Layer-by-Layer Assembled Titanium Dioxide Thin Films for Bone Tissue Engineering

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Statement of purpose: The ability to generate new bone for skeletal use is a major clinical need. Bone formation stimulating regimes, which hold the promise of significantly large increase in bone density, although attractive, have not yet become available. However bone is unique with a vast potential for regeneration. An alternative approach for skeletal repair is the selection, expansion and modulation of osteoblast cells in combination with new biocompatible, nanoporous, biomimetic scaffolds or nanoparticle culture platforms that interact and promote osteogenesis. Layer-by-layer (LbL) assembly has been used here for the preparation of scaffolds (2-D) with titanium dioxide (TiO_2) nanoparticles for optimal attachment, growth and mineralization of cells.

Methods and Materials: Mouse osteoblast cell line (D1 ORL, ATCC) was used from p2-p6 for our studies. TiO_2 nanoparticles (21nm, Degussa, Germany) were assembled as terminal layer on a glass substrate. The positively charged TiO_2 nanoparticles were coated by alternate adsorption with negatively charged poly (styrene sulfonate) sodium salt (PSS) (Sigma), above the precursor layers of PSS and positively charged Poly (dimethyldiallyl ammonium chloride) (PDDA) (Sigma) on normal microscopic glass slide using LbL technique.
Coated substrates were sterilized with 75% ethanol solution and rinsed in Hank's balanced salt solution, later cells were seeded at specified densities onto these nanoparticle coated substrates. The cell culture dishes containing the substrates in complete AMEM (Biosource) [AMEM + L-glutamine + fetal bovine serum + penstrep] were then incubated at 37°C, 5% CO_2 and 95% air within a humidified environment. After the initial attachment (2-3days) of osteoblasts, viability and proliferation studies were carried out. Later cells were supplied with osteogenic media (Cambrex) [complete AMEM + β-glycerophosphotase +ascorbate] for 14 days, which were also studied at different time periods.
Live/Dead (Molecular probes) assay was used for cell viability studies, using fluorescence microscope and fluorescence plate reader, cell attachment and proliferation studies were carried out using MTT (Sigma) assay kit and absorbance plate reader. Immunocytochemical analysis were performed for osteocalcin, osteonectin and collagen type-I bone marker proteins followed by detection of total calcium content using a standard calcium assay (StanBio Kit) and alizarin red staining (Sigma).

Results / Discussion: The substrates showed no major cytotoxicity to the cells seeded, as evidenced from plate reader and fluorescence microscope analysis. Cell attachment was greater on coated substrates than on control, but an obvious trend in the attachment of MSC was not observed with increasing number of layers of TiO_2 (increasing surface roughness), however, coating the substrates with TiO_2 clearly showed an increase in the number of cells attached after 72 hours. Cell proliferation was greater on TiO_2 coated substrate than on control at all time periods (0-24 hours), there was a statistically significant increase from control to coated substrate.
Immunocytochemical analysis of cells on substrates for bone markers proteins showed retained functionality of osteoblasts, which increased as we induced the process of osteogenesis for 14 days. This was also supported by total calcium content analysis after 14 days as seen by alizarin red staining and calcium assay.

Conclusions: In this work, we have shown retained osteoblast function on TiO_2 nanoparticle thin films with controlled nanostructured surface topography. These thin films offered very little cell death and an increased attachment and spreading of cells with increasing surface Roughness (nanoparticle coating) was observed. In conclusion, LbL assembly of TiO_2 nanoparticles offers a promising tool for the modification of implantable surfaces for increased cell attachment.
This method can be cost effectively employed for such purposes without compromising the integrity of the implant strength. LbL assembly is especially useful in these kinds of applications as the internal multilayer structure can be tailor made to include various materials like extra cellular matrix proteins or osteogenic growth factors presenting an interactive scaffold structure, which can provide positional information and a template for bone growth. Our future studies include use of LbL technique to formulate a slow release system for delivery of growth factors with in the scaffold and to design a 3-D scaffold with embedded growth factors, having slow release capabilities.

References:

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