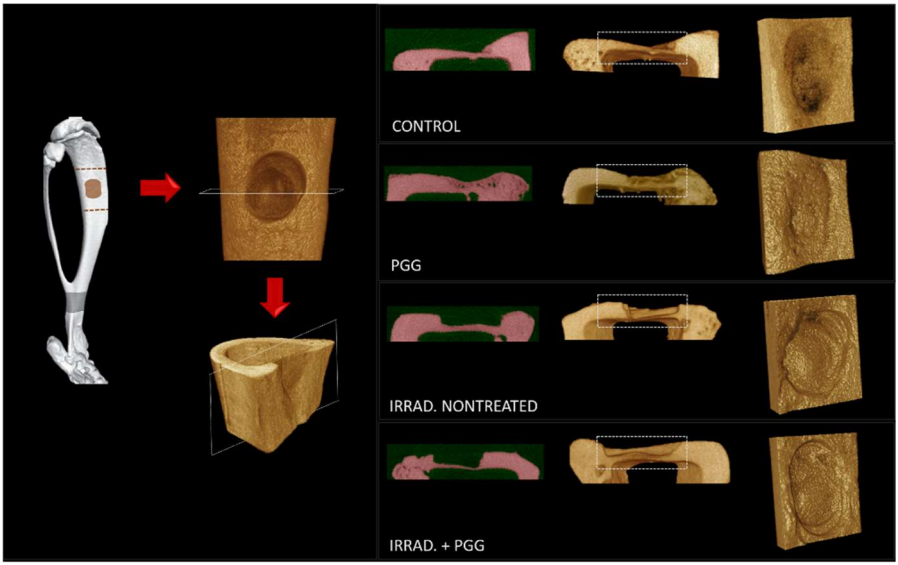


Figure 2: Slices, rendered volumes of repair regions and final VOI assessed for each group.

The PGG group presented statistically significant higher bone volume and number of closed pores ($p$<0.05) compared with control, irradiated nontreated and irradiated + PGG groups. Bone surface density was statistically significant lower on PGG group compared with control group ($p$<0,05) (Figure 3).

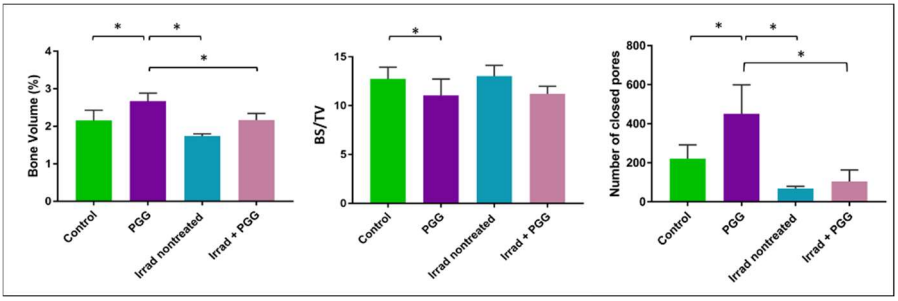


Figure 3: Graphs showing Bone Volume (%), Bone surface density and Number of closed pores for each group. An asterisk (*) indicates statistically significant difference between groups.

The mineral content mapping by μXRF from control and PGG groups showed that the elements calcium and phosphor presented higher concentration in the center of the repair region in PGG group. It was also possible to notice that the potassium distribution is homogeneous in both groups (Figure 4).

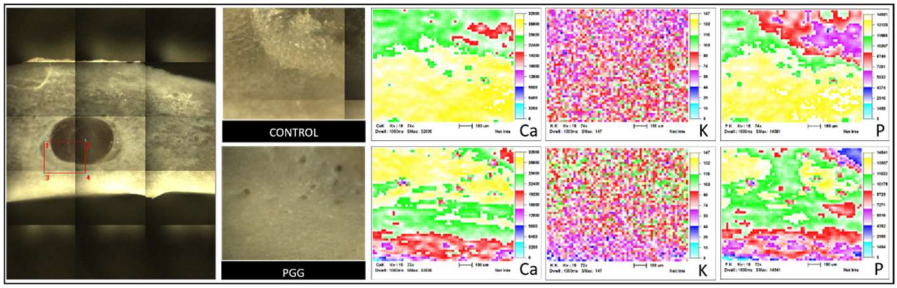


Figure 4: Calcium, Potassium and Phosphor mapping by μXRF from control and PGG groups.

## Conclusion

We concluded that PGG improves the bone regeneration process in non irradiated rats.

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