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Endothelization of a multilayered bacterial cellulose-hydroxyapatite tissue engineering scaffold

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Abstract – Multilayer bacterial cellulose-hydroxyapatite hydrogel scaffolds were synthesized by a biomimetic method. Bacterial cellulose hydrogel was soaked in alternate solutions of $CaCl_2$ and Na_2HPO_4. The Ca:P ratio in the final composite was determined by energy dispersive spectroscopy. The morphology and distribution of hydroxyapatite particles on the bacterial cellulose was analyzed by scanning electron microscopy, which revealed that hydroxyapatite was deposited on the surface and homogeneously distributed within the cellulose nanofibers. Human endothelial cells were used to evaluate the biocompability of the composite. Scaffold internal structure and surface properties were adequate for endothelial cell attachment and growth.

Angiogenesis includes endothelial cells migration, proliferation and differentiation to form new capillaries (tubulogenesis), and vascular wall remodeling [1]. Vascularization of the region of bone growth is an important factor that determines production of cartilage (chondrogenesis) and formation of bone (osteogenesis) [2], and is required for the success of bone regenerative medicine with biomaterials. Bacterial cellulose (BC) attracts great interest for numerous medical and tissue engineering applications due to its unique nanostructure and properties, including biocompability, high water retention capability, high crystallinity, ultrafine structure which allows cell growth and proliferation and high tensile strength [3,4]. On the other hand, synthetic hydroxyapatite (HAp), which is similar to bone apatite, has been used as a biomaterial to solve several biomedical problems, particularly due to its biocompatibility and osteoinductive properties.

In order to obtain a biomaterial based on nanofibrous BC and HAp, to be used in bone regenerative medicine, multilayered hydrogels of BC produced by *G. hansenii* ATCC 23769 were used as templates for precipitation and crystallization of HAp (Figure 1), by soaking the hydrogels in alternated solutions of $CaCl_2$ and Na_2HPO_4. HAp crystal growth on cellulose is feasible and has been previously reported [5]. The Ca:P ratio in the final composite was 1.47, determined by energy dispersive spectroscopy, which is lower than the Ca:P ratio for stoichiometric hydroxyapatite (1.67). HAp prepared from direct precipitation of aqueous solutions usually exhibit lower Ca:P ratios (1.33–1.66) [6]. Human endothelial cells (HUVEC) were seeded on the composite surface by immersing the multilayered hydrogels in culture medium (RPMI, with 10% fetal bovine serum) containing approximately 80,000 cells. The morphology and distribution of HAp particles on the nanofibers of the multilayered BC were analyzed by scanning electron microscopy (SEM), which revealed that HAp was deposited on the surface and homogeneously distributed within the cellulose nanofibers (Figure 2). Human endothelial cells were further seeded and bioincorporated within the composite. It was observed that the cells were attached on the multilayered surface (Figure 3), indicating that the scaffold internal structure and surface properties were adequate for endothelial cell attachment and growth and can be used in bone regenerative medicine applications.

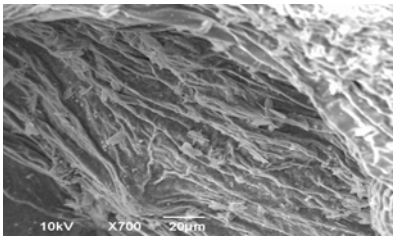

Figure 1: Multilayer bacterial cellulose-hydroxyapatite composite, SEM (700×)

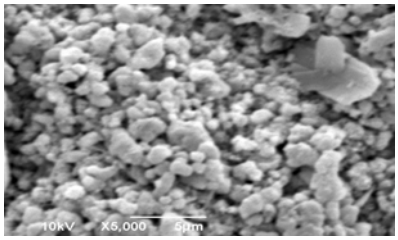

Figure 2: HAp particles on the nanofibers BC, SEM (5000×).

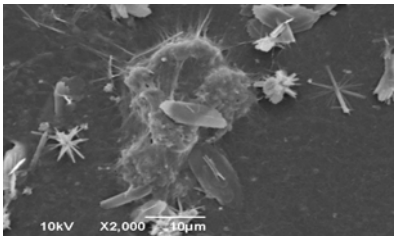

Figure 3: Human endothelial cells on biocomposite, SEM (2000×).

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