Introduction

Bone exhibits multiple levels of organization from microscopic to nanoscale. On the microscale (1-100 µm), layers of fibrils are glued together by ECM proteins to form laminated structures, called osteons with osteocytes embedded in the calcified lamellar matrix. The osteons are cylindrical in longitudinal section with concentric layers of lamellae surrounding a central Haversian canal. The objective of this work was to design and test an osteon-mimetic microtubular scaffold as a culture system for studying the effect of extracellular matrix on osteogenesis and mineralization of stromal cells isolated from the bone marrow.

Materials and Methods

PLGA fiber mesh was prepared by electrospinning. The 4-arm star ethylene oxide lactide-co-glycolide (sELG) macromer was synthesized by ring opening polymerization. The Ac-GRGD integrin-binding cell adhesion peptide was synthesized in the solid phase. The hydrogel precursor solution was prepared under sterile condition by mixing sELGA macromer, Ac-GRGD peptide, Irgacure 2959 UV initiator, and hydroxyapatite (HA). The fiber mesh was dip-coated with sELGA hydrogel precursor solution; the coated fiber mesh was placed on a Teflon plate and topped with another dip-coated fiber mesh. The uncrosslinked dip-coated fiber mesh was wrapped tightly around stainless steel rods, and the assembly was crosslinked by UV radiation.

Microtubes with inside diameter of 0.82 (10 laminated layers), 0.51 (15 laminated layers), and 0.31 (20 laminated layers) mm were produced, as shown in Fig. 1. BMS cells were isolated from the bone marrow of young adult male Wistar rats. For laminated microtubes, BMS cell suspension was injected in the tubes and allowed to incubate in osteogenic media for 21 days. At each time point, mineralization and mRNA were measured.

Results

ALPase activity of BMS cells cultured on laminated sheets changed from 0.2 to 0.5, 1.8 and 1.8 IU/mg DNA after 4, 7, 14, and 21 days, respectively, while those cultured in microtubes changed from 2.7 to 4.6, 7.1, and 2.2. The jump in ALPase activity of BMS cells cultured in microtubes, compared to sheets, was statistically significant for time points 4-14 days. Calcium content of the BMS cells on laminated sheets changed from 60 to 43, 110, and 160 mg/mg DNA after 4, 7, 14, and 21 days, respectively, while those cultured on microtubes increased from 45 to 280, 510, and 940. The jump in calcium content of BMS cells cultured in microtubes, compared to sheets, was statistically significant for time points 7-21.

Fig. 1. Image of the microtubes.

Discussion and Conclusions

The 3D osteon-mimetic laminated microtubular scaffolds can be used as a model system to study the effect of bone nano- and micro-structure on osteogenesis.

References


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Disclosures

Authors have nothing to disclose.