Engineering Micropores in Nanoparticle-bacterial Cellulose Scaffolds using a Laser-cutting Instrument: Preparation, hMSCs Proliferation and Osteogenic Differentiation for Bone Tissue Regeneration

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Abstract

Statement of purpose: Advanced biomaterials that can mimic the properties of native tissue to possess antibacterial properties, deliver drugs, and permit stem cells to adhere and differentiate are of paramount importance in the development of stem cell therapies for bone defects. Successful bone repair approaches may include an osteoconductive scaffold that permits excellent cell adhesion and proliferation, and cells with osteogenic potential. The objectives of this study were to: (1) prepare and characterize reproducible microporous nanoparticle-bacterial cellulose scaffolds using a laser-cutting instrument, and (2) evaluate cell proliferation, viability and osteocyte differentiation of human-derived bone marrow mesenchymal stem cells (hMSCs) when seeded onto such scaffolds.

Materials and methods: Biocompatible gel-like bacterial cellulose (BC) was synthesized using the bacterium *Gluconacetobacter sucrofermentans* under static culture [1]. To introduce controlled and highly reproducible micropores that will permit diffusion of nutrients into the bacterial cellulose scaffold, a laser-cutting instrument was used. CdHAP was deposited in the scaffolds to mimic native bone tissues. To improve the antimicrobial properties of the scaffold, silver nanoparticles (AgNPs) were added to one batch of the scaffolds. To assess the potential of the BC scaffold for potential nanoparticle-based drug and gene delivery applications, gold nanoparticles (AuNPs) were added to another batch of the scaffolds. The chemical, mechanical and morphological properties of the resulting composites were characterized using Fourier transform infrared spectroscopy (FTIR), mechanical testing instrument, and scanning electron microscopy (SEM), respectively. *In vitro* proliferation and viability of hMSCs (Lonza, Basel, Switzerland) on BC scaffolds and its composites were analyzed using phase contrast and fluorescent microscopy, and the colorimetric MTS assay. Additionally, the osteocyte differentiation potential of hMSCs on the scaffolds was characterized using alizarin red staining. All experiments were conducted in triplicate and repeated at least three times.

Results: Reproducible microporous BC scaffolds were generated using a laser-printing instrument (Fig. 1). hMSCs proliferated and formed multilayers on the microporous BC scaffolds, compared to cells grown exclusively on the surface of native, non-porous BC scaffolds. The various forms of BC hydrogel scaffolds exhibited distinct differences in the rate of proliferation and *in vitro* differentiation. Compared to native BC, modified BCs with only CdHAP did not affect the rate of cell proliferation after 7 days, whereas modified BCs with high concentrations of nanoparticles (AuNP > 25 μg/mL, AgNP > 5 μg/mL) were cytotoxic and reduced the rate of cell proliferation after 7 days. AgNPs indicated higher cytotoxicity than AuNPs. Cell-scaffold constructs were cultured for 14 days under osteogenic conditions and the resulting osteocytes were positive for alizarin red.

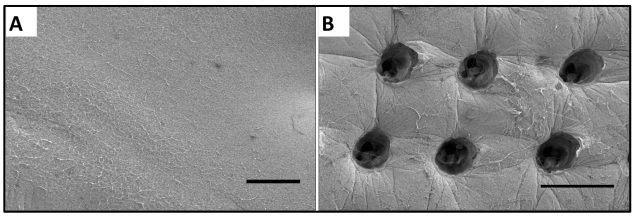

Figure 1. Scanning electron micrographs of native, non-porous BC scaffold (A) and microporous BC scaffold generated using a laser-cutting instrument (B). Scale bar = 500 μm.

Conclusions: In summary, reproducible microporous BC scaffolds were successfully generated using a laser-cutting instrument. Biocompatible and microporous nanoparticle-bacterial cellulose scaffolds supported the proliferation, viability and osteogenic differentiation of hMSCs cultured onto its surface *in vitro*, allowing for their future potential use for biomedical engineering therapies. Although the rates of cell proliferation and differentiation potentials varied between the scaffolds, cells were viable and underwent osteogenesis on the modified surfaces demonstrating their significant potential for orthopedic tissue engineering.

Reference:

[1] Favi PM. Mat Sci Eng C. 2013;33:1935-44.

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