Hydroxyapatite bioactivated bacterial cellulose as a scaffold for tissue engineering

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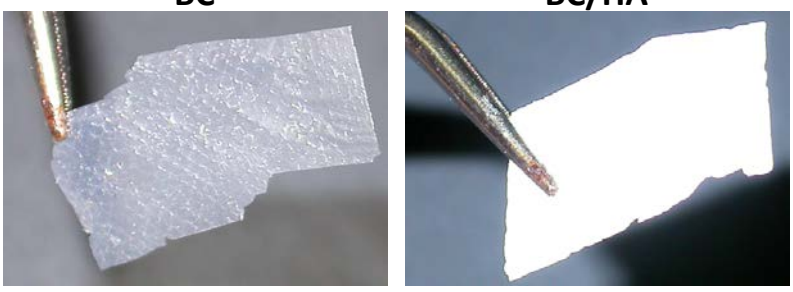
Introduction: Developing effective bone regeneration therapy is a clinically important long-term goal. Bone loss caused by trauma, neoplasia, reconstructive surgery, congenital defects, or periodontal disease is a major health problem worldwide (Franceschi 2005). Clearly, there is a need for safe, effective methods to replace and promote bone regeneration. Advances in bone regeneration therapy will require innovative molecular biology and tissue engineering-based technologies. The regeneration of complex bone structures such as joints, craniofacial structures, or even entire bones and teeth will involve vastly more complex challenges including appropriate scaffold (Bitar et al., 2008). One of the materials that can be used to design bone scaffold can be Bacterial cellulose (BC). This is produced from *Acetobacter xylinum*, and is a biocompatible polymer with excellent physical and chemical properties characterized by high tensile strength, elastic modulus and hydrophilicity (Helenius et al., 2006). Morphologically, the fibrous structure of BC is similar to the collagenous fibers of bone. These characteristics support BC as a useful scaffolding material in regenerative medicine (Andrade et al., 2010). However, study of BC as a bone regeneration scaffold is preliminary and the data are limited. The purpose of this study was to investigate whether BC and BC-hydroxyapatite (HA) can promote osteoblast growth and bone nodule formation.

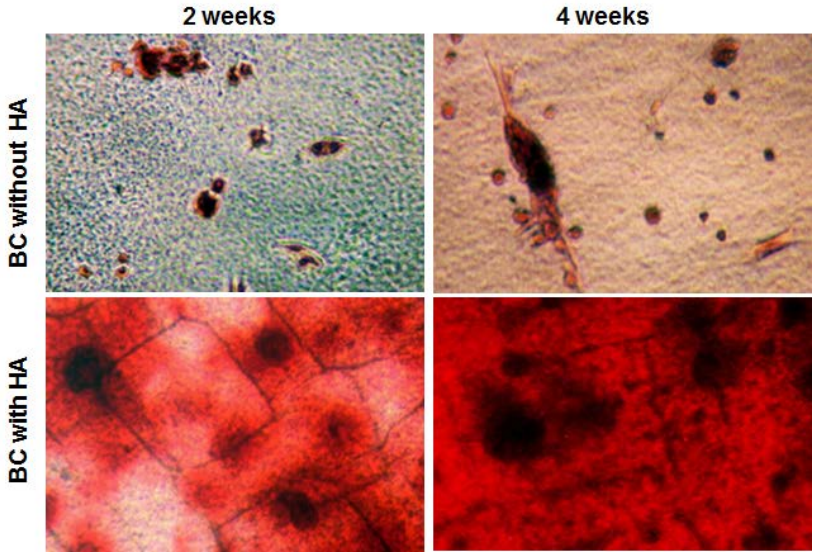
Materials and Methods: *Acetobacter xylinum* (ATCC 52582) was cultivated for 120 h at 28ºC in static culture. Bacterial cellulose pellicles were harvested and cleaned, then activated by the addition of Hydroxyapatite (HA). To do so, BC membranes were incubated in a solution of $CaCl_2$ (11 g/L) followed by an incubated in a Na_2HPO_4 solution. A second method was also used to introduce HA to the BC polymer. BC pulp of 10% cellulose was mixed with HA at a 0.33% final concentration (Varma et al., 2005). The mixture was then vigorously stirred, poured into rectangular molds and dried at room temperature before use. BC polymer with and without HA was subjected to X-ray photoelectron spectroscopy (XPS) analysis, and used for in vitro osteoblasts cultures.

Results: BC hydrogel appears as a 3-dimensional thick membrane showing a thin, opaque white BC membrane ***(Fig. 1)***. XPS analysis revealed the presence of both calcium (10%) and phosphate (10%) at the surface of the BC/HA membrane.



Osteoblast culture showed that BC alone was not toxic and could sustain osteoblast adhesion. Furthermore, osteoblast adhesion and

growth were significantly ($p \leq 0.05$) increased on BC/HA membranes as compared to BC alone. Both BC and BC/HA membranes improved osteoconductivity, as confirmed by the level of alkaline phosphatase (ALP) activity that increased from 2.5 mM with BC alone to 5.3 mM with BC/HA. BC/HA membranes also showed greater nodule formation and mineralization than the BC membrane alone ***(Fig. 2)***. This was confirmed by Alizarin red staining (ARS) and energy dispersive X-ray spectrosco py (EDX).



Conclusion: Together, these data suggest that BC could be an appropriate support for bone tissue engineering. With the incorporation of active molecules such as HA, BMP2, the osteogenic potential of bacterial cellulose polymers may be optimized for multiple tissue engineering tools.

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