Biocompatibility of PCL-HA Hybrid with Bone Marrow Derived Osteogenetic and Vasculogenetic Cells

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Statement of Purpose: Hybridization of polymer and ceramics is an attractive approach to improve the biological and mechanical properties of each component in the development of orthopedic graft. Polycaprolactone (PCL) has been implicated a potential material for tissue engineered bone substitute with vascularization. In this study, the addition of bioactive hydroxyapatite (HA) into the PCL scaffold was evaluated in regard to biocompatibility using bone marrow derived osteogenetic and vasculogenetic cells.

Methods: The HA-PCL hybrids were prepared with ratio of HA to PCL at 1:1 (group A) or 1:4 (group B) wt/wt respectively. A pure PCL (group C) was used as a control. In each group, NaCl particles were used to generate a controlled level of porosity in the matrix (212-355um pore size). PCL (Sigma USA, Mn 80000) was dissolved in tetrahydrofuran (Sigma, USA) at 40˚C. HA powder and NaCl particles were homogeneously mixed in the PCL solution until viscous slurry developed. Mixtures with thickness of 4mm were dried in glass mode at 37˚C. The composites with dimension of 1.5×1.5cm were cut out and washed in excessive distilled water to leach out the NaCl. All materials were then sterilized in 70% ethanol and dried before biological evaluation. Bone marrow cells were obtained from 6-8 week-old BALB/c mice and induced to osteoblasts and endothelial cells respectively as described previously [1, 2, 3]. The phenotypes of induced osteoblasts or endothelial cells were evaluated by the expression of osteocalcin and osteopontin or VEGFR-2 and VE-cadherin respectively. The cells were seeded on the scaffolds and cultured for one week. Scanning electron microscopy (SEM) was performed to examine osteoblasts morphology on scaffolds. The Alamar blue assay (Biosource, USA) was used to determine viability of the cells on the materials. Alkaline phosphatase (ALP) activity of osteoblasts was measured by mixing 100µl culture supernatants with p-nitrophenyl phosphate solution (Sigma, USA). Nitric oxide (NO) productions of endothelial cells were tested using NO assay kit (Calbiochem, USA). Total DNA of each sample was determined using the Hoechst 33258 DNA Assay (Sigma, USA). All the data from the viability assay, ALP activities, and NO production were normalized to DNA standard. One-way analysis of variance (ANOVA) was used, and $P \leq 0.05$ was considered significant difference.

Results: After one-week of incubation on HA-PCL hybrid scaffolds, the osteoblasts exhibited spread morphology, and paved the surfaces of scaffolds in groups A (Figure 1) and B, while few cells proliferated on the group C. The concentration of HA addition clearly influenced the viability of osteoblasts and their ALP expression with sequence of group A>B>C ($p<0.05$) (Figure 2). However, the viability of endothelial cells on the scaffolds were not markedly influenced by the amount of HA in scaffolds, although a elevated release of NO was observed in the group B compared with group A or C ($p<0.05$) (Figure 3).

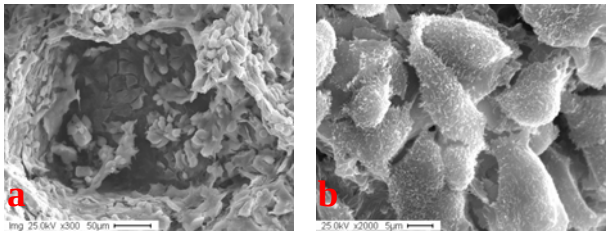

Figure 1: SEM images show osteoblasts grew on the scaffold of group A. a: 300×; b: 2000×

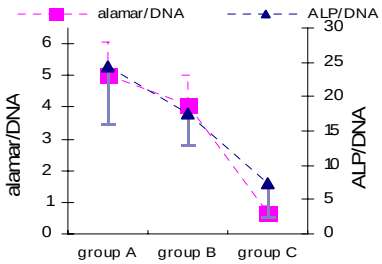

Figure 2: activities of osteoblasts cultured on PCL+HA scaffolds

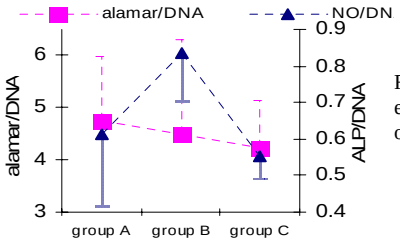

Figure 3: activities of endothelial cells cultured on PCL+HA scaffolds

Discussion/Conclusions: HA is considered to be biocompatible and bioactive due to the similarity of its chemical composition to the mineral phase of bone. The addition of HA to PCL effectively improve the bioactivity of PCL in this study. Results indicated that HA incorporated in PCL significantly increased the viability of osteoblasts and expression of osteoblastic markers (ALP) in a dose-dependent manner. Endothelial progenitor cells, the precursor of endothelial cells, persist in bone marrow stroma normally, and being considered potential seeding cells for vascularization of tissue engineered organs. The capacity of endothelial cells releasing NO, which play an important role in anti-thrombosis and anti-atherogenesis, acted as a criterion of endothelial activity. A low concentration of HA (HA:PCL=1:4) raised NO production in comparison with groups of using higher HA concentrations (HA:PCL=1:1) or the HA-free group, although the endothelial cells viability of all groups were equivalent, which indicated possibly the activity of endothelial cells is independent of the concentration of HA involved in PCL.

References:

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