Bioabsorbable Hydrogel Made of Hydroxyapatite Functionalized Microspherelike Bacterial Cellulose Particles for Cartilage and Bone Tissue Regeneration

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Statement of Purpose: A novel hydrogel based on microspherelike bacterial cellulose particles and hydroxyapatite that could be applied to cartilage and bone tissue regeneration was developed in this work. The unique microspherelike structure of bacterial cellulose was synthesized in the orbitally agitated culture by the specific cellulose producing bacteria *Gluconacetobacter xylinum* (JCM 9730 strain, ATCC 700178). The use of current tissue scaffolds can promote adhesion, migration and proliferation of cultured cells. However, limitations caused by immune rejection and non-degradation still exist. The incorporation of cellulases into hydroxyapatite functionalized microspherelike bacterial cellulose (HFMBC) particles was achieved by a double lyophilisation methodology after the functionalization of hydroxyapatite onto the microspherelike bacterial cellulose particles. The creation of engineered bioabsorbable HFMBC hydrogel will not only carry hydroxyapatite to the site where the tissue lost by using microspherelike particles as substrates, but also resolve the problem caused by non-degradation of traditional bone scaffolds. In this work, the *in-vitro* biocompatibility of HFMBC particles to osteoblast cultures from the human embryo (ATCC CRL 11372) and mouse (MC3T3-E1) was explored. Prior testing on nonfunctionalized bacterial cellulose incorporating cellulases in animal models demonstrated a gradual degradation over time until virtually no material could be observed in the wound area. In addition, no adverse response to the cellulases was observed.

Methods: *Gluconacetobacter xylinum* (JCM 9730 strain, ATCC 700178) was utilized to produce microspherlike bacterial cellulose particles. Carboxylmethylcellulose (CMC) and chitosan were used to make the hydrogel with microspherelike particles. Functionalization of hydroxyapatite onto the micropsherelike particles was performed in SBF (simulated body fluid) suspension containing 1 mg/ml hydroxyapatite nanopowder. Human obsteoblast (hOB, ATCC CRL 11372) and mouse (mOB, MC3T3-E1) osteoblast was used to evaluate the *in-vitro* biocompatibility of microspherelike particles and incorporated cellulases.

Results: The orbital agitation speed was constrained to a range of 100 to 200 rpm, which produced 10 mm to <0.5 mm spherelike particles, respectively, as the speed increased. Spherelike particles with a size of <0.5 mm and CMC or chitosan were used to make the hydrogel. Functionalization of hydroxyapatite onto the micropsherelike particles in SBF solution showed that the HFMBC particles exhibited a denser structure than non-functionalized particles. Better bioaffinity to the non-functionalized bacterial cellulose was observed for hOB cell culture than mOB.

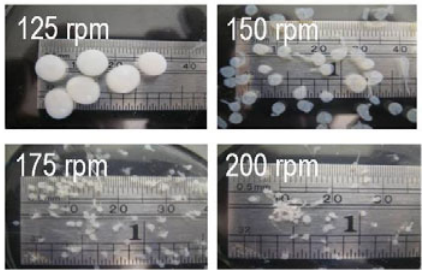

Figure 1. Spherelike bacterial cellulose particles.

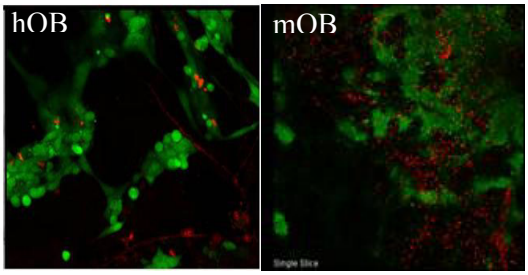

Figure 2. Images of hOB (left) and mOB (right) on the unfunctionalized bacterial cellulose under the confocal microscope.

Conclusions: Microspherelike cellulose particles (<0.5 mm) with highly pure cellulose component were obtained from the culture medium. Incorporation of cellulases into HFMBC particles can result in the degradation of microspherelike particles while the hydroxyapatite can be retained at the damaged tissue site. Good *in-vitro* biocompatibility of hOB implies the specific application of HFMBC in the repair and regeneration of cartilage/bone tissue. No adverse response of cellulases to the host wound occurred based on results of nonfunctionalized bacterial cellulose incorporating cellulases in animal models.

References:

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