Biocompatibility and Bioactivity of Novel Composite for Prosthesis Femoral Stems

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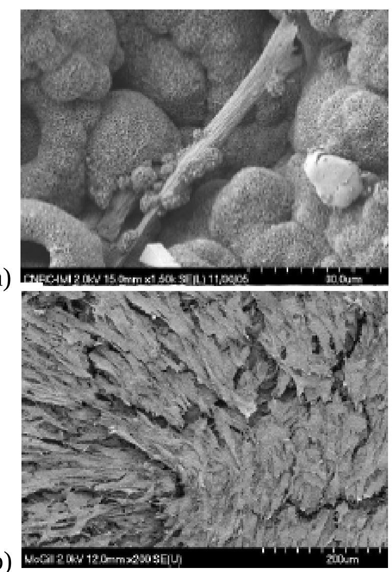
Statement of Purpose: Although survival rates of total hip replacement (THR) are acceptable for middle-aged or elderly patients, lower survival rates for younger patients and higher life expectancy call for improved longevity THR prosthesis. As introduced in previous works [1,2], we have developed a novel THR stem based on carbon fiber-reinforced polymer composite with an atmospheric plasma sprayed (APS) bone-like, bioactive hydroxyapatite (HA) coatings. Such coatings are already used successfully for metallic implants, but they have yet to be developed for temperature sensitive polymer-based materials. The aim of this study is to investigate the *in vitro* effects of HA coated polymer composite on osteoblasts and fibroblasts differentiation, morphologies and adhesion; as well as the cytotoxicity of the base materials employed for the THR prosthesis stem expressed on fibroblasts.

Methods: The MTT assay was performed with the extracts of the polymer composite in order to evaluate the short-term effects of the degradation or leachable products. The cell viability of L929 mouse fibroblast cell line was analyzed after direct contact with the THR polymer composite by the Alamar Blue assay. Fibroblasts adherence and morphology were qualified through Field Emission Gun Scanning Electron Microscope (FEG-SEM) observations. Also, osteoblasts were isolated from the calvariae Spargue Dawley rat fetuses [3]. The alkaline phosphatase (ALP) activity test was performed after 15 days of osteoblasts cells cultured on the HA coated polymer composite with the use of tissue culture plate (TCP) as negative and APS treated medical Ti as positive control. Using the same positive control, FEG-SEM observations were used to determine the adherence, morphology and growth of the osteoblasts on the HA coated polymer composite at different time points.

Results/Discussion: MTT results showed that the polymer composite extracts had no effect on the viability, morphology and proliferation of L929 fibroblasts up to 3 days in culture. While they indicate the lack of generation of degradation or leachable products, the conditions employed (250 rpm agitation for 24 h at 37°C) to obtain these results cannot be considered as representative of an *in vivo* environment with mechanical constraint. The indirect cytotoxicity contact (MTT) tests were complemented with direct contact tests by culturing L929 fibroblasts directly on the polymer composite. A material-dependent proliferation was quantified by the Alamar Blue assay; the HA coated polymer composite reduced the metabolic activity of L929 fibroblasts to 87% of control and the polymer composite supported 118% of control metabolic activity at day 3.

The HA coated polymer composite was then evaluated to determine the stem material bioactivity potential. The ALP activity test after 15 days was quantified by color image analysis. The HA coated composite resulted in 36% increase in ALP activity (t-test, $p=0.007$) as compared to identically APS treated HA coated medical Ti substrate. This intimate response was qualified by FEG-SEM. The novel APS coated polymer composite not only supported healthy attachment and spreading but it also showed partly covered osteoblast cells by mineral concretion (Fig. 1a), associated to apatite crystal precipitation from the culture media [2]. After 15 days, osteoblast cells also covered the entire surface of HA coated polymer composite (Fig 1b). This different tendency of fibroblasts and osteoblasts adhesion and spreading could be related to the surface roughness, as several studies show that fibroblasts spread more on smooth surfaces than roughened surfaces, whereas studies on osteoblasts describe preferences for rougher surfaces. It is not possible to rule out the effect of surface chemistry as a factor affecting fibroblasts and osteoblasts.



a) b)

Fig 1. Osteoblast morphology after a) day 1 and b) day 15.

Conclusions: Our *in vitro* results indicate that the HA coated composite surfaces have the potential to improve osseointegration and soft tissue adhesion. The ability of the base polymer composite to support fibroblast activity *in vitro*, combined with its compatible extracts suggests potential use of these composites as load-bearing bone biomaterials.

References: 1. Campbell M, Bougherara HA, Bureau MN, Denault J, Yahia L'H ASM Mater. Proc. Med. Dev. 2005, Boston, MA (USA), Nov. 14-16 2005. **2.** Auclair-Daigle C, Bureau MN, Legoux J-G, Yahia L'H, J Biomed Mater Res A. 2005;73(4):398-408. **3.** Bellows CG, Aubin JE, Heersche JN, Antosz ME, Calcif Tissue Int, 1986;38:143-154.