"Bioinspired Approach towards Bone Grafts based on Nano- and Micro-fabrication of Phosphorylated Polymers"

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Abstract

Replacing lost bone through grafting of autologous or synthetic materials is an age old practice which however, does not provide holistic solution to the burgeoning problem of bone disorders. While autografts are limited by problems with donor site, synthetic grafts do not meet all the structural and functional requirements for successful healing. Present knowledge of bone formation indicates a critical role of biomineralization in ossification process and also in osseointegration of grafts at injury site. The present thesis draws inspiration from the non-collagenous proteins which are bound to collagen nanofibrils in native bone and are intricately involved in regulating the vital process of biomineralization. However, isolating phosphoproteins is an expensive proposition. In this context, though phosphorylated polymers (PP) are known to mimic the function of the phosphoproteins, thorough investigations on bone graft scaffolds based on biomimetic fabrication like electrospinning of PP has not been reported till date.

In this work, two polymers with different electrospinning and biological properties are modified into their phosphorylated derivatives and interrogated for suitability as polymer based bone scaffold materials. Several of observed properties in PP were found to be correlated with their degree of modification. First one, polyvinyl alcohol was selected as model polymer due to its convenience in electrospinning and phosphorylation. The derivative exhibited reduction in electrospinning window of PVA which was attributed to increased conductivities and viscosity of phosphorylated polymer. However, once formed these nanofibers showed higher mechanical strength and enhanced proliferation as well as function of pre-osteoblast cells cultured on them compared to unmodified PVA. Second polymer selected was N-methylene phosphonic chitosan, as chitosan based nanofibers have proven osteoconduction properties. A strong *in vitro* osteoinductive effect was observed on phosphorylated chitosan matrices by analysis of *in vitro* biomarkers like ALP, osteocalcin as well as by PCR analysis of their genetic expression. Subsequently, *in vivo* implantation of phosphorylated chitosan nanofibers was carried out in a rabbit tibial defect wherein a one point histological examination revealed no adverse tissue reaction of grafts while radiological evidence suggested improvement in bone healing compared to untreated controls. Moreover, it was encountered that nano-scale pore size in nanofibers is not suitable for generating 3D grafts of electrospun sheets. Therein, macro-porosity was designed and engineered in nanofibrous scaffold by a laser engraving followed by rolling up approach to obtain 3D grafts which demonstrated promise for increased infiltration of cells. Further, phosphate functionalization with a hydrophobic polymer was also explored by synthesis of phosphorylated polyvinyl-alcohol-graft-polylactic acid which showed improvement in contact angles and subsequent cell responses. Overall, the experiments and results together illustrate nano/micro fabrication of PP can be a promising approach to design bone grafts.

Keywords: Phosphorylation, PVA, Chitosan, PLA, Electrospinning, Laser engraving, Osteoinduction, MG-63, Bone grafts.