CHAPTER 8
SUMMARY AND CONCLUSIONS

8.1 CONCLUSIONS

To develop a biomaterial for bone regeneration, the scaffold must have structural and functional similarity with the native extracellular matrix (ECM). Nanofibrous matrices fabricated via electrospinning technique closely mimics the morphology of extracellular matrix hence is the appropriate method to generate polymeric biomaterial. Ideal scaffold for tissue engineering should meet various design criteria: the scaffold should be biocompatible and biodegradable should have appropriate porosity to provide sufficient space for cell adherence, nutrient diffusion and extracellular matrix regeneration, it should allow the cells to adhere and promote its growth and the material should be reproducible into desired three dimensional structures. Hence, a polymer composite system based on polyvinylalcohol -polyvinylpyrrolidone (PVA-PVP) blends and polyvinylalcohol-polycaprolactone (PVA-PCL) bilayers were theorized to be the basic scaffold structure for the present research study.

PVA-PVP blends were electrospun in different ratios of 25/75, 50/50 and 75/25 by varying applied voltage and keeping the tip-target distance constant at 20 cm and maintaining the flow rate uniform at 5 µl/min. Uniform and submicron fibers were observed for all the ratios of PVA-PVP blend. Increase in voltage resulted in thinning of fiber diameter further generating ultrafine fibers while optimizing average fiber diameters (AFD) for individual ratios. The AFD of blends in various ratios was found in the range of 140 nm to 230 nm. PVA-PVP blend system exhibited good thermal stability and was found to have single glass transition temperature attributing to its highly miscible behavior. Also, it was observed that the blend with high PVP content had the maximum T<sub>g</sub> around 159 °C. Blend with high PVA content showed good porosity and water retention
ratios as the fine hydrogel behavior of PVA enhances hydrophilicity of the scaffolds. XRD pattern revealed that the prominent peaks for PVA-PVP blend nanofibers occurred nearly at 11.20°-11.60° and 20.65°-21.60°. All the corresponding peaks were short and broadened pointing to the highly amorphous nature of blend nanofibers and to some extent responsible for poor mechanical property of prepared blend films. The reason for highly miscible behavior of blend comes from the intermolecular secondary interaction happening between the carbonyl oxygen on a PVP chain and a hydroxyl group along a PVA chain and this bond formation was revealed during FTIR studies. PVA/PVP scaffold showed better adhesion and proliferation of cells compared to PVA samples. Pore diameter of PVA/PVP scaffold was in the optimum range of 0.2-0.4 µm which is highly suitable for better adhesion of cells on the scaffold.

Similarly, PVA-PCL bilayer samples were prepared by varying the thickness of PCL layer over PVA membrane which was spun for constant time for all the bilayer samples. Not much change was observed in the AFD’s of bilayer sample as they lied in the range of 220 to 250 nm. The spinning parameters for bilayer samples were optimized as follows: applied voltage being kept at 15 kV, flow rate at 5 µl/min and tip-target distance at 15 cm. Prominent effects caused by spinning PCL over PVA for bilayer formation were a slight depression of T_m and substantial drop in crystallinity of PVA when compared to the values reported for PVA alone. Surprisingly, T_m values for PCL remains the same at 62 °C for the entire bilayer sample and the sharp peaks demonstrate the retention of crystalline behaviour of PCL. Bilayer fibers exhibit better thermal stability and biocompatibility with NIH 3T3 mouse fibroblasts when compared to PVA-PVP blend nanofibers for which one of the reasons may be good mechanical strength of the bilayer scaffolds and enhanced porosity which supports the cell adhesion in a more efficient manner leading to better proliferation and growth of seeded cells.

The PVA/PCL bilayer incorporated with HAp was prepared by depositing PCL-HAp nanofibers over PVA-HAp nanofibrous membranes. In order to
prepare the PVA-HAp solution for electrospinning, PVA granules and HAp were mixed in the ratio of 8:1 in deionized water to give a 8.75 wt% solution (with respect to the solvent). Similarly, to prepare PCL-HAp solution, PCL granules and HAp were mixed in the ratio of 12:1 in a 4:1 mixture of chloroform/DMF to give a 9 wt% solution (with respect to the solvent). SEM analysis of synthesized HAp ceramic revealed that grain size ranged from 150 to 300 nm. EDAX results showed that the HAp particles had a Ca/P ratio of 1.62, which was very close to that of natural bone. The morphology of bilayer scaffolds showed better fiber formation with AFD in the range of 340 nm. Structural characterization using XRD indicates the broadening of PVA peaks in the (PVA: PCL)-HAp scaffolds reiterating the fact that addition of ceramic particle in the polymer matrix diminishes its crystalline behavior as diffraction of filler particle dominates in polymer ceramic composites. Thermal studies of composite scaffolds using DSC revealed enhanced T\text{g} of PVA, T\text{m} of PCL and higher enthalpy of fusion in the HAp loaded bilayer scaffold which proves the ameliorated thermal stability of the matrix above the threshold limits to perform as a potential scaffold in tissue engineering applications. Improved hydrophilicity (141%) was observed for bilayer composite scaffold due to the presence of PVA and a porosity of 60 % was observed. Greater cell viability of MG-63 cell lines was observed for PVA-HAp and (PVA:PCL)-HAp scaffolds owing to smaller pore diameters of 0.3 and 0.5 µm which establishes its mettle as potential scaffold for bone tissue engineering applications.

In similar studies wet precipitation route was used to synthesize β-TCP with CaCl$_2$ and Na$_3$PO$_4$ as precursor material. The PVA-PCL bilayer incorporated with TCP was prepared by depositing PCL-TCP nanofibers over PVA-TCP nanofibrous membranes. In order to prepare the PVA-TCP and PCL-TCP solutions, PVA granules and TCP were mixed in the ratio of 8:1 in deionized water to give a 8.75 wt% solution (with respect to the solvent). Similarly, to prepare the PCL-TCP solution, PCL granules and TCP were mixed in the ratio 12:1 in a 8:2 mixture of chloroform/DMF to give a 9 wt% solution.
(with respect to the solvent). The morphology of β-TCP powder consisted of highly agglomerated particles having an average size of 50-70 nm. Ca/P ratio for β-TCP was found to be 1.49, exactly matching the standard literature value. SEM images for bilayer nanocomposites scaffolds depict ultrafine submicron fiber formation with AFD around 102 nm. XRD pattern substantiated that the obtained precipitate calcined at 900 ºC had a pure β-TCP phase with high crystallinity according to JCPDS 70-2065 and 09-0169 cards. The corresponding reflection peaks for β-TCP in the bilayer composite occurred at 2θ = 31.28º, 33.34º and 46.26º. Insignificant changes in the characteristic peaks of β-TCP were observed in all the composites indicating the presence of same crystal structure of added ceramic in all the matrices. The thermal properties of the pure PVA, PCL, PCL-TCP and (PVA: PCL)-TCP composites were studied by DSC. The glass transition temperature (T_g) and melt peak (T_m) of pure PVA were observed at ~123 ºC and ~224 ºC respectively. The T_m for pure PCL was observed at 62.4 ºC. Shift in T_m of PCL towards slightly higher temperature (~63 ºC for PCL-TCP and ~64.5 ºC for (PVA: PCL)-TCP) was observed in both the composites. Similar result was observed for PVA as its T_g substantially enhances to 136.9 ºC in (PVA: PCL)-TCP composite. These results indicate the existence of strong interaction between polymer matrix and nanoscale filler. The porosity of PCL-TCP was found to be around 57% and (PVA: PCL)-TCP composite was found to be 63%. PVA-TCP scaffold was not studied due to its poor mechanical properties. Greater porosity of bilayer scaffold was found more appropriate for cell adhesion. The composite scaffold also showed excellent cell viability for MG-63 cell lines and the fibrous mesh exhibited normal morphology.

In comparison with (PVA: PCL)-HAp membranes, (PVA: PCL)-TCP membranes exhibited better cell viability for MG-63 osteoblast cell line. Though HAp is highly osteoconductive in nature, its non resorbable and poor dispersion behaviour in PCL may affect the cell adhesion and growth in bilayer scaffolds PVA-PVP blend composites incorporated with HAp and β-TCP were fabricated by electrospinning. 0.5 g of HAp and β-TCP were added separately to 10 ml of
8% PVA: 26% PVP (1:1 ratio) blend solution with constant stirring and sonication. Electrospinning parameters were optimized to 10 cm tip-target distance, 12 KV applied voltage and 0.5 µl/min flow rate for all the scaffolds. SEM results showed a homogenous smooth nanofibrous morphology with AFD in the range of 250-400 nm. XRD results demonstrated the presence of all the peaks corresponding to HAp and β-TCP in the scaffolds which finely corroborated with JCPDS file no (09-0432) and (09-0169). HA peaks retained their crystalline behaviour in composite scaffolds to some extent. Except some sharp crystalline peaks, mostly broad and amorphous peaks were observed indicating the increment of amorphous nature of scaffold with incorporation of β-TCP. DSC thermograms were obtained for pure and composite blends and glass transition temperature (Tg) was analyzed. Single glass transition temperature was observed for pure and composite blends at 66 ºC for (PVA:PVP), 81 ºC and 86 ºC for (PVA: PVP)-HAp and (PVA:PVP)-β-TCP scaffolds, indicating the hydrogen bond formation between hydroxyl groups of PVA and carbonyl groups of PVP resulting in highly miscible blends. Increment in Tg was observed with addition of HAp and β-TCP attributing to enhanced thermal stability of composite scaffolds. Excellent cell viability was observed for (PVA: PVP)-HAp scaffolds compared to (PVA: PVP)-β-TCP scaffolds with normal morphology of MG-63 cell lines.

8.2 FUTURE SCOPE OF THE DEVELOPED BONE SCAFFOLD

Bone is prone to a range of disease like other part of body tissues. Generally it’s a difficult task to deliver drugs to bone. Hydroxyapatite has already been identified as a promising target for selective drug delivery to bone. One of the major future directions of this research is to fabricate core shell nanofibers using co-axial electrospinning. PVA, PCl co-axial nanofibers incorporated with HAp can be used as target site for loading drug which can be systematically and gradually delivered to bone as the remodeling process is successfully carried out.
The factors which are to be controlled to attain the desired result include encapsulation and controlled release of drug without pronounced effect on the tensile properties of the optimized scaffold. Secondly, the activity and incorporation of lowest quantity of drug levels at molecular level needs to be standardized. Further the core shell nanofibers loaded with HAp and binded with drug must be evaluated for \textit{in vivo} performance.

Development of three dimensional aligned co-axial nanofibers using PVA-PVP and PVA-PCL system must be carried out for better cell adherence and proliferation. Another important course of further studies would be to develop strategies for fabricating commercially viable scaffolds.

Biomedical atomic force microscopes (bio-AFM) is an important tool that paves the way for a multitude of applications in Soft Matter and Life Science research. As a complementary study to AFM imaging, force spectra (FS) analysis can be carried out for cells treated with materials to observe biophysical and biomechanical changes of cells before and after the treatment. Overall, this study attempts to unravel the material interactions with cells, the resulting cytotoxicity and cellular properties. The focus will be to use this tool to assess cell growth on prepared scaffolds and get better idea about their adherence and growth.