ABSTRACT

The overall goal of this research project is to develop polymeric nanocomposite scaffolds as extracellular matrix to engineer soft tissue. Tissue engineering can be defined as the use of physical, chemical, biological and engineering processes to control and direct the aggregate behavior of cells. The envisioned tissue engineering strategy contains following steps: to create a biocompatible, biodegradable and mechanically facile scaffold structure using electrospinning technique, to seed MG-63 bone like cells on these scaffolds and assess their biocompatibility in vitro. These steps were carried out in various consecutive studies to engineer suitable nanocomposite scaffold which mimics the exact function of extracellular matrix for bone. Basically the extracellular matrix comprises nanoscale fibers that offer structural integrity to tissues. Various processing techniques such as drawing, template synthesis, phase separation, self-assembly and electrospinning have been used to prepare nanofibers in recent years. Electrospinning method has drawn considerable attraction for its simple, elegant and cost effective techniques. We can synthesize fine uniform nanofibers either using a polymeric solution or its melt part. Also the porosity, pore diameter of the polymeric nanofibers can be controlled according to the need using this technique. In the first study polyvinyl alcohol (PVA) and polyvinylpyrrolidone (PVP) were blended in various ratios and electrospun to get bead free uniform nanofibers. Water and ethanol were used as
solvents for PVA and PVP which was found to be prerequisite in getting smooth blend nanofibers. Electrospinning parameters viz. concentration of polymer and applied were investigated and optimized. Consecutively in the second study composites based on a hydrophobic polymer poly (ε-caprolactone) (PCL) and hydrophilic polymer polyvinylalcohol (PVA) were fabricated by preparing bilayer samples using electrospinning technique and spinning parameters were optimized as stated above to get ultrafine submicron fibers. Further the blend and bilayer studies were characterized using scanning electron microscopy (SEM), X-Ray diffractometer (XRD), Fourier transform infrared spectroscopy (FTIR) and differential scanning calorimeter to investigate their morphological, structural, chemical and thermal behavior. The fabricated scaffolds were found to be smooth and beadless with highly uniform morphology. The blend fibers were highly amorphous in nature having single glass transition temperature reiterating their highly miscible blend formation. Comparatively the bilayer scaffolds were found to be semicrystalline in nature due to the presence of highly crystalline PCL polymer. All the scaffolds had competent thermal stability for their use in tissue engineering applications. Image J (4.1) version software was used to calculate porosity and pore diameter of prepared scaffolds. The scaffolds had appropriate porosity in the range of 55-65 % and suitable pore diameter making them feasible for better cell infiltration properties. To prove their preclinical relevance the scaffolds were seeded with NIH 3T3 cell lines and their in vitro biocompatibility was assessed using MTT assay. In comparison
with PVA-PVP blend system PVA-PCL bilayer system was found to be more optimal in supporting NIH 3T3 cell lines.

In the third study scaffolds were fabricated to produce more clinically relevant and specific bone matrices. Ceramic filler particle like Hydroxyapatite (HAp) and beta tricalcium phosphate (β-TCP) were synthesized and characterized for their particle size, phase and purity using SEM, EDAX, XRD and FTIR. The ceramic particles were incorporated in the PVA-PCL bilayer system individually and electrospun to fabricate polymer-ceramic nanocomposites. The prepared composites were extensively characterized to validate their crystalline behavior, uniform morphology and thermal stability. Water retention capacity, porosity and pore diameter of the scaffolds were assessed to improve their cell infiltration properties. (PVA-PCL)-HAp scaffolds had an edge over (PVA-PCL)-TCP scaffolds as they had enhanced crystalline behavior and thermal stability. Biocompatibility of bilayer composite scaffolds was assessed using MG-63 osteoblast like cell line for 7 days in three intervals (1\textsuperscript{st}, 4\textsuperscript{th} and 7\textsuperscript{th} day). HAp and β-TCP incorporated bilayer scaffolds were found to have suitable biocompatibility to prove their mettle as potential scaffold for bone tissue engineering.

In the final study PVA-PVP blend composite solution was prepared with adding HAp and β-TCP individually and studied in detail for their morphological nature, crystalline behavior, thermal stability, porosity and water retention capacity. Both the blend composites had almost similar characteristic
except crystalline property which was observed more for HAp incorporated blend composites. MTT assay was carried out to find the cell viability percentage for the prepared scaffolds seeded with MG-63 osteoblast cell lines. Again HAp added blend composite scaffolds were more proficient compared to TCP composites as they had better cell viability percentage and showed good adherence and proliferation of seeded cells. The cells had elongated spindle morphology and were dense and agglomerated proving the good adherence and growth capability of HAp blend composite scaffolds.

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