

## Characterization and Cytocompatibility of Doxycycline Composite Scaffolds

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**Statement of Purpose:** Composite scaffolds of biodegradable polymers and bioceramics are an alternative in the tissue engineering area for bone regeneration have been widely-used as bioreabsorbable material. Doxycycline (DOX) is a large spectrum antibiotic, has wide use as an aid in periodontal therapy. Nevertheless, it is important to prolong their therapeutic effect to allow the regeneration of periodontal tissues. In this work the aim was to evaluate composites of polycaprolactone (PCL), biphasic calcium phosphate (BC) loaded with DOX to evaluate their biocompatibility and activity on osteoblasts cells.

**Methods:** The BC/PCL composite was prepared using the dual-phase mixing method. The PCL was dissolved in dichloromethane at 1.25, 2.5 and 3.75% w/v under stirring for 1 h at room temperature and then added the biphasic calcium phosphate Osteosynt® (BCP) with granulometry varying from 60 to 80 Mesh, 65% of hydroxyapatite- $\text{Ca}_5(\text{PO}_4)_3\text{OH}$  (w/w)- and 35% of  $\beta$ -tricalcium phosphate ( $\beta$ -TCP)- $\text{Ca}_3(\text{PO}_4)_2$  (w/w)- was gently supplied by EInco Biomaterial, Brazil: BCP/PCL = 0.25, 1 and 4 w/v remained under agitation for 6h at room temperature. Finally, DOX was added in the ratio DOX/(BCP + PCL) = 0.1 w / w, being in agitation for 24h. Thus, nine composites were produced with different concentrations.

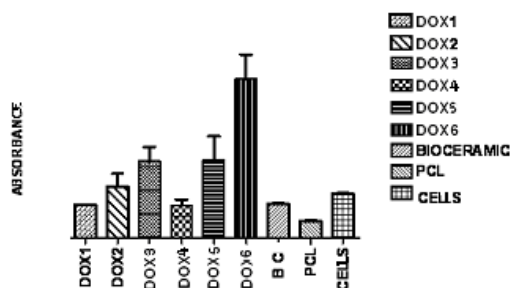
Characterization studies: composites and free substances were characterized by FTIR and X ray powder diffraction method. The absorption spectra in the infrared region of 4000-400  $\text{cm}^{-1}$ , FTIR were obtained from KBr pellets and recorded on Perkin Elmer Espectrum GX spectrophotometer. The X-ray powder diffraction patterns of the samples were obtained in a Rigaku Geigerflex 2037 diffractometer, using copper pipe and  $\text{CuK}_\alpha$  ( $\lambda = 1.54051$ ) radiation in the  $2\theta$  angles ranging from 4 to 60 degrees and a scan rate of 40  $\text{min}^{-1}$ , the voltage and current per step were 30kV and 5 mA, respective<sup>29</sup>.

### MTT-based cytotoxicity assay

Osteoblasts proliferation was evaluate using the methyl-thiazol-diphenyl-tetrazolium (MTT) assay for cell viability/proliferation. The MTT assay is based on the reductive cleavage of yellow tetrazolium salt to a purple formazan compound by the dehydrogenase activity in intact mitochondria by viable cells. 37°C, The osteoblasts were plated ( $10^6$  cells/ml) at 5% CO<sub>2</sub> for 48h. DMEM medium containing BCP, PCL, BCP/PCL and the BCP/PCL loaded with DOX were then distributed to the wells in triplicate. After 48 h of incubation the medium was aspirated, the cells washed twice with PBS and the MTT assay was carried out. The optical density was determined at 595 nm on a plate reader (ELISA Thermo Plate), and data was expressed as absorbance. Results were analyzed statistically using variance test (ANOVA). Differences were considered significant at  $p < 0.05$ .

**Results:** XRD patterns of PCL and BC showed a semi crystalline profile. Diffractograms of composites PCL/BCP, PCL/BCP/DOX, represent the overlapped of the patterns of free compounds. The FTIR spectrum of BC shown a strong absorption peak at 3500  $\text{cm}^{-1}$  assigned to  $\nu\text{OH}$ , a peak at 1050  $\text{cm}^{-1}$  attributed to  $\nu_{\text{as}} \text{PO}_4^{3-}$  and the peaks 960; 600 and 570  $\text{cm}^{-1}$  assigned to  $\nu\text{S-P-O}$  from  $\text{PO}_4^{3-}$  ions. The PCL FTIR spectrum shown a peak at 2949  $\text{cm}^{-1}$  and 2865  $\text{cm}^{-1}$  assigned to  $\nu_{\text{as}}$  and  $\nu_{\text{S-CH}_2}$  respectively. At 1727  $\text{cm}^{-1}$  was observed a peak assigned to carbonyl stretching vibration and in 1170  $\text{cm}^{-1}$  a peak of the  $\nu\text{C-O-C}$ . The PCL/BCP/DOX composites shown that the spectrum followed of original of free compounds. The FTIR spectra of these scaffolds suggests that the composite is a solid solution of free compound and did not show new peaks in the spectra.

The results of cells proliferation are shown in the Fig 1. Cultures grown in control condition showed a significant increased growth in compare with the control cultures. The viability and proliferation of osteoblasts onto BC/PCL loaded with DOX were greater than in the control, reflecting the osteoconductive property of BCP. DOX 6, as well as containing a higher concentration of the drug and PCL in his groups, also contained higher amount of bioceramics ( $p < 0.05$ ). In MTT assay, osteoblasts grown in proportion to the drug concentration. The exposure to doxycycline caused evident induction of cell proliferation from the first 48 h.



**Fig.1.** Proliferation of in vitro cultured osteoblast cells: composites of BC/PCL loaded with DOX after MTT assay

**Conclusions:** The results indicate that Dx even at high concentrations was not cytotoxic, and increases cell proliferation.

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