11th International Conference on Advanced Materials
Rio de Janeiro Brazil
September 20 - 25
ICAM2009

Osteointegration of poly-(3-hydroxybutyrate-*co*-3-hydroxyvalerate) pharmacological scaffolds incorporated with violacein: an *in vivo* study

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Abstract – This work reports on the production and *in vivo* evaluation of scaffolds of poly-(3-hydroxybutirate-*co*-3-hydroxyvalerate) incorporated with violacein (PHBV-vio). PHBV-vio was sinthesized in a bioreactor by *Chromobacterium violaceum*, pressed and partially sintered to produce scaffolds with 3mm of diameter and 3mm of height. The scaffolds were microstructurally characterized, implanted in femur of Wistar rats, extracted and analyzed after 30-60 days of surgery. Histological evaluation revealed that no inflammatory reaction occurred and new bone tissue was formed in the implant, without any signal of infection. The results indicated that PHBVs with violacein are potential candidates for application in regenerative bone tissue engineering.

Biopolymers have attracted great interest as alternative materials for wound and lost tissues treatments [1]. The objective of this work is the study of the interaction between biological tissues and scaffolds with an incorporated antibiotic, violacein, produced from biosynthetic polyhydroxyalkanoates, which are biodegradable and bioresorbable polymers [1,2].

Scaffolds of poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) incorporated with violacein (PHBV-vio) were produced in a bioreactor by *Chromobacterium violaceum* [3]. The material was pressed and partially sintered to produce pharmacological scaffolds with 3 mm of diameter and 3 mm of height. The scaffolds were implanted in femur of Wistar rats according to a protocol developed in this work. The PHBVs were extracted and analyzed after 30 and 60 days of surgery. Microstructural characterization and chemical analysis to determine the inorganic constituents and the Ca:P ratio of the implanted biopolymers were evaluated by scanning electron microscopy (SEM) coupled with X-ray dispersive energy spectroscopy (EDS). Osteointegration was evaluated by optical microscopy observations on histological sections of the implants (5 µm thick) stained with hematoxilyn-eosin and Tricromic of Masson.

The microstructure of the scaffolds is characterized by a homogeneous surface and pre-sintered grains with sizes of approximately 30 µm. The microstructure of the scaffolds is characterized by interconnected pores with sizes between 50 and 100 µm and open porosity of 61 ± 5% (Figure 1). These features are necessary for cell migration and osteogenesis in the implant [4]. After 30 days the presence of 5wt.% of Ca (bone mineral matrix) was detected by EDS in the center of the implant, which implies that osteointegration occurred even 1.5 mm distant from the bone/implant interface (Figure 2). Histological evaluation revealed that no inflammatory reaction occurred and new bone tissue was formed in the implant (Figure 3). The arrows indicate the new formed bone matrix. No post-operative infection was observed. The results indicated that PHBVs with violacein are potential candidates for application in regenerative medicine, especially in bone tissue engineering.

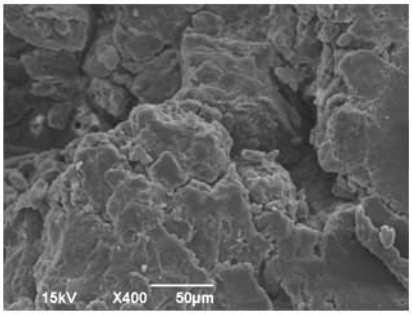

Figure 1: SEM micrograph of the PHBV-vio scaffold.

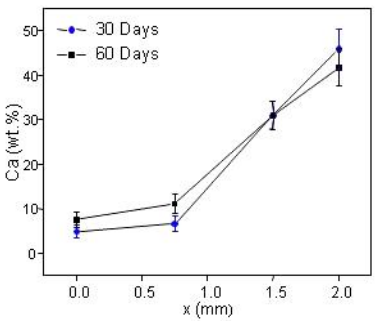

Figure 2: Ca content *versus* the distance from the center of the implant, x.

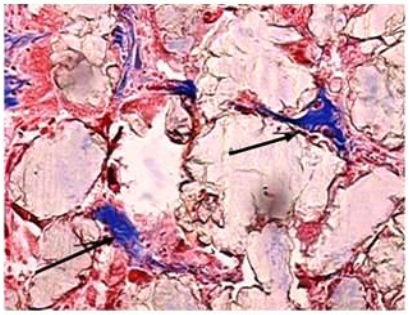

Figure 3: Histological section of the implant after 30 days.

References

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