Designing Degradable Microporous Bacterial Cellulose Scaffolds and its Biomimetic Composites for Bone and Cartilage Tissue Engineering

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Statement of Purpose: Bacterial cellulose (BC) is a natural polysaccharide with promise as a scaffold for tissue engineering. It is synthesized extracellularly by non-pathogenic bacterium such as *Gluconacetobacter sp* in the form of a three-dimensional nanofibrous hydrogel. BC is highly pure and crystalline cellulose with high mechanical strength, hydrophilicity and biocompatibility. We have previously demonstrated that native BC scaffolds support the proliferation and viability, osteogenic and chondrogenic differentiation of equine-derived bone marrow mesenchymal stem cells (EqMSCs) cultured onto its surface *in-vitro*. Native BC however, is nanoporous and non-degradable *in vivo*. Therefore, in this study, biodegradable microporous BC and its biomimetic composites were prepared to render the scaffold more suitable for bone and cartilage tissue engineering.

Methods: Natural wax particles were placed in the BC growing culture to prepare microporous BC (*1*). BC was then modified using a periodate oxidation to yield dialdehyde cellulose which degrades at physiological pH (*2*). The scaffolds were prepared to mimic native bone and cartilage. Calcium-deficient hydroxyapatite was biomimetically deposited in the scaffolds, and amination and carboxylation of scaffolds were performed to mimic bone and cartilaginous tissues, respectively, in the animal body (*2, 3*). Resulting scaffolds were then characterized for their ability to support and maintain growth, proliferation, osteogenic and chondrogenic differentiation of EqMSCs *in vitro*. These parameters were compared to cells grown on tissue culture treated plastic (TCP).

Results: EqMSCs formed multilayers on the BC scaffold surface (Fig 1 and 2). Various forms of BC hydrogel scaffolds exhibited distinct differences on the rate of proliferation and *in vitro* differentiation potential of EqMSCs. Compared to native BC, non-degradable modified BCs did not enhance the rate of cell proliferation after 14 days. However, degradable modified BCs did enhance the rate of cell proliferation after 14 days compared to native BC. Overall, cells cultured on the degradable modified BCs showed excellent extension, adhesion and presented the best osteo- and chondrogenic potential for differentiation. Although proliferation rate and differentiation potentials varied between the scaffolds, cells were viable on all modified scaffolds.

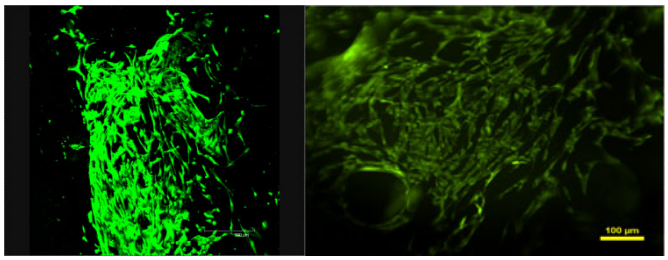

Figure 1. Two-dimensional confocal microscopy image (left) and fluorescent microscopy image (right) views of degradable microporous BC and EqMSCs stained using calcein-AM and illustrating cellular adhesion and viability.

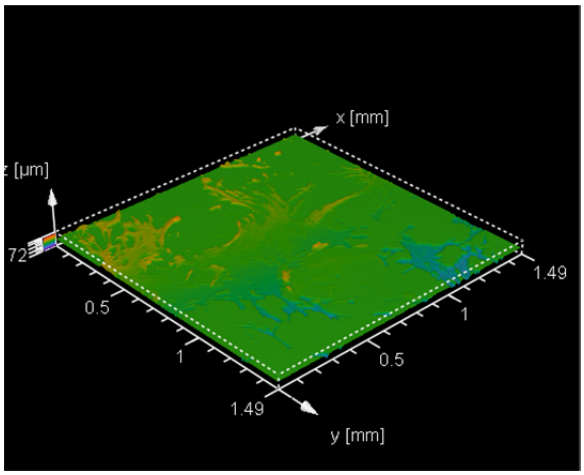

Figure 2. Three-dimensional confocal microscopy image view of degradable microporous BC and EqMSCs stained using calcein-AM.

Conclusions: These findings demonstrate that native and specifically modified BC scaffolds are promising constructs for bone and cartilage tissue engineering therapies.

References:

1. H. Bäckdahl, M. Esguerra, D. Delbro, B. Risberg, P. Gatenholm, *Journal of Tissue Engineering and Regenerative Medicine* **2**, 320 (2008).
2. S. Hutchens, R. Benson, B. Evans, C. Rawn, H. O'Neill, *Cellulose* **16**, 887 (2009).
3. K. Watanabe *et al.*, *Cytotechnology* **13**, 107 (1993).