CHAPTER VII

CONCLUSIONS AND FUTURE PROSPECTS

This chapter summarizes the major conclusions drawn from the thesis work. The conclusions drawn from the each of the systems have been recapitulated in regard to their biomedical applications. An interpretation has been tried to figure out the best biomaterial scaffold out of the three systems of the synthesized material in the present work in terms of their physicochemical properties and biological performance in modulating the cell behaviour. At the end of this chapter, the future scope of research in the field of conducting polymer based biomaterials has been briefly elucidated.

7.1 Conclusions

The present thesis is a detailed description of synthesis and modification strategies of nanostructured conducting polymer (CP) based biomaterials for their tissue engineering applications. Effect of surface functionalization of nanostructured CP based biomaterials through incorporation of the different polar functionalities on its physicochemical properties and its consequences on cell-biomaterial interactions have been studied extensively. The present thesis also deals with the electrical stimulation of neuronal like PC12 cells cultured on the as-synthesized CP based biomaterials to modulate the neurite characteristics. The major conclusions drawn from each of the working systems of the present thesis are summarized in the subsequent subsections.

7.1.1 Conclusions from Chapter IV

PAni nanofibers (PNFs) have been synthesized by dilute polymerization method using HCl as dopant and APS as the oxidant but without the use of any organic solvent, which make them environment friendly and may be useful for biological applications. PNFs have been surface functionalized by 1% glutaraldehyde solution in phosphate buffer solution (PBS) solution (pH =7.4) to introduce polar functionalities such as aldehyde (-CHO), hydroxyl (-OH) groups on the surface. The physicochemical properties of the as-synthesized nanofibers have been characterized TEM, SEM, XRD, *I-V* characteristics, tensile strength test, TGA, UV-Vis absorption spectroscopy,

fluorescence spectroscopy, FT-IR, ^{1}H NMR spectroscopy and contact angle analysis using sessile liquid drop technique. The interaction mechanisms of surface functionalized PAni nanofibers (SF-PNFs) with building blocks of proteins such as essential amino acids have been investigated in order to predict the favorable cell-biomaterial interactions. The biological characterizations of the materials have been performed with the help of hemolysis assay, MTS assay, acridine orange/ethidium bromide (AO/EtBr) staining of MDA-MB-231 cells and cell adhesion test. The major conclusions of **Chapter IV** have been summarized as:

1. TEM confirms the synthesis of PNFs with an average diameter of 35.66 nm. SEM images confirm nanofibrous structures with interconnected networks and highly entangled morphology of PAni powder and film, respectively. SEM and TEM demonstrate the formation of nanofibers of PAni by dilute polymerization without secondary growth, since no particles or granules have been observed.
2. The XRD patterns reveal two major reflection peaks at 2θ=19^{o} and 25^{o} for pristine PNFs and SF-PNFs at 2θ=19^{o} and 25^{o} corresponding to (100) and (110) reflections. The XRD results indicate improvement in crystallinity after surface functionalization due to possible cross-linking between the polymer chains, which contributes towards longer domain length (L) and lower strain (ε) than that of the non-functionalized PNFs.
3. The *I-V* measurement demonstrates that surface functionalization has been accomplished without much affecting the conductive properties of PAni nanofibers films as there is only slight increase in the sheet resistance of PNFs after functionalization from 1.23 ± 0.48 × 10^{5}Ω.cm (PNFs) to 1.33 ± 0.55 × 10^{5} Ω.cm (SF-PNFs) due to the possible loss of π–π conjugation during functionalization process. Moreover, PNFs and SF-PNFs show Ohmic conduction up to 3 V of applied bias and beyond 3 V, SCLC takes place due to injected charge carriers from the electrodes. The non-linear I-V characteristics of PNFs and SF-PNFs follow *Kaiser Equation* and suggest slightly easier hopping of charge carriers in PAni chain before surface functionalization.
4. Thermogravimetric analysis (TGA) indicates enhanced thermal stability of PNFs after functionalization. The major degradation temperature for the polymer backbone has been observed to be shifted from 519˚C to 538˚C after

surface functionalization owing to the different chemical structure of benzenoid and quinoid units in PAni chains after functionalization. It futher indicates actual glutaldehyde mass fraction introduced on the surface of PNFs is about 1% of total weight of the polymer.

5. Tensile strength test reveals that after surface functionalization, thin film of PNFs has become mechanically stronger than pristine, which is attributed to the cross-linking between the polymer chains after surface functionalization as indicated by the XRD results. The stiffer surface of the PAni film suggests its potential to support cell adhesion, spreading and migration for tissue engineering applications.
6. The PAni nanofibers were found to be stable in physiological condition since there was no significant change in sheet resistance and fiber diameters after keeping non-functionalized and functionalized PAni film in PBS (pH=7.4) for 30 days.
7. UV-visible absorption and photoluminescence studies depict that PNFs are partially in emeraldine base and emeraldine salt form before and after functionalization. Two clear broad absorption bands in the region 300 nm and 600 nm appear in the absorption spectra of pristine and functionalized samples, whereas the band at 430 nm corresponding to emeraldine salt of PAni is not distinct. Both PNFs and SF-PNFs exhibit a distinct photoluminesnce peak at 385 nm, with the intensity of the peak for SF-PNFs is higher. The enhancement in the photoluminescence intensity after surface functionalization is ascribed to increased number of benzenoid like units in the polymer chain due to partial reduction at the imine sites as well as incorporation of functional oxygenated species like aldehyde (-CHO), hydroxyl (-OH) functionalities in the polymer by functionalization of glutaraldehyde through grafting, which is supported by FT-IR and ^{1}H NMR results.
8. FT-IR and 1H NMR spectroscopic studies reveal successful incorporation of polar aldehyde (-CHO) and hydroxyl (-OH) functionalities into PNFs through amide bond and Schiff base formation between PNFs and glutaraldehyde. Typically, the vibrational bands at 1720 cm^{-1} (C=O stretching in non-conjugated aldehyde or in amide) and 1636 cm^{-1} (C=N stretching vibrations in Schiff base) in FT-IR spectrum of SF-PNFs suggest cross-linking of

glutaraldehyde to PNFs through amide bond and Schiff base formation. The ^{1}H NMR signals of SF-PNFs in the range 4.7-4.9 ppm corresponding to cyclic hemiacetal or/and oligomeric form of glutaraldehyde and at 9.7 ppm due toaldehydicproton, further confirm incorporation of polar aldehyde and hydroxyl functionality on the surface of PNFs.

9. Contact angle measurements demonstrate an increase in wettability of PAni films after surface functionalization as contact angle decreases from 76.2° to 52.1°. Surface energy calculations along with its components using OWRK (Owens, Went, Rabel and Kaelble) and AB (Acid-base) methods show increase in surface energy, surface polarity and hydrophilicity owing to hydroxyl and aldehyde functional groups introduced onto the surface through covalent bonding between glutaraldehyde and PAni. Surface energy calculations by OWRK and AB method demonstrate up to 6-9% enhancement in surface polarity of the PAni film after surface functionalization by glutaraldehyde. More specifically, the enhancement in the basic component of surface energy $\left(\gamma_s^-\right)$ evaluated by AB method indirectly indicates the introduction basic aldehyde and hydroxyl groups on the surface of PNFs after surface functionalization.
10. Fluorescence spectroscopy has been used as an analytical tool with SF-PNFs as a substrate to monitor any biochemical reactions involving three aromatic amino acids viz. Tyrosine (Tyr), Tryptophan (Trp) and Phenylalanine (Phe). The appearance of broad vibartional bands around 1640 cm^{-1} attributed to C=O stretching vibrations in amide bond often overlapped with C=N stretching vibrations in Schiff base, at 1240 cm^{-1}, 1304-1354 cm^{-1} (with splitting and shifting) assigned to C-N strecthing vibration as indicated by FT-IR spectroscopy, reveals strong covalent binding between SF-PNFs and the three amino acids. It is well supported by ^{1}H NMR spectroscopy as the evolution of the peaks in the regions 7.92-8.22 ppm due to amide proton, 7.85-7.88 ppm due to Schiff base proton are clearly observed along with the peaks in the region of 4.22-5.02 ppm due to methylene protons in cyclic hemiacetal and its oligomeric form of SF-PNFs conjugated with the amino acids.Remarkable enhancement in fluorescence signals of SF-PNFs in presence of aromatic amino acids has been observed and the apparent binding

constant (K_A) and the number of binding sites (n) have been calculated using fluorescence enhancement equation. The K_A value has been found to be highest for SF-PNFs+Tyr complex and n is two for all the polymer amino acid complexes, which are in agreement with the FT-IR and 1H NMR results. Fluorescence resonance energy transfer (FRET) efficiency has been found to be highest for SF-PNFs+Tyr complex giving maximum fluorescence enhancement. This study of interaction mechanisms by means of an extremely sensitive technique like fluorescence spectroscopy using SF-PNFs as a substrate may provide a promising analytical tool for detection and monitoring any biochemical reactions involving these three aromatic amino acids that are precursors to several proteins like hromones, melanin, thyroid, serotonin, epinephrine in the human body.

11. SF-PNFs have been found to be biocompatible as revealed from hemolysis assay and MTS assay. SF-PNFs have been found to exhibit hemolysis activity less than 1% and hence, they are non-hemolytic. Fluorescence microscopy of AO/EtBr stained MDA-MB-231 cells shows enhanced cell adhesion, spreading and proliferation on SF-PNFs when compared to PNFs, whereas cell attachment on the PAni scaffold was confirmed by SEM.
12. The enhanced cell adhesion, spreading and proliferation on SF-PNFs suggest favorable cell-biomaterial interactions owing to improved surface hydrophilicity as a result of incorporation of polar hydroxyl and aldehyde functionality after surface functionalization by glutaraldehyde. The results suggest that improved bioactivity and biocompatibility of SF-PNFs can make it a promising candidate for biomedical applications such as tissue engineering and biosensing applications.

7.1.2 Conclusions from Chapter V

Herein, PAni nanofibers synthesized by dilute polymerization method have been blended with chitosan (Ch), a natural biopolymer, at a concentration of 4 % and 6 % (w/v) to confer bioactivity and biodegradability for improved function as biomaterial scaffold. The as-synthesized PAni:Ch nanocomposites were surface functionalized with glutaraldehyde and glycine N-hydroxysuccinimide (NHS) ester, separately, in order to investigate the hypothesis that surface functionalization of these conductive materials will enhance bioactivity and improve cell-biomaterial interactions. The

physicochemical properties were investigated with the help of SEM, XRD, I-V characteristics, tensile strength test, TGA, FT-IR, XPS and contact angle analysis using sessile liquid drop technique. In order to predict the bioactivity of the nanocomposites, urease was immobilized and kinetics of immobilized urease was investigated. Subsequently, the biological characterizations were accomplished with the help of hemolysis assay and with MDA-MB-231 cells, 3T3 fibroblasts and neuronal model PC 12 cells. The nanocomposites were further investigated for electrical stimulation of neuronal model PC 12 cells under constant electrical potential.

1. SEM reveals layers of interconnected networks of PAni nanofibers soaked in chitosan matrix with rough and porous surface when compared to smooth pure chitosan.
2. XRD results suggest that the nanocomposites are more or less amorphous in nature. Interestingly, enhancement in crystallinity of the nanocomposites has been observed with increasing PAni content due to the possible electrostatic interactions between the abundant amino and hydroxyl groups in chitosan with the primary and secondary amines of PAni.
3. The *I-V* characteristics of PAni:Ch nanocomposites with 6 wt% PAni content show improved I-V characteristics than the nanocomposites with 4 wt% PAni content, while all the materials demonstrate Ohmic conductive behavior at lower voltage region ($0<V<4$) and space charge limited non-linear conductive behavior at higher voltage region ($4<V<10$). Sheet resistance (R_s) calculations derived from I-V characteristics reveal no significant change in the conductive properties of the glutaraldehyde functionalized and glycine NHS ester functionalized nanocomposites from their non-functionalized counterparts and suggest that surface functionalization has been performed without much affecting the conductive properties of the nanocomposites.
4. The thermal stability of the non-functionalized nanocomposites has been observed to be increased with increasing concentration of PAni in the nanocomposites as demonstrated by TGA analysis. However, the thermal stability of the nanocomposites after surface functionalization has been found to be decreased.
5. The mechanical strength test reveals that tensile strengths of all the nanocomposites match the tensile strength of softer tissues. The stiffness

constants (*E*) and ultimate tensile strength (UTS) of the both types of the surface functionalized nanocomposites have been found to be lower when compared to non-functionalized nanocomposites due to possible hydrolysis of chitosan during surface functionalization process. However, glutaraldehyde functionalized nanocomposites exhibit improved mechanical properties than glycine NHS ester functionalized nanocomposites.

6. Stability test demonstrates that after incubation in physiological solution (PBS, pH=7.4) for 15 days, the nanocomposites reveal remarkable enhancement in porous morphology on the surface due to degradation of chitosan matrix as confirmed from SEM analysis, whereas the measurement of sheet resistance reveals no significant change in conductive properties indicating no degradation occurs to PAni nanofibers in the nanocomposites. The distribution of pore diameters after 15 days of incubation in PBS has been increased from 20-100 nm to 50-1000 nm, providing an essential porosity of tissue engineered scaffolds. Therefore, we speculate that PAni nanofibers take a lead role in maintaining the electrical and structural stability of the nanocomposites as compared to chitosan.

7. Incorporation of aldehyde functionality on the surface of PAni:Ch nanocomposites after functionalization by glutaraldehyde has been confirmed from FT-IR and XPS results. In the case of glutaraldehyde functionalized nanocomposites (GFPAni:Ch-4 wt% and GFPAni:Ch-6 wt%), FT-IR results indicate the presence of aldehyde (1720 cm^{-1}) and hydroxyl (1040 and 3440 cm^{-1}) functionalities after surface functionalization. This is further confirmed by XPS results, which show nearly 4 times higher fractions of C=O, C-OH and higher atomic percent of O1s on the surface of the nanocomposites after functionalization. The FT-IR and XPS results suggest incorporation of aldehyde functionality in two possible ways: firstly, through amide bond formation between the amino group of chitosan in PAni:Ch nanocomposites and aldehyde group of monomeric glutaraldehyde; secondly, through Schiff base formation between aldehyde group of monomeric glutaraldehyde and primary amine of chitosan in the nanocomposites. The possible functionalization mechanisms have been proposed on these results and shown in **Figure 5.10** in **Chapter V**.

8. The appearance of C=O stretching at about 1724 cm^{-1} and a broad band centered at about 3017 cm^{-1} corresponding to strongly H-bonded O-H stretching vibration, in the FT-IR spectra of glycine NHS ester functionalized nanocomposites (EFPAni:Ch-4 wt% and EFPAni:Ch-6 wt%), confirms the presence of carboxylic acid. XPS results suggest the enrichment of the surface of PAni:Ch nanocomposites with nearly 6 times higher fractions of O-C=O, C-OH along with the higher percentage of O1s and N1s confirming the incorporation of glycine onto the surface of nanocomposites making it a carboxyl functionalized surface after functionalization by glycine NHS ester. Incorporation of glycine onto the surface of PAni:Ch nanocomposites has been confirmed in two ways: by interaction with the carboxylic acid site of glycine keeping the amine site free and vice versa as confirmed from FT-IR and XPS results. Probable interaction mechanisms between unprotected glycine NHS and the nanocomposite surface have been also proposed based on FT-IR and XPS results and shown in **Figure 5.12** in **Chapter V**.

9. Contact angle analysis by OWRK and AB method indicates improvement in wettability and surface polarity of the nanocomposite after surface functionalization glutaraldehyde and glycine NHS ester. The OWRK method demonstrates enhancement in total surface energy (γ_s), polar component of surface energy (γ_s^p) and percentage of surface polarity of PAni:Ch nanocomposites after surface functionalization glutaraldehyde and glycine NHS ester. Similar observation has been also confirmed from AB method. In fact, the AB method further confirm the FT-IR and XPS results by demonstrating in the enhancement of the Lewis basic (γ_s^-) component of surface energy of the nanocomposites due to basic aldehyde incorporation after surface functionalization by glutaraldehyde, whereas the enhancement in the Lewis acid (γ_s^+) component of surface energy of the glycine NHS ester functionalized nanocomposites indicates the incorporation carboxylic acid functionality on the surface.

10. The glutaraldehyde functionalized nanocomposites exhibit higher urease activity immobilized on it owing to improved surface hydrophilicity due to the incorporation of polar aldehyde (-CHO) and hydroxyl (-OH) functionality

when compared to its non-functionalized counterpart. The Michaelis constant (K_m) has been determined to be 5.41 mM, 13.93 mM and 21.5 mM from the Lineweaver-Burk plot for free and immobilized urease on glutaraldehyde treated and untreated films, respectively, indicating improved kinetics of urease immobilized on glutaraldehyde functionalized nanocomposites.

11. Both types of surface functionalized nanocomposites show very less hemolytic activity (less than 5%) when compared to the non-functionalized counterparts, indicating improved blood compatibility of the materials.

12. MTS proliferation assay demonstrate improved MDA-MB-231 cell viability on glutaraldehyde functionalized nanocomposites. Furthermore, MDA-MB-231 cell seeded on glutaraldehyde functionalized nanocomposites exhibit a higher percentage of viability, adhesion, spreading and morphology as confirmed after acridine orange staining and cell adhesion test by SEM.

13. MTS proliferation assay indicates the improved viability of 3T3 fibroblasts and a neuronal rat pheochromocytoma (PC12) cells on glycine NHS ester functionalized nanocomposites, indicating their non-cytotoxic effect. Surface functionalized nanocomposites demonstrate improved 3T3 cell adhesion, spreading, proliferation and morphology as confirmed by calcein-AM/ethidium homodimer live/dead assay and SEM analysis.

14. The study further demonstrates that several factors such as surface functional groups and charge as well as wettability and material stiffness, can be moderated by surface functionalization of PAni:Ch nanocomposites with polar functional groups to improve cell adhesion, spreading, and growth on conductive polyaniline based biomaterials.

15. The collagen coated PAni:Ch nanocomposites support the growth and differentiation of PC12 cells to sympathetic neurons comparable to control collagen-coated glass plate. Electrical stimulation of PC12 cells under potential of 500 mV/cm for 2h/day through the conductive collagen coated PAni:Ch nanocomposites demonstrates more neurite formation and longer neurite outgrowth than the unstimulated cells on the same scaffolds and indicates PAni:Ch nanocomposites may be suitable as conductive scaffold for nerve repair.

7.1.3 Conclusions from Chapter VI

In **Chapter VI**, We have shown the synthesis and optimization of electrically conductive, porous, mechanically strong and bioactive MEH-PPV:PCL nanofibers with blended form with variation in the volume ratio of the constituents by simple electrospinning process and core-sheath morphology with variation in diameter with varying flow rate by coaxial electrospinning along with post-synthesis surface functionalization using APTES and 1,6-Hexanediamine. We have carried out physico-chemical and biological characterization of the blended electrospun meshes and confirmed that increasing concentrations of MEH-PPV in a PCL blend improve biocompatibility of MEH-PPV alone, reduced nanofibrillar diameter and tensile strength but increased conductivity and subsequent differentiated neuronal growth characteristics on cell seeded surface functionalized and collagen coated meshes under electrical stimulation. We have shown that a core-shell synthesis route with MEH-PPV shell increased fibrillar diameter and tensile strength characteristics whilst improving conductive growth stimulus characteristics for neurite outgrowth on surface functionalized and collagen coated meshes. Electrospun meshes prepared by simple electrospinning and coaxial electrospinning demonstrate enhanced 3T3 fibroblasts adhesion, spreading, proliferation and migration after surface functionalization by APTES and 1,6-Hexanediamine, separartely. Briefly, the synergistic effect of nanofiber feature, surface functionalization of electrospun MEH-PPV based materials and electrical stimulation in neuronal growth has been investigated for potential application of these materials in neural tissue engineering. The major results of this study have been summarized below.

1. Coaxial electrospinning produces uniform nanofibers with larger diameters and better conductive and mechanical properties than the electrospun nanofibers produced by simple electrospinning process as confirmed by SEM, TEM and tensile strength measurements. The diameter of the electrospun nanofibers [324 ± 70 nm (20:80), 280 ± 82 nm (40:60), 198 ± 30 nm (50:50) and 132 ± 53 nm (60:40)] obtained from a blend of MEH-PPV and PCL decreased with increasing concentration of MEH-PPV as confirmed by SEM. The core-sheath morphology of the nanofibers have been confirmed by TEM

and SEM, which indicate the production of larger diameter nanofibers (630 ± 137 nm) using higher flow rate than lower flow rate (526 ± 60 nm).

2. The highly porous electrospun meshes exhibit higher mechanical properties such stiffness constant *(E)* and ultimate tensile strength (UTS). Surface functionalized meshes demonstrate enhanced stiffness constant *(E)* and UTS as compared to their non-functionalized counterparts due to the cross-linking between the polymer chains occurred after functionalization. The core-sheath nanofibers are mechanically strong when compared to the nanofibers prepared from a blend of MEH-PPV and PCL, which is assigned to larger diameters of the core-sheath fibers.
3. The core-sheath electrospun meshes exhibit improved *I-V* characteristics than the nanofibers prepared by simple electrospinning of blend of MEH-PPV and PCL, which can be attributed to the presence of conductive $FeCl_3$ doped MEH-PPV in the sheath of core-sheath nanofibers. No significant changes in conductive properties of the electrospun meshes after surface functionalization by APTES and 1,6-Hexanediamine have been noticed indicating that functionalization has been performed without much affecting the conductive properties of MEH-PPV. *I-V* characteristics of all the electrospun meshes demonstrate Ohmic conductive behaviour at lower voltage region (up to 2-3 V) and space charge limited non-linear conductive behaviour at higher voltage region (beyond 2-3 V). Analysis of *I-V* measurements results further reveals that all the core-sheath nanofibers with $FeCl_3$ doped MEH-PPV in the shell of the core-sheath nanofibers and all the blended electrospun nanofibers with higher MEH-PPV concentration possess lower values of critical voltage (V_c) due to higher density of free charge carriers *(p)*. Furthermore, the non-linear behaviour of the *I-V* characteristics of all the electrospun meshes has been explained with the help of *Kaiser Equation*, which also demonstrate easier hopping of charge carriers in the electrospun core-sheath nanofibers and blended electrospun nanofibers with higher MEH-PPV concentration.
4. Stability test results suggest that all the electrospun nanofibers were found to be stable enough in physiological solution due to the non-degradable nature of MEH-PPV and slow degradation rate of PCL, where fibrillar diameter and

surface resistivity have been found almost constant after 45 days incubation in PBS (pH =7.4).

5. FTIR and XPS show successful incorporation of amine functionality after surface functionalization. Particularly, the bands around 1560 cm^{-1} and 3500 cm^{-1} corresponding to C-N-H stretching/N-H bending and N-H stretching vibrations in FT-IR spectra of APTES functionalized electrospun meshes and the bands around 1550 cm^{-1} and 3400 cm^{-1} corresponding to N-H bending in secondary amine and N-H stretching in primary amine along with the C=O stretching vibration in the FT-IR spectra of 1,6-Hexanediamine functionalized electrospun meshes, clearly indicate incorporation of amine functionality onto the blended nanofibers after surface functionalization. The C1s core-level XPS spectra and XPS survey scans of APTES and 1,6-Hexanediamine functionalized electrospun meshes confirm the presence of nitrogenous chemical groups such as C-N (BE at about 285.91 eV) and atomic nitrogen (N1s) on the surface of the functionalized electrospun meshes, which are not present in the non-functionalized meshes. XPS results support higher doping levels in the core-sheath nanofibers leading to better conductive properties which also confirm the presence of MEH-PPV in sheath.
6. Hemolysis activity assay demonstrates the improved hemocompatibility of the 1,6-Hexanediamine functionalized electrospun meshes than that of the APTES functionalized and non-functionalized electrospun meshes.
7. The surface functionalized electrospun meshes showed significant improvement in viability of 3T3 fibroblasts and a neuronal model rat pheochromocytoma 12 (PC12) cells as confirmed by MTS proliferation assay. Live-dead assay and cell adhesion study by SEM demonstrate that 3T3 fibroblasts adhered, spread, proliferated and migrated well on the surface functionalized electrospun meshes as compared to non-functionalized meshes. It is worthy to be noted that surface functionalization by 1,6-Hexanedamine slightly more effective in the modulation of 3T3 cell behavior, however, this effect is not significant with PC12 cells. PC12 cells were found to adhere and differentiated well on collagen I coated meshes followed by surface functionalized meshes as compared to non-functionalized meshes, as demonstrated by beta (III) tubulin immunochemistry and cell adhesion test (by SEM).

8. Electrical stimulation of PC12 cells through the electrically conductive electrospun nanofibers under the potential of 500 mV/cm for 2 h for 3 consecutive days demonstrates significant improvement in neurite formation and outgrowth than the unstimulated PC12 cells. However, the effect of electrical stimulation on PC12 cells cultured on core-sheath nanofibrous meshes has been found to be more prominent owing to their better conductive properties than the nanofibers prepared from blended MEH-PPV with PCL. It is also noteworthy that surface amination of the core-sheath nanofibers along with electrical stimulation comes out as a promising scaffold to replace the need of coating the scaffold with costly biomolecules such as collagen, laminin, fibronectin etc.

9. The results indicate the potential of MEH-PPV based biomaterial scaffolds in fabrication of nerve guidance channels to bridge the gap for directive growth of damaged nerves in peripheral nervous system (PNS) as an alternative to conventional nerve grafts such as autograft and allograft. It also provides a new additional option using CPs in neural tissue engineering applications as an alternative to widely investigated PPy, PANI and PEDOT. The poor solubility exhibited by these polymers inhibits nanofibril formation by electrospinning whereas MEH-PPV with PCL has been shown to produce nanofibrous scaffolds with varying morphology for potential neuronal stimulation.

7.2 Future prospects

We have developed an electrically conductive, biocompatible, porous, nanofibrous, bioactive and mechanically strong biomaterial scaffolds viz. PAni nanaofibers, PAni:Ch nanocomposites and electrospun MEH-PPV:PCL nanofibers and we have carried out physico-chemical and biological characterization of these materials. We have shown surface functionalization as an attractive technique to improve cell-biomaterial interaction using simplest wet chemical method through incorporation of polar functionalities such as carboxyl (-COOH), amine ($-NH_2$), aldehyde (-CHO), hydroxyl (-OH) groups. It has the potential to replace the need of coating the biomaterial scaffold with costly biomolecules such as collagen, laminin, fibronectin etc. Out of all the three systems, electrospun MEH-PPV:PCL nanofibers comes out to be an ideal tissue engineered scaffold for potential application in nerve repairing. The

combined effect of nanofiber feature, surface amination and electrical stimulation along with good electrical conductivity, mechanical stability, porosity makes it a potential alternative to the conventional technique for nerve regeneration. However, there are further scopes in research using these materials regarding the following concerns:

- There is still need of some more research in the effect of surface modification on neuronal growth.
- Optimization of electrical stimulation with different form of electrical signal is still undone using these materials, particularly MEH-PPV system.
- Biodegradability of these materials is still unexplored.
- May become suitable material for nerve tissue engineering.
- In addition to its utility for nerve regeneration, the polymer systems, particularly MEH-PPV system, could also be applied to other areas of tissue engineering as well, such as skin and connective tissue, wound healing, bone repair, cartilage and muscle tissue engineering etc.