CHAPTER I

INTRODUCTION

This chapter embodies an overview of the exciting and emerging field of conducting polymers (CPs) with an emphasis on conducting polymer based biomaterials. The present chapter begins with a general introduction to conducting polymers followed by primary features of 1D CP nanostructures and their advantages with regard to biomedical applications. The synthesis and surface modification strategies of 1D CP for biomedical applications particularly for improved performance as a tissue engineered scaffold and biomolecule immobilization have been described. A substantial literature review on biomedical applications of CPs and their nanostructures with special focus on tissue engineering has been presented. At the end, the motivation of the thesis, scope of the thesis and statement of the thesis problems are presented.

1.1 General introduction to conducting polymers (CPs) and its interesting features

1.1.1 Conducting polymers (CPs)

Conducting polymers (CPs) are the recent generation of organic polymers, which combine the electrical, electronic, magnetic, and optical properties of metals and inorganic semiconductors with the mechanical properties, processability, etc. of traditional polymers [1]. The structure of this special class of polymers, considered as fourth generation of polymers, is entirely different from traditional polymers or blend of insulating polymer with a conducting material such as a metal or carbon powder. The alternating single and double bonds along the highly conjugated backbone in CPs aid electron mobility and charge transport within and between the polymer chains, leading to high electrical conductivity [2, 3]. The conductivities of the CPs are in the range 1-10^3 S/cm, while the traditional insulating polymers have much fewer conductivities in the range of 10^{-20} to 10^{-6} S/cm [4].

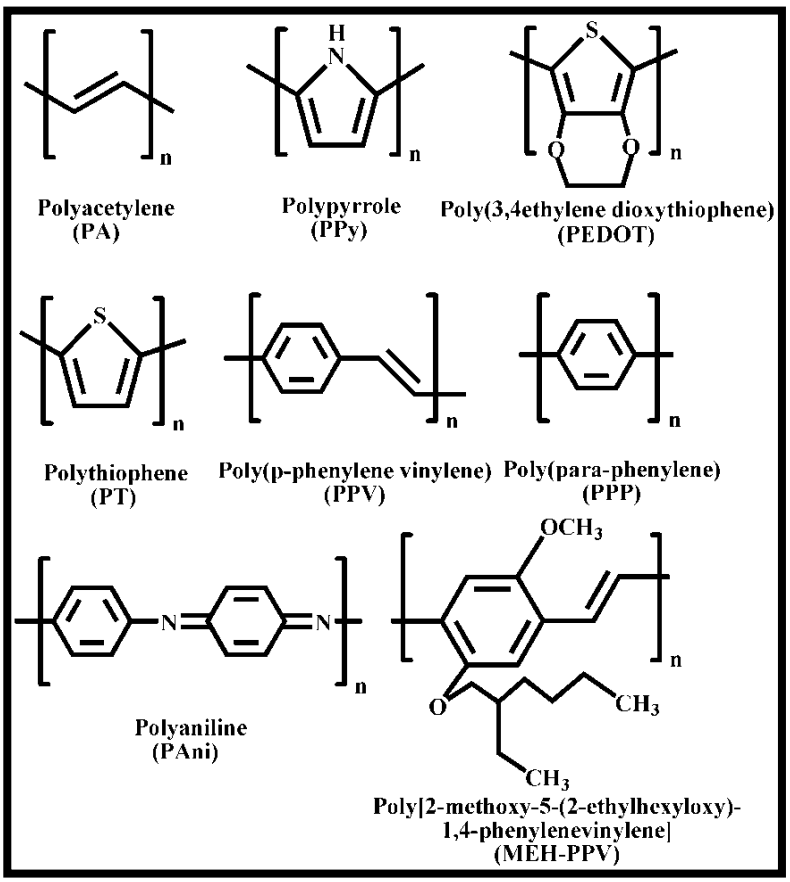

Figure 1.1. Molecular structures of some of the widely used conducting polymers (CPs).

The first reference of the synthesis of CP was reported by Letherby in 1862, where the anodic oxidation of aniline in dilute sulphuric acid yielded an insoluble deep-blue shiny powder deposition on a platinum electrode [5, 6]. Although further studies were reported on this material known as "aniline black" at that time, due to lack of understanding about CP, the discovery was disregarded [7-9]. A significant step towards the discovery of CP is the discovery of the interesting electrical properties of an inorganic polymer, poly (sulfurnitride) $(SN)_x$ during 1973-1975 [10, 11]. Finally in 1976, Alan MacDiarmid, Hideki Shirakawa, and A. J. Heeger produced conjugated conducting polyacetylene doped with bromine and iodine vapor resulting 10 times higher electrical conductivity than the undoped monomers [12, 13]. Their path breaking discovery and development of CPs was distinguished with the Nobel Prize in chemistry in 2000. The electrochemical synthesis of CP was started by Diaz *et al.* in 1979 to achieve conducting polypyrrole (PPy) films [14, 15]. Concurrently, Heeger found that CPs synthesized through chemical and electrochemical redox processes

have relatively higher electrical conductivities [16]. Thus, the flexibility of tuning the conductivity of CPs under oxidation has made them to attract the interest of the scientific world. A tremendous amount of work has been devoted to the development of CPs with important electronic and optical properties of semiconductors and metals and with the attractive mechanical properties and processing advantages of organic polymers with an aim to replace the needs of non-environment friendly and toxic metals in several critical areas such as optoelectronics such as organic light-emitting diodes and field-effect transistors [17-19], energy storage devices such as supercapacitors [20, 21] and solar cells [22, 23], electromechanical actuators [24, 25], electronic textile [26, 27], and anticorrosive coatings [28, 29]. Some examples of conjugated CPs are polyacetylene, polypyrrole, polyaniline, polythiophene, poly(3,4-ethylenedioxythiophene) (PEDOT), poly(p-phenylene vinylene) (PPV) and its derivatives. **Figure 1.1** shows the molecular structures of some of the widely used intrinsic CPs that have been synthesized during the last few decades and applied in different application areas.

1.1.2 Requirements of electrical conductivity in CPs

Electrical conductivity of a material describes how well that material allows electrons to flow through it. In CPs, electrical conductivity arises mainly due to two factors and they are as [30]:

- Conjugated alternation of double bonds and
- Doping

1.1.2.1 Conjugated alternation of double bonds

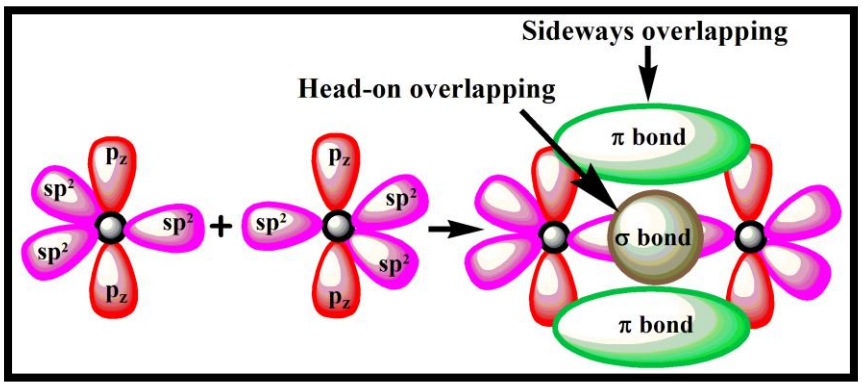

Figure 1.2. Schematic illustration of the formation of σ and π molecular orbitals from two sp^2 hybridized carbon atoms in conducting polymers (CPs).

In CPs, the electronic configuration of the polymer backbone with conjugated alternate single-double carbon-carbon or carbon-nitrogen bonds makes them fundamentally different from other insulating polymers [31]. The backbone in CPs consists of a localized strong 'sigma' (σ) bond and a less strongly localized 'pi' (π) bond withsp^2 hybridized atoms [31, 32]. The sp^2 hybridized carbon atoms consist of one *s* and two *p*-orbitals, leading to one unpaired electron (π electron) per carbon atom as shown in **Figure 1.2**. The remaining out-of-plane p_z orbitals overlap to form a π-band leading to electron delocalization along the polymer backbone. Typically, the *2s* orbital hybridizes with two of the available *2p* orbitals, which results in three sp^2 hybridized orbitals with one unhybridized p_z orbital remaining unaffected. The sp^2 hybridized orbitals are coplanar with angles of 120° among them, whereas the unhybridized p^z orbital is perpendicular to the plane of hybridized orbitals. The overlapping of the hybridized orbitals along the nuclear axis produces strong σ (sigma) bonds accounting for the formulation of polymer chains, whereas, the p_z orbital, perpendicular to the plane of the polymer chain, overlaps laterally with p_z orbital of another carbon atom to from π (pi) bonds as shown in **Figure 1.2**. The delocalization of the electron clouds in the π (pi) bond allows charge mobility along the backbone of the polymer chain and between adjacent chains. Thus, these characteristics of the π-band play a significant role in determining the semi-conducting or in some cases metallic behavior in CPs.

1.1.2.2 Doping

CPs are either intrinsically insulators even after the presence of conjugated alternating double bonds, since the covalently bonded polymer does not contain valence band like pure metals. The universally accepted effective method to impart electrical conductivity to CP is doping by simple anionic or cationic chemical species called dopants [31, 33]. The concept of doping is the unique, central, underlying, and unifying theme which does not distinguish only CPs from all other types of polymers but also it is much different than that in case of inorganic semiconductors [33-35]. Doping in CPs is interstitial whereas in inorganic semiconductors the doping is substitutional. In CPs, doping is nothing but a charge transfer reaction, resulting in the partial oxidation (or less frequently reduction) of the polymer. Most importantly, doping process in CPs is reversible because the original polymer can be obtained with

little or no degradation of the polymer backbone by chemically and electrochemically through the process of dedoping [36]. Doping with suitable dopant counterions can convert an insulating or semiconducting polymer of conductivity 10^{-10}-10^{-5} S/cm to a polymer with conductivity in the metallic regime (~1-10^{4} S/cm) [31, 33]. Thus, the electronic, electrical, magnetic, optical, and structural properties of CPs can be modulated through the controlled addition of small (<10%) nonstoichiometric quantities of suitable dopants [33].

Doped conjugated polymers, when in appropriate oxidized or reduced states are semiconductors and in some cases exhibit metallic behavior due to their unique π-conjugation. Doping creates conduction bands in CP allowing electron flow along the backbone. During doping, the loosely bound electrons in the conjugated system jump around the polymer chain. The unique bond conjugation throughout the backbone of CP, causes delocalization of the electrons, resulting themselves being shared by many atoms [33]. Consequently, the delocalized electrons act as charge carriers making the polymer conductive. Thus, when electrons can be removed or added through creation of cations or anions, respectively, which can hop from one site to another in the polymer chain under effect of an electrical field resulting in increased conductivity of the polymer.

1.1.3 Charge carriers in CPs

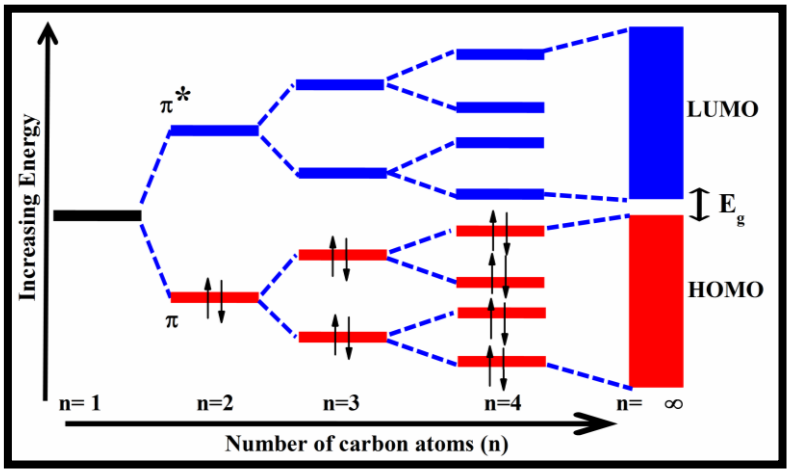

Figure 1.3. Scheme of HOMO and LUMO band formation in conducting polymers (CPs).

In metals or inorganic semiconductors, the overlapping of the atomic orbitals of each atom gives rise to a number of continuous energy bands providing delocalized electrons throughout the entire array of atoms [37]. In CPs, it is the interaction between the two unhybridized p_z orbitals in two sp^2 hybridized carbon atoms of the repeating units throughout the chain, which form the band structure. Thus, the combination of two or more adjacent *p* orbitals generates a set of bonding (π) and anti-bonding (π*) molecular orbitals in which the electron pairs are shared by more than two atoms resulting in a delocalized π-band. The bonding π-orbital is the highest occupied molecular orbital (HOMO) and the anti-bonding π-orbital is the lowest unoccupied molecular orbital (LUMO) [38]. The HOMO and LUMO can be considered to be analogous to the valence and conduction band in case of solid state materials.The difference between the energy of HOMO and LUMO is the energy gap (E_g) of the polymer. The formation of HOMO and LUMO in CPs is shown in **Figure 1.3**.

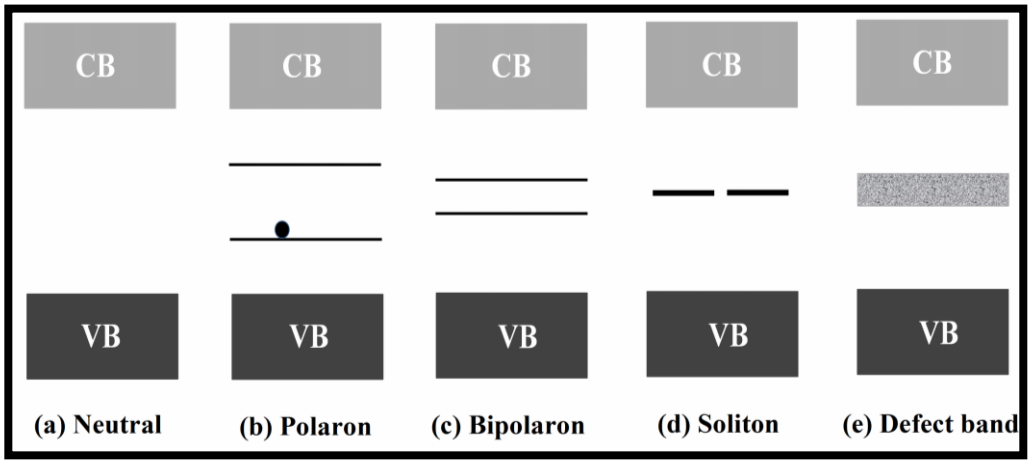

Figure 1.4. Schematic representation of band structure of CPs after the formation of localized defects (charge carriers) viz., polaron, bipolaron, soliton and defect band upon doping.

In CPs, localization of charge and a local distortion (relaxation) of the lattice around that charge, are energetically favorable, leading to the formation of localized electronic state in the band gap [38-40]. The modification in the band structure of CPs after the formation of localized defects such as polarons, bipolarons, solitons and defect bands in CPs is shown in **Figure 1.4.** According to the charge transfer

mechanism, charged defect observed in CPs such as polyacetylene (PA) with degenerate ground state structures and in CPs such as polyaniline (PAni) with non degenerate structures are different [39]. The two geometric resonance structures of PA are of the same energy. The defect either it is a radical (neutral), cation (positive), or anion (negative), on the backbone of PA divides the polymer into sections that are mirror image of each other [**Figure 1.5 (a-c)**]. The movement of such defects in either direction with no effect on the energy of the backbone can be described as a solitary wave or soliton. The radical defect is a soliton with spin ½, while spinless anions and the cations are charged solitons [**Figure 1.4 (a)**] [39-41]. The formation of neutral, positively charged (cation defect) and negatively charged (anion defect) in *trans*-PA is shown in **Figure 1.5 (d)**.

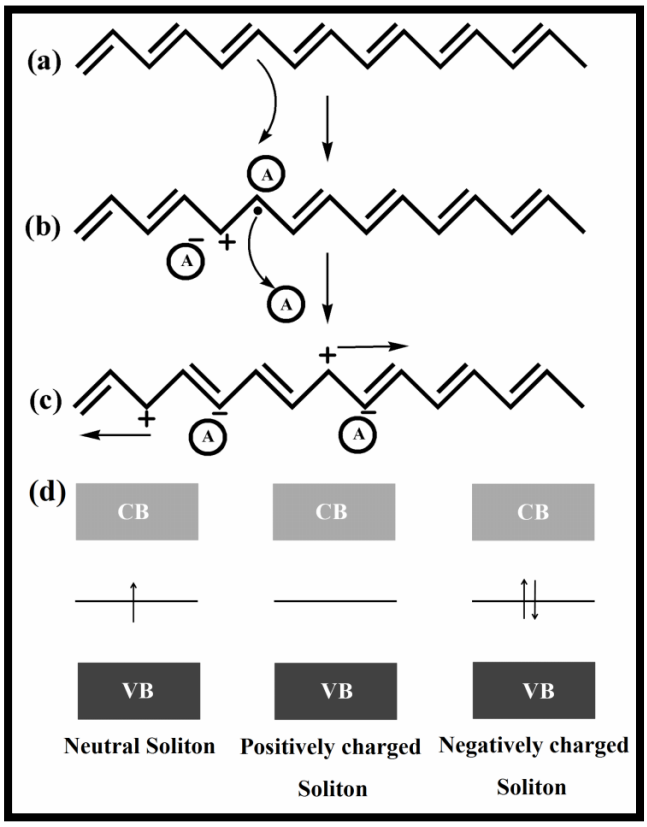

Figure 1.5. Formation of polaron and solitons in *trans*-PA (a) neutral chain, (b) polaron and (c) soliton. (d) Schematic illustration of the modifications in the band structure of *trans*-PA after the creation of neutral, positively charged and negatively charged soliton upon doping.

The ground state of CPs with non-degenerate ground state structures corresponds to a single geometric structure (aromatic-like structure) with lower energy than that of the

corresponding resonance quinoid-like structure. Oxidation (removal of electrons) of CPs creates holes or radical cations along the backbone of the polymer. When an electron is removed from the π-system of a non-degenerate polymer such as polyaniline (PAni) or poly[2-methoxy-5-(2-ethylhexyloxy)-1,4-phenylenevinylene] (MEH-PPV) via chemical oxidation, a free radical (an unpaired electron with spin ½) and a spinless positive charge (cation) are formed [39, 42, 43]. This facilitates the radical ion in the polymer chain with charge or cation localization around it. The radical and cation are coupled to each other via a local bond rearrangement, creating a non-linear defect called polaron which appears in the band structure as localized electronic states symmetrically located within the gap with the lower energy states being occupied by a single unpaired electron [**Figure 1.6 & Figure 1.7**].

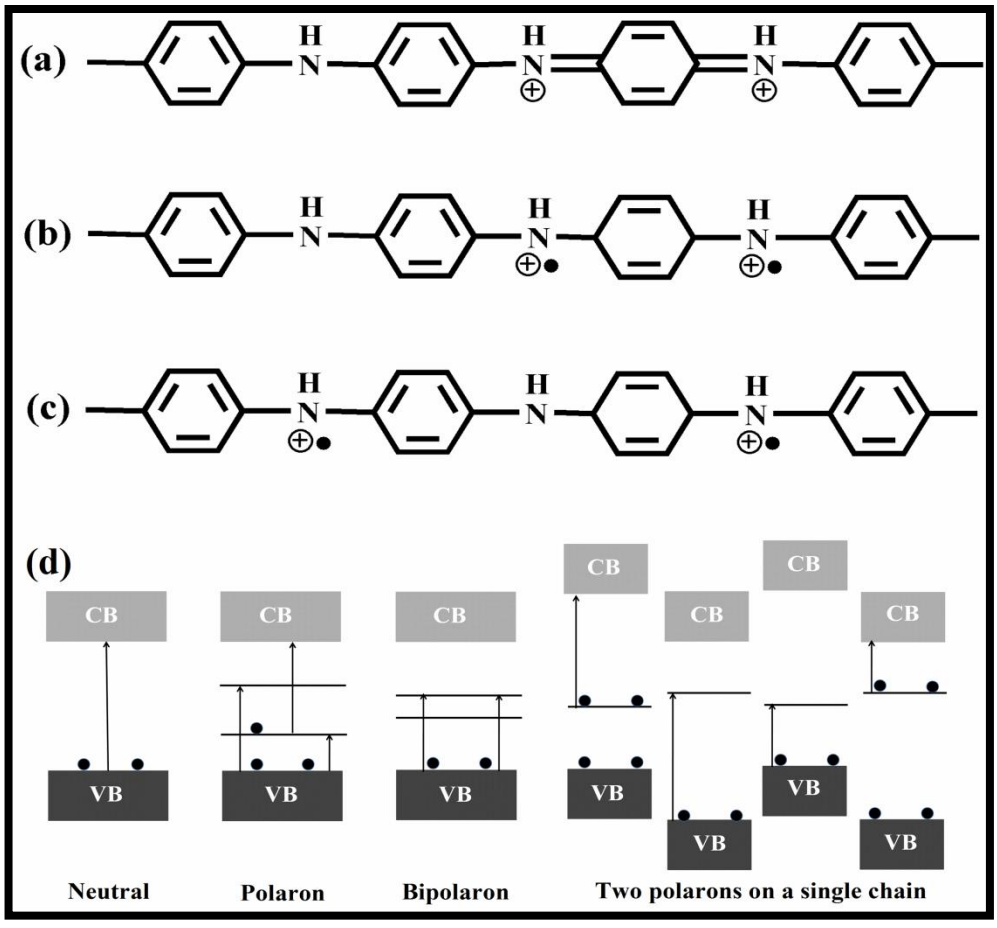

Figure 1.6. Phase transition of localized bipolaron to ordered polaron lattice in PAni. Formation of (a) bipolaron, (b) two polarons (after disassociation of bipolaron) and (c) polaron lattice in PAni. (d) Schematic illustration of energy levels of neutral polymer, positive polaron, bipolaron, and polaron pair of PAni.

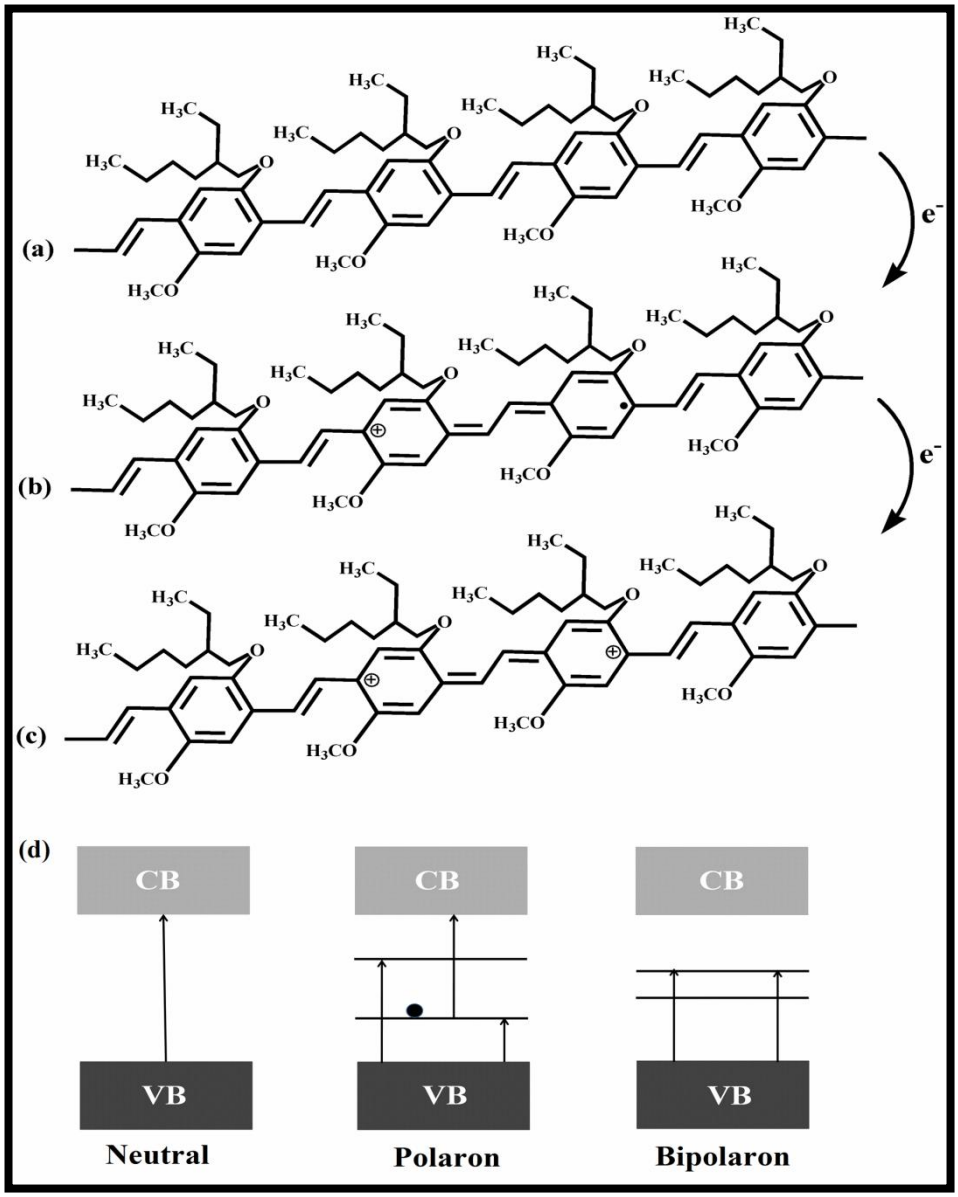

Figure 1.7. Formation of localized defects in MEH-PPV: (a) neutral chain, (b) polaron and (c) bipolaron, (d) Schematic illustration in the band modifications of MEH-PPV upon formation of localized defects, i.e., polaron and bipolaron.

During further oxidation upon higher levels of doping, an electron can be removed from either the polaron or the remaining neutral portion of the polymer chain. When an electron is removed from polaron lattice, the free radical nature of the polaron is lost and a dication is created comprised of two positive charges coupled through the lattice distortion, creating a new spinless defect known as the bipolaron [**Figure 1.6 (a)&Figure 1.7 (b)**] [39, 42, 43]. Removal of an additional electron from a neutral portion of the chain creates two polarons as in case of PAni [**Figure 1.6 (b)**]. Because

bipolaron formation is energetically more favorable than two polaron formation as the former causes a higher decrease in ionization energy. These new empty bipolaron states are also located symmetrically within the band gap. Additional localized bipolaron states are formed upon further doping. At higher doping levels, these bipolaron states overlap with each other and create continuous bipolaron bands. A bipolaron is a spinless pair of same type of charges associated with a strong local distortion. Due to highly energetic nature, the bipolaron charge carrier is short lived. Hence, charge and spin are redistributed resulting in more stable charge carrier, i.e., polaron. Thus, during the doping process, non-linear defects such as polarons, bipolarons and solitons are produced within the polymeric backbone, which are considered as the source of the electrical conduction in CPs [39, 42-44].

1.1.4 Polyaniline (PAni)

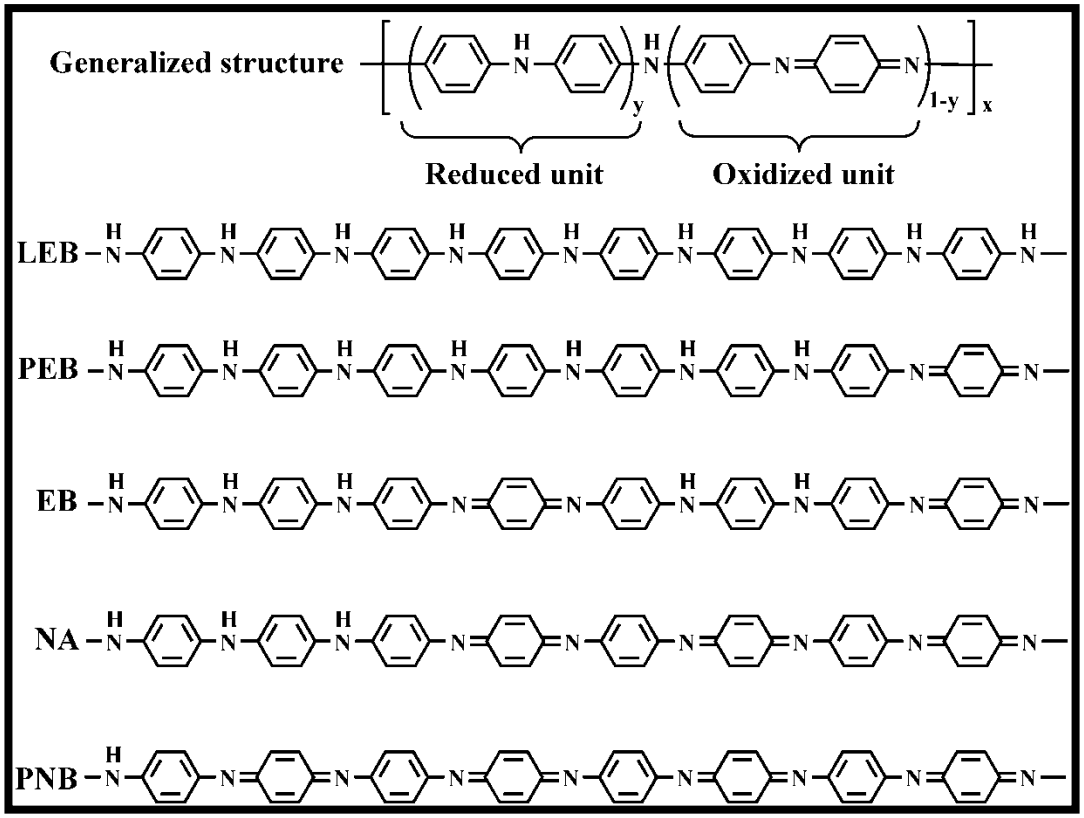

Figure 1.8. Generalized molecular structure of PAni along with typical intrinsic oxidation states of PAni viz. leucoemeraldine (LEB), protoemeradine (PEB), nigraniline (NA), emeraldine (EB), and pernigraniline (PNB).

Polyaniline (PAni) was first synthesized more than 150 years ago by Runge in 1834 [16]. Thereafter, Letheby discovered that the aniline black as a dark brown precipitate after anodic oxidation of aniline at a platinum electrode in presence of sulphuric acid [5]. The first systematic studies of synthesis and characterization of the aniline black were reported by Green and Woodhead in 1910, however, the electrical properties were not measured at that time [8]. In 1986, MacDiarmid *et al*. first reported the conductivity of PAni synthesized by chemical polymerization method [36]. Since then, PAni has been synthesized by various chemical and electrochemical methods [45-48].

PAni is a unique in the family of CPs, which has gained scientific interest in the last few decades owing to its good environmental stability, controllable electrical conductivity, intriguing redox properties and sufficient mechanical properties [16, 49-52]. Besides, the electronic structure and electrical properties of PAni can be reversibly controlled both by charge transfer doping to vary in the oxidation state of the main chain and by acid protonation [53]. PAni has a complex molecular structure consisting of benzoid and oxidized quinoid units, which is dominated by a mixed oxidation state.The generalized molecular structure of PAni has been shown in **Figure 1.8**, where the average oxidation state is denoted by 1-y. The value of y determines the existence of each of the oxidation states of PAni and the different oxidation states of PAni are shown in **Figure 1.8** as reported by Green and co-workers [54, 55]. Thus, depending on the synthesis conditions and doping methods, PAni can exist as fully reduced leucoemeraldine (LEB) where 1-y = 0, half oxidized emeraldine base (EB) where 1-y = 0.5, 75% oxidized nigraniline (NA) where 1-y=0.75 and fully oxidized pernigraniline (PEB) where 1-y = 1. The conducting form of PAni is emeraldine salt (ES) form, which has a half-oxidized and half reduced structure. The emeraldine form is regarded as the most utile form of PAni due to its higher stability at room temperature, consisting of alternately located two benzoid units and one quinoid unit that and it is considered as a semiconductor [53]. Furthermore, LEB can be easily oxidized while the PEB can be easily degraded [53-55]. Thus, due to its vast versatility as described, PAni and PAni based materials have gained the tremendous research interest.

1.1.5 Poly[2-methoxy-5-(2-ethylhexyloxy)-1,4-phenylenevinylene] (MEH-PPV)

Since the discovery of the potential of CPs in light emitting devices in 1990, poly(p-phenylenevinylene) (PPV) and its derivatives have been investigated extensively for optoelectronics applications [56-58]. PPV is the only CP of the rigid-rod polymer family that can be processed into a highly ordered crystalline thin film. It is established that solution processability of polymeric materials is highly desirable for fabrication of thin films for various applications. One of the drawbacks associated with the parent unsubstituted PPV is its poor solubility. Moreover, this parent PPV is infusible and difficult to process.

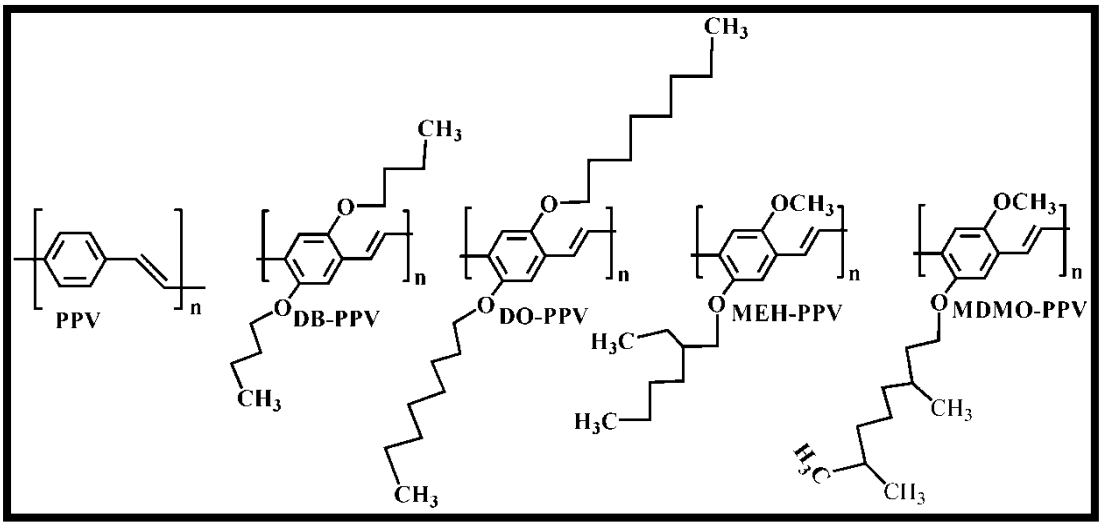

Figure 1.9.Molecular structure of PPV and its various alkoxy-substituted derivatives in increasing order of their solubility.

To overcome the poor solubility, Wessling and others reported synthesis of the soluble precursors, which can be processed into thin films prior to thermal conversion to PPV [59-61]. However, control over polydispersity and molecular weight of the synthesized polymer is the major concerns of the precursor routes. Thereafter, Ohnishi *et al.* and Wudl *et al.* first showed the synthesis of soluble PPV derivatives by grafting long alkyloxy chains into PPV ring, which causes some conformational mobility of the polymer [62-64]. Thus, the incorporation of long alkoxy side chains makes the PPV derivatives soluble in common organic solvents such as chloroform,

dichloromethane, tetrahydrofuran, toluene, or chlorobenzene [62-66]. Consequently, the soluble derivatives have lower glass transition temperatures than parent PPV. Some soluble derivatives of PPV are shown in **Figure 1.9** with increasing order of their solubility. The soluble derivatives of PPV have provided the platform to study the mechanical and electrical properties of the luminescent CPs. Poly[2-methoxy-5-(2-ethylhexyloxy)-1,4-phenylenevinylene] (MEH-PPV) is one of the extensively studied soluble PPV derivatives for its ease of processing, reproducibility, and versatility for various applications such as organic polymer photovoltaics [67-69], light-emitting diodes [56, 57, 65], and light emitting electrochemical cells [70-72], as well as in biosensor applications [73]. Among the soluble derivatives of PPV, MEH-PPV and MDMO-PPV have the highest solubility due to their branched alkoxy chains [**Figure 1.9**] [74]. In fact, MEH-PPV is the first soluble derivative of PPV that was successfully synthesized. MEH-PPV can be synthesized by several procedures such as Wittig reaction [75], the dehydrohalogenation of α,α'-dichloro-*p*-xylene [76], Heck reaction [77], Suzuki reaction [78], and Gilch polymerization [79]. Most of these synthesis procedures are multistep and the bases such potassium *tert*-butoxide, used in Gilch polymerization are costly and unstable. Moreover, the gelation or microgelation occurred due to use of the bases in Gilch polymerization is a serious problem, which restricts its various applications [80]. Wu *et al*. reported a novel and simple liquid-solid two-phase reaction for the one step synthesis of MEH-PPV free from gelation [80].

MEH-PPV is a p-type semiconducting polymer that has low conductivity due to its low hole and electron nobilities. However, suitable doping can improve its conductivity for desired applications. For example, Shin Sakiyama *et al*. demonstrated remarkable improvement in the conductivity of MEH-PPV using $FeCl_3$ (p-type dopant) and Cs_2CO_3 (n-type dopant) [81-83]. The charge carrier formations in MEH-PPV are shown in **Figure 1.7**. Amidst the versatile applications of MEH-PPV in optoelectronics and solar photovoltaic, it offers an interesting property for biological application since it allows the immobilization of biomolecules due to its high density holes-traps. Moreover, the solubility of MEH-PPV in common organic solvents provides an extra advantage over poorly soluble polymer in processability into desired forms for specific applications [84].

1.2 Tissue engineering and biomaterials

Tissue engineering is an interdisciplinary field that applies the principles and innovations from engineering and life-sciences toward the development of biological substitutes that restore, maintain or improve tissue function [85-87]. In recent years, the multidisciplinary knowledge obtained from micro and nanoscience and technology are being also involved in tissue engineering [88, 89].

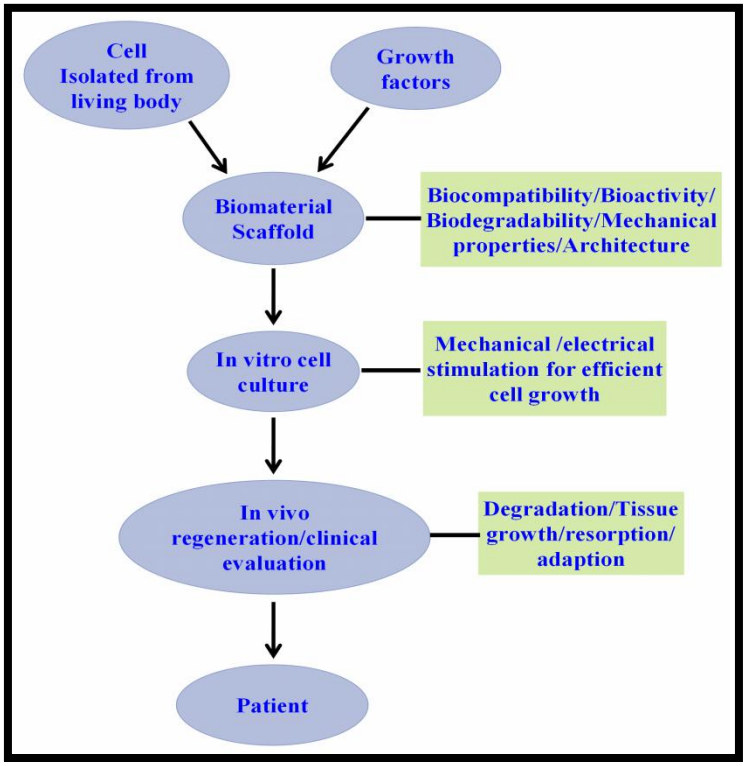

Figure 1.10. Principle of tissue engineering. General process of tissue engineering involves seed cells on biomaterial scaffold, culturing *in vitro* and implant into the patient showing the necessary characteristics that an ideal scaffold should possess.

Typically, three approaches are investigated singularly or in combination: cells that create tissue, biomaterial scaffolds that gives structural support to cells, and growth factors and cell-matrix (scaffold) interactions to create an environment that promotes the regeneration of functional tissues and organs [**Figure 1.10**] [88-90].Therefore, one of the basic and sensitive strategies of the tissue engineering is the selection and construction of a biomaterial scaffold with the desired features for in the design and fabrication of neotissues/organs. International Union of Pure and Applied Chemistry (IUPAC) defined biomaterial as a material exploited in contact with living tissues,

organisms or microorganisms [91]. Biomaterials facilitate exciting new opportunities for repairing and reconstruction of damaged tissues by fabrication of biomimetic, biocomposite scaffolds upon which new cells can regenerate *in vivo*. Therefore, the ideal biomaterial should be biocompatible, biodegradable, highly porous with a large surface area to volume ratio, mechanically strong and capable of being formed into desired shapes. The biomaterial should possess appropriate surface properties to favor cellular attachment, proliferation, and differentiation [92, 93]. Moreover, the biomaterial scaffold should degrade at the rate of tissue formation as the cells synthesize natural matrix structure around themselves, the scaffold shall provide structural integrity within the body and eventually break down leaving the neo-tissue [92, 93].

Since the first use of biomaterial based medical devices on human in the late 1940s and early 1950, the biomaterials field has acquired widespread scientific and technological applications such as cardiovascular prostheses, intraocular lenses, joint replacements, dental implants, scaffolds for *in vivo* and *in vitro* cell growth, skin substitutes, sutures, blood bags, bone cement, etc [94]. With the advancement in the last few decades in areas such as medicine, cell and molecular biology, chemistry, materials science and engineering, biomaterials research has significantly evolved. The stages of development of biomaterials research can be classified in three generations, where each generation differs from the previous one by the specific purpose it tries to accomplish, thus enlarging the field of applications, and temporal overlapping can occur. Till now, the field is continuously updating with the design of novel biomaterials and emerging technologies [93, 94].

The 1st generation of biomaterials during 1950-1960, comprised of widely available industrial materials which, although not being designed for a specific medical application. These materials induced minimal foreign body response and indicated potential in tissue replacement due to their suitable chemical and physical properties [95]. For example, silicone rubber used in medical components and assemblies or pyrolytic carbon for mechanical heart valves are examples of 1st generation biomaterials.

During 1980-1990, the 2nd generation of biomaterials was designed with an aim to achieve bioactivity [96] for favorable cell-biomaterial interactions. Hence, biomaterials are conceptualized as systems for controlled drug release and gene therapy, delivering pharmacologic agents such as drugs, active proteins, growth

factors, and other macromolecules of interest to localized areas. For example, bioactive glasses and ceramics are widely used in orthopedic and dental procedures [97]. Drug-eluting stent coatings designed to prevent endovascular restenosis (blood vessel closure) or biodegradable sutures are also found among this group [98].

From 2000 onwards, the 3rdgeneration biomaterials research is focused on developing novel biomaterials with innovative strategies to support functional tissue regeneration as opposed to replacement (1st generation), or either resorbability or bioactivity (2nd generation) [99]. Herein, the designing of the biomaterial scaffold involves the reassemble the complexity of natural tissue assemblages to mimic the native extracellular matrix (ECM), and stimulate specific cellular responses at the biointerfaces to promote cell adhesion, proliferation, migration and differentiation processes [100]. Several new strategies are adapted to design as a smart interface or proactive cell-instructive material including the use a series of chemical, biochemical and biophysical signals that actively guide the whole tissue repairing process [101-103].

Various materials such as metals (titanium, stainless steel, platinum, platinum-iridium), ceramics (calcium phosphates), glasses, carbons, polymers (polyester, poly(lactic acid), polyurethane, silicone, polypropylene, Teflon®, poly(methyl methacrylate), nylon) and biopolymers have been utilized as biomaterials throughout these three generations [104, 105]. Out of which polymeric biomaterials are idiosyncratic due to ease of the flexibility in formulation into a wide range of shapes, viz., coatings, fibers, films, membranes, foams, nanoparticles or hydrogels. Although the polymeric biomaterials may be either synthetic or natural, the synthetic polymers offer some extraordinary advantages such as the potential of being customized within a wide range of physicochemical properties, being fabricated into various shapes with desired morphologic features and uniformity.

1.3 CPs in biomedical applications

As stated in the previous section, from 2000 onwards, the polymeric materials have the edge over all other materials in biomaterials research and the list has become extensive by now. Regarding tissue engineering applications, most of them have been utilized in a passive way, just as support for the cells and tissues. However, the active behaviour or unique properties intrinsic to the material can be taken into account for

some specific applications as an advantage to the conventional polymeric biomaterials. This idea initiated the need for the development of smart biomaterials.

In this regard, with the similar electrical and optical properties of metals and inorganic semiconductors and flexibility in ease of synthesis and processing of conventional polymers, CPs have been proved to be innovative bioactive materials or functional biointerfaces. It has been verified that CPs have all the desired features of intelligent materials, because [106]:

- ❖ CPs can be engineered at the molecular level to recognize specific stimuli.
- ❖ They are conductive, they facilitate transport of electrical information.
- ❖ They are capable of localized processing as well as actuation of response mechanisms.

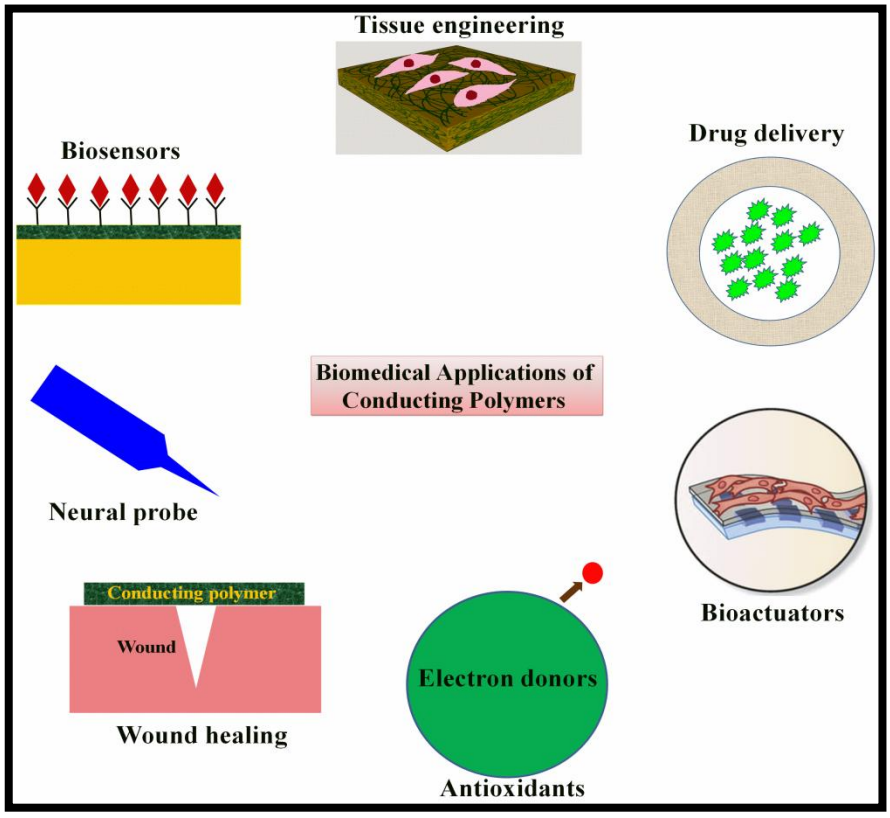

Figure 1.11. Biomedical applications of CPs.

Due to this unique combination of beneficial properties, CPs have found a diverse range of applications in the microelectronics industry [16-19], battery technology [20, 21, 107, 108], photovoltaic devices [22, 23, 109, 110], light emitting diodes [65, 111], and electrochromic displays [112, 113], and more recently in the biomedical field

[114-126]. The evidence of compatibility of CPs with many biological molecules in 1980s initiated the research on biomedical applications of CPs. Since then, CPs have been investigated extensively for evaluation of their biocompatibility due to their intrinsic the ability to entrap and controllably release biological molecules through reversible doping, ability to charge transfer from a biochemical reaction, and the flexibility to easily alter the electrical, chemical, physical, optical and other physicochemical properties of the CPs to better fit the nature of the desired application. After the assessment of the biocompatibility of CPs, their utilization in biomedical applications has been remarkably increased. Thus, the combination of their outstanding properties (good stability, intrinsic high conductivities and ease of synthesis) with additional features such as biocompatibility, high sensitivity/selectivity towards specific analyte, reproducibility of the electrode response and redox stability, turned them into potential candidates in biosensors [127-129], tissue engineering [118-123], neural probes [130-132], drug delivery [117, 133-135], bioactuator devices [136, 137] **[Figure1.11]**. Thereby, during past 20 years, CPs such as polyaniline (PAni), polypyrrole (PPy), polythiophene (PT) and poly(3,4-ethylenedioxythiophene) (PEDOT) have gained significant importance in fabrication of biomaterials for biomedical applications. Particularly for tissue engineering applications, CP based biomaterials are important in that they can induce electrical, electrochemical and electromechanical stimulation of cellular activities [118-122]. Different biomedical applications of CPs have been reviewed in the following subsections.

1.3.1 Biosensors

CPs lend themselves as a potential choice as electronic transducer material in biosensor due to efficient charge transfer from biochemical reaction, electrochemical synthesis in situ on electrodes, the ability to entrap biomolecules and ease of surface modification [138]. CP based biosensors have been fabricated and utilized in various fields such as health care (in medical diagnosis such as glucose, fructose, lactate, ethanol, cholesterol, urea detection), immunosensors (in medical diagnostics and environmental sensors), DNA sensors (in the detection of various genetic disorders), environmental monitoring (to control of pollution and detection of hazardous chemicals) and food analysis (for detection of glucose, fructose, ethanol, sucrose, lactate, malate, galactose, citrate, lactose, urea, starch etc. in food industries) [128].

Depending on the nature of sensing the chemical event, CP based biosensors can be divided mainly into amperometric (measures current), potentiometric (measures potential), conductometric (measures change in conductivity) and optical (measures light absorbance or emission) biosensors [128]. The immobilization of biological sensing elements such as enzymes, antibodies, growth factors, DNA or RNA fragments including polar functionality such as carboxyl (COOH), amine (NH_2) etc., is one of the prerequisites for fabrication of highly sensitive and selective biosensors. There are two main approaches for immobilization of biological sensing elements on CPs: covalent immobilization, which includes all techniques that create a covalent bond between the conducting substrate and the biomolecule via functional moieties and non-covalent immobilization, which includes adsorption, physical entrapment, and affinity binding [139]. For example, glucose oxidase and lactate oxidase were covalently immobilized on carboxyl acid functionalized PT for the detection of glucose and lactic acid, respectively [139-141]. Carboxylic acid substituted PT was used for P-aminophenyl-a-D-mannopyranose immobilization,that selectively binds to viruses [142]. PPy based immunosensor was fabricated by immobilizing Listeria monoclonal antibody on carboxylic acid-functionalized PPy for detection of Listeria monocytogenes [143, 144]. In another example, DNA has been immobilized by physical adsorption method on PPy surfaces via mercapto-oligonucleotide probe immobilization onto Au-Ag nanocomposites for fabrication of DNA biosensor [145, 146].

1.3.2 Tissue engineering

The evidence of success of the applicability of CPs in biosensors has laid the foundation of its applicability in tissue engineering. The growing interest for electrical and electromagnetic stimulation in the medical field arises from the understandings of the intrinsic functionalities of living tissues. Living tissues produce electromotive forces, maintain a required potential differences, and switch current on and off by controlling current flow and store charge [120]. It is well established that electrical voltage exists across the plasma membrane, while the inner part of cells are more negative than the outer part. This potential difference, namely resting potential, is continued at a steady level when excitable cells are inactive. Hence, the application of electrical signal influences strongly the cellular activity in modulating the intracellular signal transduction mechanisms. In this context, the use of electrical signals to control

the local microenvironment for cells is crucial to trigger basic cellular activities towards specific phenotypes to attain a long-term functionality of tissues during the *in vitro* regeneration processes. In current tissue engineering practices, cells are generally forced to survive in a dynamic environment reproduced by custom-made scaffolds that mimic the composition and topography of a natural ECM, by the support of physical factors, such as mechanical and electrical signals. Currently, CP based biomaterials offer remarkable scaffold functionalities by perfectly supporting the electrical stimulation among cells that is mandatory for promoting regeneration mechanisms in the case of specific stimuli responsive cells (i.e., neurons, myotubes, cardiomyocites) [118-120, 122, 147, 148]. CPs have been demonstrated to positively influence cellular activities, including cell adhesion and migration [121-126, 149], DNA synthesis [150, 151] and protein secretion [153].Considering these versatility, several CPs such as polypyrrole (PPy), polythiophene (PT), polyaniline (PAni) and poly(3,4-ethylenedioxythiophene) (PEDOT)) have been investigated since the discovery of CP in 1977 for numerous applications. These CPs were studied with various cell types including endothelial cells [154], rat pheochromocytoma (PC12) cells [155, 156], cardiac myoblasts [157], neurons and support cells (i.e., glia, fibroblasts) associated with dorsal root ganglion (DRG) [155, 158], primary neurons [158, 159], keratinocytes [118, 160], and mesenchymal stem cells (MSC) [119, 161]. The beneficial effect of electrical stimulation on neurite formation and neurite outgrowth was shown using PPy, PAni and PEDOT with PC12 cells [155, 162], retinal ganglion cell (RGC) [130, 163], dorsal root ganglion (DRG) [155, 164], and nerve stem cells [165, 166].

1.3.3 Neural probe

For neural probe applications, the required properties are high surface to volume ratio, hydrophobicity and cell specificity to improve and maintain good signal to noise ratio for detection of neuron signals [167]. The efficiency of the conventional neuroprosthetic implants fabricated from platinum, gold or alloys of these metals and iridium oxide are limited by the minimal interaction of these materials with neural tissue and thereby, limit their ability to provide optimal stimulation and recording from neural cells [168]. However, the flexibility offered by CP based biomaterials such as ease of processing into various shapes and surface modification of biomaterials surface and intrinsic higher charge injection capacity of CPs, make them

a suitable candidate for neural probe applications. Neurite growth was demonstrated to be improved on PPy in presence of neurotrophins like NT-3 and brain-derived neurotrophic factor (BDNF) for cochlear explants [169]. A study examined the effect of entrapping nerve growth factor (NGF) within the PEDOT during electrodeposition to create a polymer capable of stimulating neurite outgrowth from proximal neural tissue [170].

1.3.4 Drug delivery

Currently available drug delivery systems are effective at the controlled release of drugs. However, targeting the individual cells is still a challenge. In this context, the utility of CP based biomaterials gains immense importance since their biocompatibility opens up the possibility of using them for *in vivo* biosensor applications for continuous monitoring of drugs or metabolites in biological fluids [171], or as a means of opening up the field to a variety of new analytes [129, 133]. The CPs such as PAni, PPy and poly(aniline sulphonic acid) have been proved as effective scavengers of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical indicating potential as antioxidants in tissues suffering from oxidative stress, where the ability to lower the excessive levels of reactive radical species is desirable [129].The excellent redox behaviour of CP based biomaterials has been used to increase permeation of drugs such as dexamethasone [172]. Moreover, electrical stimulation of CP based biomaterials such as PPy, PEDOT, has been used to accelerate the release of a number of therapeutic proteins and drugs like nerve growth factor (NGF), dexamethasone and heparin [133]. However, CPs have certain disadvantages attributed to the initial burst release of the drug and the hydrophobic nature of the polymer, which limits their application [129, 133]. Nevertheless, the drug delivery systems based on CP are of immense scientific interest and have potential for the treatment of cancer and also minimum invasive techniques for several neural and cardiovascular applications.

1.3.5 Bioactuators

Bioactuators are used to produce mechanical force, which in turn can be used as artificial muscles. The phenomenon of change in the volume of the CP scaffold upon electrical stimulation has proven to be beneficial in the construction of bioactuators. Bioactuators based on CP have many advantages such as they can be electrically

controlled, have a large strain which is favorable for linear, volumetric or bending actuators, possess high strength, require low voltage for actuation (1 V or less), can be positioned continuously between minimum and maximum values, work at room/body temperature, can be readily microfabricated and are light weight, and can operate in body fluids, which make them an ideal candidates in the fabrication of bioactuators [167]. In artificial muscle applications, CPs have been used in a triple layer arrangement, where the middle layer comprises a non-conductive material [173, 174]. When current is applied across the two CP films, one of the films is oxidized and the other is reduced. PPy, PAni and PPy-PAni composites and composites of these polymers with carbon nanotubes (CNT), i.e. PAni-CNT and PAni-CNT-PPy, have all been explored for their ability to function as actuators [173-177].

1.4 CP based biomaterial scaffolds: Biomimetic properties

The design of CP based scaffolds for tissue engineering purpose is a challenging task. The characteristics of the biomaterial scaffolds are governed by the fabrication technique. Therefore, during design, it is the utmost importance to incorporate all well defined features to the biomaterial scaffold that mimic the native ECM. By definition, the biomaterial scaffold is a temporary supporting structure for growing cells and tissues also called as synthetic or artificial ECM, where cells can undergo proliferation, migration, and differentiation in three dimensions, which eventually leads to the formation of a specific tissue with appropriate functions as would be found in the human body. The native ECM in the body is a complex and dynamic arrangement of proteins and polysaccharides such as collagen, glycosaminoglycans, hyaluronic acid, proteoglycans, elastin along with a complex mixture of nano features of pores, ridges and fibers of nano-scaled sizes, from several to more than 100 nm and thereby, provides substrates with specific ligands for cell adhesion and migration, regulates cellular proliferation and function by providing various growth factors [121, 178-181]. Therefore, mimicking both the nanofibrillar structure and the complex function of native ECM are required to create an ideal biomaterial scaffold capable of regeneration of fully functional tissues. The CP based biomaterial scaffold should possess some few basic characteristics such as biocompatibility, surface roughness, porosity, hydrophilicity, three-dimensional geometry with nanofiber feature, redox stability, biomolecule functionalization, degradability and elasticity to function in the same way as that of native ECM under physiological conditions. Moreover, the CP

based biomaterial scaffold should be conductive enough to deliver electrical stimulation to the cells to function as smart biomaterial.

Biocompatibility is the first and foremost required characteristics of any biomaterial scaffolds such that it does not provoke any rejection, inflammation, and immune responses when in contact with the living system [178-181]. One of the essential properties of an artificial ECM is the biological activity to facilitate the necessary cell-biomaterial interaction to mediate cellular adhesion, migration, and proliferation [181, 182]. Bioactivity of CP based scaffolds can be achieved by the biofunctionalization of CPs using bio-regulative cues such as collagen [183], gelatin [184], laminin [169], lysozyme [185], polysaccharides [186], dextrin molecules [187], RGD (Arg-Gly-Asp) [188] or doping with heparin [189], hyaluronic acid (HA) and chondroitin sulphate A [190] to promote cellular adhesion, while nerve growth factors (NGF) have been used to improve compatibility with neural cells [188, 191]. It should provide a 3D template in nanofibriliar feature with the surface to volume ratio for maximum accommodation of cells and cell-biomaterial interaction [121]. The biomaterial scaffold should possess a high level of porosity (with pore size for efficient influx of anabolic nutrients and outflow of catabolic waste (approx. 10-1000 nm) [121, 180]. Furthermore, the scaffold should be biodegradable such that the degradation rate of the scaffolds matches the rate of tissue regeneration to maintain the tissue functionality [121, 180]. It is worth pointing out the necessary mechanical stability and elasticity of the scaffold to withstand *in vivo* biological forces, and to support the cells to synthesize specific proteins including other biochemical and biological processes needed for a healthy tissue function and growth [121, 180]. In addition, it should be sterilizable as well to avoid toxic contaminations without compromising any physicochemical properties [121, 180]. Specifically, to utilize the conductive property of CP based scaffold as a smart biomaterial, it should be designed in such a manner that charge transfer takes place efficiently between the plasma membrane of cells and the biomaterial to stimulate the normal cellular activities just like longer neurite outgrowth of neurons under electrical stimulation. Moreover, the stability of the CP based biomaterials in physiological condition required without any affect on its conductivities. Last but not least, the production process CP based biomaterial scaffold incorporating all the above essential features must be performed in a reproducible, economical, and scalable manner.

In the present thesis, the synthesis and modification of one dimensional (1D) CP nanostructures have been emphasized to use as potential biomaterial scaffold and accordingly, the following sections, subsections of this chapter deals with the brief discussion of the advantages of 1D CP nanostructures over other nanostructures and its bulk counterpart followed by the synthesis and modification strategies of 1D CP nanostructured biomaterials.

1.5 One dimensional (1D) CP based nanostructures

In last few decades, nanotechnology has been rapidly emerging as one of the most active interdisciplinary research area because of its astounding potential for a variety of applications. When one of the dimensions of the many well-studied materials is reduced to nanoscale (less than 100 nm), radically improved or new surprising properties often emerge. Nanostructured materials may be zero dimensional (0D) such as quantum dots, one dimensional (1D) such as nanofibers, nanowires, nanotubes, nanorods, two dimensional (2D) such as nanosheets and three dimensional (2D) such as nanoparticles. Nanomaterials become a fascinating playground for scientists and engineers primarily because of their unusual but fascinating chemical/physical properties in reduced dimensionality and unique applications. With remarkable enhancement in optical, electronic, chemical and mechanical properties from their bulk counterpart, nanomaterials have a deep impact on both fundamental research and potential applications in nanoelectronics or molecular electronics, nanodevices and systems, nanocomposite materials, bio-nanotechnology, and medicine [192-194]. The enhanced properties of nanomaterials when compared to the bulk counterpart are mainly due to their large surface to volume ratio, which is a crucial factor in determining device efficiency. Moreover, the variation in the energy band gap in reduced dimensionality causes a remarkable change in optical and electrical properties in nanomaterials from the bulk counterpart. Among the different types of nanostructures, 1D nanostructures have attracted a great interest owing to their smaller dimension and high aspect ratio, which offer efficient charge transport along one controllable direction and thereby, makes it highly suitable for moving charges in integrated nanoscale systems [192-194]. Due to their unique physical/chemical properties, 1D nanostructures can be exploited as device elements in many kinds of nanodevices. Therefore, in recent years, an enormous amount of research has been focussed on the development of 1D organic semiconductor such as CPs due to the

potential advantages of combining an organic semiconductor with low dimensionality [192-194]. The 1D nanostructures of CPs can be an excellent complement to the conventional inorganic semiconductors owing to their readily tunable band gaps, rich redox chemistry (or electroactivity), excellent flexibility and/or good processibility and greater biocompatibility over conventional inorganic nanomaterials. The 1D nanostructured CPs possess some unique properties such as π-conjugated polymeric chains, metal/semiconductor like conductivity, reversible doping/de-doping processes, making them potential candidates for various applications such as nanoelectronics or molecular electronics, nanodevices and systems, nanocomposite materials, bio-nanotechnology, and medicine.

However, the development of nanostructured CPs with tunable microstructures and controllable chemical/physical properties still remains a challenge. In regard to tissue engineering applications, an ideal scaffold for regeneration of tissue should be biocompatible, biodegradable, bioactive, highly porous with a large surface area to volume ratio, mechanically strong and capable of being formed into desired shapes. There are several issues such as toxicity, poor cell-biomaterial interactions due to the absence of cell interaction sites, poor hydrophilicity, non-biodegradability poor solubility, and processability, as well as uncontrollable mechanical properties to mimic native ECM that need to be improved for realizing the true potential of these materials in tissue engineering applications.

Additionally, nanocomposite with incorporation of at least one secondary component into 1D nanostructures of CPs have been shown to improve or extend the functionality of CPs [194]. The synergistic effect of each component contributes towards the improved chemical properties or combined multi-functionalized chemical/physical/biological properties of the CP based nanocomposites. Therefore, nanocomposites have also attracted extensive interest because of their potential to combine different building blocks to improve a variety of physico-chemical and biological properties [194, 195]. Nanocomposites are a class of materials originating from appropriate combinations of two or more nano dimensional objects by using some suitable techniques, resulting in materials having unique physical properties. Composite materials are naturally occurring or engineered materials made from two or more constituent materials with significantly different physical or chemical properties as compared to their individual components which remain separate and

distinct at the macroscopic or microscopic scale. A common example is "Concrete", a composite construction material composed of cement (commonly Portland cement) and other materials such as fly ash, slag cement, limestone, granite, fine aggregates such as sand, water, and chemical admixtures. Concrete is used more than any other man-made material in the world. However, in the microscopic world, due to the reduced particle size of the components, the interface interactions play a pivotal role in composite mixtures and this is especially important for a new class of recently developed materials named the nanocomposites. Thus, CP based nanostructured materials may be broadly classified into two categories:

- CP nanostructures such as nanofibers, nanoparticles, nanowires, nanotubes and
- CP nanocomposites, which are mixtures of metal/metal oxide/ceramic nanoparticles with CP at nanoscale or nanoscale mixture of CP nanostructures with another polymer.

1.6 Synthesis and modification strategies of nanostructured CP based biomaterials

1.6.1 Synthesis of 1D CP nanostructures

In the previous sections, the potential of CPs in biomaterial fabrication has been demonstrated. CPs represent an excellent choice to electrically stimulate local tissue is of great potential, while CP based biomaterials can also support cell adhesion, migration, and proliferation [196]. In contrast their vast potential, their application is constrained by their non-biodegradable properties, low porosity, hydrophobicity and poor mechanical integrity. A common strategy to overcome such limitations is the processing of the CPs into nanofiber morphology and blending with natural biopolymers. As discussed above, nanofibrillar morphology of CP based biomaterial can contribute towards facilitation of higher surface area for maximum cell loading, porosity, elasticity, and most importantly, controlled conductivity in one particular direction, while blending with natural biopolymers provides biodegradability, bioactivity to mimic native ECM. Accordingly, CP based biomaterials scaffolds can effectively interface with biological systems and act as cell-instructive platforms, thus contributing enormously to the field of tissue engineering and regenerative medicine.

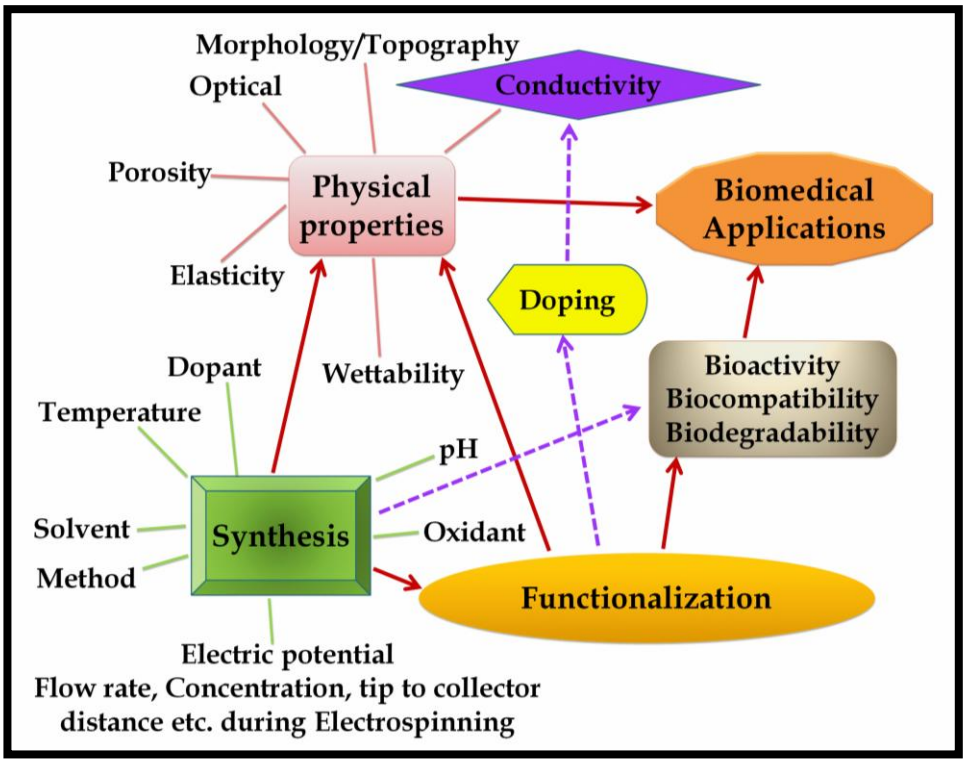

Figure 1.12. Various parameters and factors in fabrication of electrically conductive CP based biomaterials.

Taking the advantages of the unique properties CPs in combination with reduced dimensionality, as well as tailored physicochemical properties, efforts have been focussed to produce suitable conductive biomaterial scaffold. While synthesis of 1D CP based nanostructures is highly sensitive to the choice, it is important to bear in mind that due to the vast versatility, a number of influencing parameters such as purity of the dopant, solvent, the oxidant, the relative concentration of the reagents, reaction time, temperature, stirring rate, etc. are interconnected in the synthesis of 1D CP based nanostructures [**Figure 1.12**] [196]. The challenge is to balance all these parameters to fabricate a functional CP based biomaterial. The selection of dopant determines the properties of a CP for a specific application. Using electrical stimulation, the dopant can be expelled and incorporated again into the polymer, allowing the control of these preset physical properties. If the chosen biomolecule cannot be used as the dopant, it can still be incorporated using an intermediating doping molecule. If doping is not the right way to make the conductive polymer better suited for an application, physical adsorption, entrapment and covalent bonding offer alternative routes. Synthesizing chemically or electrochemically, perhaps as

composites, electrospun fibers or hydrogels, can also be used to improve the usefulness of the end product [196]. Many synthetic strategies (both physical and chemical) have been adopted for the preparation of 1D CP based nanostructures viz. electrospinning, hard physical template-guided synthesis and soft chemical template synthesis (e.g., interfacial polymerization, template-free method, dilute polymerization, reverse emulsion polymerization, etc.), and a variety of lithography techniques, which are discussed below.

1.6.1.1 Hard physical template method

The template method of polymerization proposed by Martin *et al.* [197] is an effective technique, which involves the use of a template membrane to guide the growth of arrays of aligned polymer micro/nanotubes and wires with controlled length and diameter. There are several advantages such as excellent control over the dimensions and the geometry nanostructures through proper selection of suitable template, production of highly ordered nanostructures [195]. However, a post synthesis step is needed to remove the template, which may result in drastic alteration or even destruction of the synthesized nanostructures [195]. Additionally, the production of nanostructures is limited by the number of pores that are present in the membrane. Anodic aluminium oxide (AAO) templates and particle track-etched membranes (PTM) are the examples of the most commonly used porous materials as templates for the synthesis of nanofibers and tubes. By now, nanotubes/wires of a variety of CPs such as PAni, PPy, PEDOT, PPV, poly(3-methylthiophene) (P3MT), have been synthesized via chemical or electrochemical route inside the pores of these template membranes [197-200]. Furthermore, CdSPPy heterojunction nanowires [201], multi-segmented Au-PEDOT-Au [202] and Au-PEDOT-PPY-Au nanowires [203], MnO_2/PEDOT [204] and Ni/PPV coaxial nanowires [205] have also been prepared by the hard template method. Besides these hard templates with channels inside pores, many kinds of pre-existing nanostructures can serve as seeds or templates to synthesize CP nanostructures. For example, PAni, PPy, and PEDOT nanofibers/tubes have been prepared by using V_2O_5 nanofibers or MnO_2 nanowires as seeds [206-208].

1.6.1.2 Soft chemical template method

Soft-template method, also called as the template-free or self-assembly method, is a relatively simple, low cost but powerful approach for synthesizing various nanostructures of CPs [192]. Till now, surface micelles, surfactants, colloidal

particles, liquid crystalline phases, structure-directing molecules, and aniline oligomers have been reported to serve as soft templates, and accordingly, a number of soft-template methods have been reported, viz., interfacial polymerization [209], dilute polymerization [210], template-free method [211], rapidly mixed reactions [212], reverse emulsion polymerization [213], ultrasonic irradiation [214], and radiolytic synthesis [215]. Kaner *et al.*, first, reported the interfacial polymerization, which involves polymerization of two monomers or agents, dissolved separately in two immiscible phases so that the reaction takes place at the interface between the two liquids [209]. These methods are usually based on self-assembly approaches due to hydrogen bonding, π–π stacking, Van der Waals forces, and electrostatic interactions as driving forces. However, poor control on the morphology, orientation, and diameter of the 1D CP nanostructures are the demerits of the soft-template method. The template-free method developed by Wan *et al.* [211] is a simple self-assembly method with no use of external template. By controlling synthesis conditions, such as temperature and molar ratio of monomer to dopant, the nanostructures of PAni and PPy can be prepared by *in situ* polymerization in the presence of protonic acids as dopants. In this method, the micelles formed by dopant and/or monomer, act as soft templates in the formation of 1 D nanostructures such as tubes and wires. Up to now, a variety of PAni nanostructures such as micro/nanotubes, nanowires/fibers, hollow microspheres, nanotube junctions and dendrites have been prepared by the template-free method.

1.6.1.3 Reactive template method

In this method, a reactive template is utilized to guide the growth of CP nanostructures which also simultaneously initiates the polymerization process through oxidation [195]. Most importantly, the reactive template can be converted to soluble ion in redox reaction after polymerization. Therefore, the pure polymeric nanostructures can be obtained without further post-polymerization treatment. Besides, the shape and size of the reactive template can control the shape and size of the CP nanostructures. Li *et al.* synthesized nanotubes of PAni by using Mn_2O_3 nanowires as reactive template [216]. Methyl orange-ferric chloride (MO-$FeCl_3$) was used as a reactive template for the synthesis of PPy nanotubes [217].

1.6.1.4 Nanoimprint lithography or embossing

Soft lithography or embossing is a rapid and low-cost technique to shape an initially

flat polymer film by using a micro-mold with the assistance of temperature or solvent vapours [192]. Recently, it was reported that CP nanowires can easily be synthesized by this technique. For example, poly(4-styrenesulfonate) doped PEDOT was patterned in the form of nanowires on a glass or a Si wafer by micro-molding in capillaries [218]. Hu *et al*. [219] demonstrated a simple embossing protocol to produce arrays of CP nanowires with internal preferential alignment. Recently, Huang *et al.* proposed a technique based on nanoimprint lithography and a lift-off process for patterning CPs [220].

1.6.1.5 Electrochemical approach

The electrochemical approach is an another method to produce 1D CP nanostructures. During electrochemical polymerization, three electrodes such as working, reference and counter electrode are used. Monomer is dissolved in appropriate solvent and is polymerized at the surface of the working electrode. CP polymerization can be accomplished using different electrochemical techniques such as (i) constant current i.e., galvanostatic, (ii) constant potential i.e., potentiostatic or (iii) potential scanning/cycling and sweeping methods where the deposition is done by continuous cycling between the predetermined potential [192, 195]. The morphology of the electrochemically polymerized CP nanostructures is determined by the electrochemical conditions such as charge density, time, potential as well as surfactants, dopants, and the buffer solution used during electrochemical polymerization. When compared with chemical template free methods, electrochemical template free method has better controllability in the nanostructure morphology. The electrochemical polymerization of CP nanostructures exhibits the advantage over chemical method because in this method parameters like film thickness, film morphology, doping level etc. can be controlled by controlling current density, applied potential or current and polymerization time. However, the quantity of the final product is limited by the size of the working electrode. Large arrays of uniform and oriented nanowires of CPs were produced using a step-wise electrochemical polymerization with diameters much smaller than 100 nm on a variety of substrates such as Pt, Si, Au, carbon, silica. PPy nanofibers were produced using a novel electrochemical method in combination with interfacial polymerization as reported by Wei *et al.* [221]. Although this method produces highly conductive 1D CP based nanostructures, the examples of fabrication of stable nanofibrous scaffold

for tissue regeneration is limited [222].

1.6.1.6 Directed electrochemical nanowire assembly

This technique has been developed to grow metal nanowires as well as CP micro/nanowires [192, 223]. In this method, monomers are electrochemically polymerized for CP nanowires and assembled onto two biased electrodes (anode and cathode) immersed in aqueous monomer solutions. The basis of this method is an electrode-wire-electrode or electrode-wire-target assembly. Directional growth of PPy, PAni, and PEDOT micro/nanowires with knobby structures between electrodes by this technique was reported recently for pH sensors and in cell stimulation studies [192, 224]. However, again, this method is not suitable to incorporate all the well defined features of an ideal biomaterial scaffold.

1.6.1.7 Electrospinning

Even though the methods discussed above produced nanofibrous scaffolds, they have often encountered some limitations in controlling the fiber orientation. Furthermore, the nanofibers of CP produced by these methods are relatively mechanically weaker, smaller in length, non-uniformly distributed in terms of diameters and of less porosity. For successful tissue engineering, fiber orientation has also same importance as the fiber diameter, because alignment of the fibers predominantly influences the cellular growth. There are also difficulties in the fabrication of free standing membranes of the produced nanofibers by these methods. In this regard, electrospinning emerges as a versatile process to produce the nanofibrous scaffolds that mimic the structural features of native ECM using variety polymeric materials [225]. During electrospinning, a high-voltage electrostatic field is used to draw a jet from a polymer solution. As this jet travels toward a collector electrode, the solvent evaporates and a polymer fiber is formed [192]. The theory of electrospinning has been discussed in detail in **Chapter II** [**Section 2.2**]. Electrospinning allows the fabrication of 3D matrices with tunable fiber features, such as the degree of fiber density, size, and alignment. Thereby, electrospinning produces scaffolds with interconnected porous networks of enhanced surface area with bountiful binding sites for protein absorption and favorable interactions with receptor proteins on cell membrane for promoting cell adhesion and cell in-growth [121]. Moreover, the porous morphology of electrospun scaffolds enables the efficient exchange nutrients and wastes during anabolic and metabolic activities in cells. Thus, electrospinning can produce nanofibrous mats

mimicking topographically the native ECM collagen with 3D networks of nanofibers with diameters between 50-500 nm [121, 192].

Thus, electrospinning has gained huge attention in several biomedical applications such as drug gene/cell delivery, artificial blood vessels, wound dressings, tissue engineered scaffolds, enzyme immobilization and development of catalyst systems due to the flexibility of fabricating composites and incorporate drugs, ability to control the topographical features, viz., nanoscale size, alignment and orientation, of nanofibers, feasibility to render 3D scaffolds with the porosity required for effective tissue regeneration application, encapsulation and local sustained release of drugs (e.g. growth factors, antioxidants, anti-inflammatory agents), and ease of surface functionalization. CPs can be also electrospun alone, but this requires organic solvent PPy or in case of PAni, chemical conditions after doping and dissolution in hot sulphuric acid created nanofibers unsuitable for biological applications [121, 192]. Therefore, CPs are usually combined with spinnable polymers (e.g. polyethylene oxide, polystyrene) for electrospinning. Another way to produce conductive nanofibers is to coat the nanofibers of electrospinnable polymer with CP. For example, PPy coated fibroin fibers were demonstrated to support the adherence and proliferation of MSCs and fibroblasts [226]. PPy was also grown on PLGA fibers, and was shown to allow the growth and differentiation of PC-12 cells and hippocampal neurons [155]. Another method is to blend with a carrier material such as poly(ethylene oxide) (PEO) before the electrospinning process [227]. Using this method, PAni has been electrospun with polycaprolactone (PCL) to create a substrate for cardiac and skeletal muscle tissue engineering [228]. Nanofibers of a blend of PAni and gelatin were the first novel conductive biocompatible scaffold prepared by electrospinning, which was shown for attachment and proliferation of H9c2 rat cardiac myoblasts [229].

1.6.2 Surface functionalization of CP based biomaterials

It is universally accepted that the “bio-recognition processes”, which the specific binding of the receptors on the cell surface with their corresponding ligands, control the interactions between the cells with their surrounding environments [182]. The normal cellular activities in native tissues are mediated by the interactions between the receptor proteins on the cell surface and cell adhesion proteins such as fibronectin, vitronectin, laminin and collagen present on native ECM. The native ECM does not

only provide the physical support but also controls the cellular activities by presenting various kinds of growth factors. Following the similar mechanisms, the interactions between the cell surface and biomaterial surface regulate the anchorage dependent cellular activities such as cell adhesion, spreading, migration, and proliferation. Any materials come into contact with body fluid or cell culture medium *in vitro*, the proteins in the medium get adsorbed atop the materials' surface and subsequently, the materials interact with the cells through the adsorbed protein layer on its surface. Thus, the surface is the first point of contact between the living tissue and the biomaterial when a certain device is implanted into the body and therefore, surface chemistry of biomaterials play critical roles in determining subsequent cell behaviors. In addition, CPs are often considered as inert synthetic hydrophobic materials. In this regard, surface functionalization of CPs is the essential step to confer bioactivity. Generally, several approaches are adapted for surface engineering of CP based biomaterials, which are discussed below.

1.6.2.1 Biomolecule immobilization

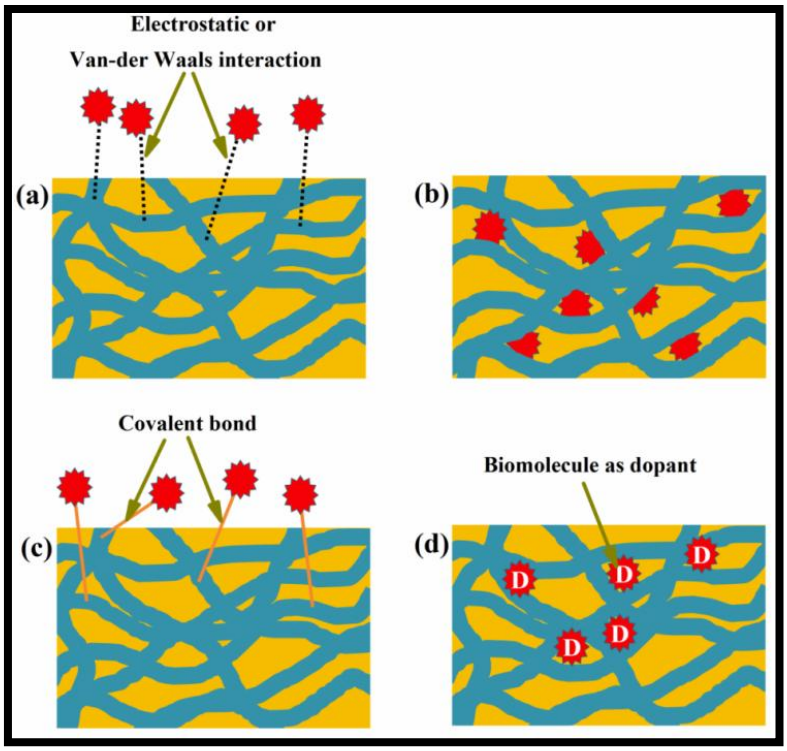

Figure 1.13. Common modification strategies of CPs for biomedical applications: (a) physical adsorption, (b) entrapping, (c) covalent modification and (d) exploiting the doping mechanism.

The first approach is the immobilization of certain biomolecules on the biomaterial surfaces, which can be achieved four major ways as discussed below [**Figure 1.13**].

1.6.2.1.1 Physical adsorption

It is a simple and direct route of post synthesis modification of CP. The biomolecule of interest is physically adsorbed on the polymer surface due to weak static forces between polymer surface and charge of the biomolecule surface [139, 196, 230]. This method is highly sensitive to external factors such as pH and the adsorbed biomolecule is prone to leaching out. The adsorption of biomolecule also affects the conductivity of the polymer. Due to such shortcomings, the method is not suitable for tissue engineering. However, several reports can be found on enzyme immobilization on CP surface for biosensor applications. For example, glucose oxidase was immobilized CPs such as PPy, PAni by physical adsorption method for fabrication of glucose biosensors [139]. The schematic representation of this method is shown in **Figure 1.13 (a)**.

1.6.2.1.2 Entrapment

In physical entrapment method, the desired biomolecule is entrapped in the growing CP during electropolymerization [139, 196].The monomer, dopant, and biomolecules are mixed simultaneously in one solution and polymerization is performed under mild conditions (i.e., neutral pH, aqueous, low oxidation potentials). Various large biomolecules such as enzyme, DNA can be entrapped within CP matrices preventing their leakage [231]. However, this method can alter the protein activity as well and therefore, restricted to biosensor applications only. It has been utilized to immobilize enzymes such as glucose oxidase to create glucose sensors [232], and to bind DNA to detect aromatic amines, cDNA and Hep C virus [139, 231] on various CP matrices. The schematic representation of this method is shown in **Figure 1.13 (b)**.

1.6.2.1.3 Covalent modification

It is a robust and stable immobilization method when compared to the above two methods. The desired biomolecule can be strongly bound to CP and therefore, improves the stability of the polymer along with higher loading of the biomolecule [139, 196]. However, the conductivity is generally reduced once again as CP chains adopt new conformations that alter the conjugation system. Although it can be

achieved both during the synthesis of the polymer or post polymerization, most of CPs have poor solubility in most of the common organic solvents, which constraints post-polymerization covalent modification. Moreover, after functionalization, the conjugation system in CP may be disturbed leading to decrease in their conductivity. The schematic representation of this method is shown in **Figure 1.13 (c)**.

1.6.2.1.4 Doping

Doping of CP using various biomolecules as long as they charged is another way to impart bioactivity. For example, growth factors, collagen, heparin, hyaluronic acid (HA), chondroitin sulphate A, chitosan and ATP have already been successfully bound to various CPs via doping for enhanced cellular activities [120,196]. Unfortunately, this method allows only a relatively small amount of the molecules to be bound, while also having a greater negative effect on the polymer's conductivity than covalent bonding [1939, 196]. The schematic representation of this method is shown in **Figure 1.13 (d)**.

1.6.2.2 Surface functionalization by polar groups

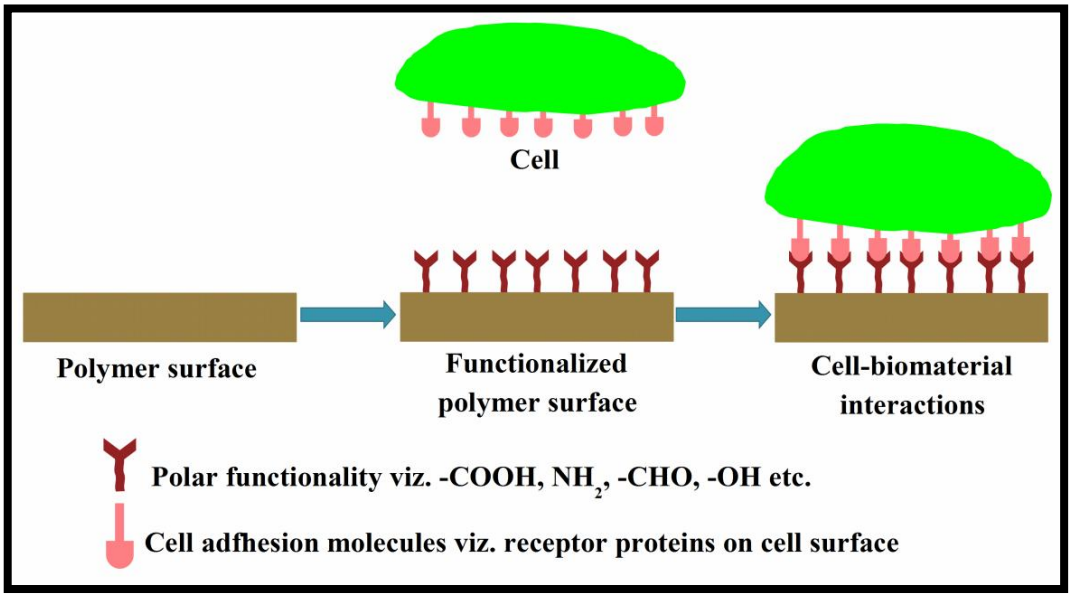

Figure 1.14. Schematic representation of surface functionalization of CP based biomaterials through incorporation of polar functionalities such carboxyl (-COOH), amine (-NH_2), aldehyde (-CHO), hydroxyl (-OH) etc. for favorable cell-biomaterial interaction.

Although, some of the above methods were shown in promoting cellular activities, all these methods are complex and use of biomolecules is itself a costly affair. Further, above discussions demonstrate ineffectiveness some of the methods such as reduction in conductivity and the stability of the polymer and most importantly, loss of activity of the biomolecule. Therefore, an easy and inexpensive way is highly desirable to overcome these demerits as shown in **Figure 1.14**.

In this approach, the material surface properties such as surface chemistry, hydrophilicity/hydrophobicity, surface energy, surface charge, and roughness etc. are improved in such a way that the adsorbed proteins can maintain their normal bioactivities and the material can interact with the cells through that adsorbed protein layer [233, 234]. The most important parameter influencing cell biomaterial interaction through adsorption of proteins is surface hydrophilicity, which in fact allows covalent attachment of proteins on the material surface [233]. Although the protein adsorption on hydrophobic surfaces is possible thermodynamically, but it may be irreversible, which causes protein denaturation leading to loss in bioactivity. On the other hand, a higher hydrophilicity may also limit protein adsorption. Therefore, surfaces with moderate hydrophilicity and hydrophobicity are preferred, which can absorb required amount of proteins retaining their natural conformation, which can respond positively towards cellular activities [233].

Since, CPs are hydrophobic materials, efforts have been devoted towards the improvement of the biomaterial's surface hydrophilicity, although, till date, this approach has not been extensively studied. This can be achieved through incorporation of polar groups like hydroxyl, carboxylic, aldehyde, amino and sulphate groups on top of a materials' surface by wet chemical surface functionalization techniques [233, 234]. The wettability of a material's surface, which is directly related to surface hydrophilicity and consequently, the surface free energy, can be modulated through the variation of the density of polar functionalities on the surface, leading to adjustment of surface biocompatibility depending on the applications.

However, this method is not novel; rather, it uses the same concept of covalent modification in introducing the functional groups which can easily interact covalently with the receptor proteins on the cell surface and conductivity, stability of the polymer affected at a lesser extent when compared to the physical adsorption, entrapment and covalent modification. Several surface functionalization techniques have been developed to improve surface hydrophilicity, wettability, the surface energy of

polymer surfaces by incorporating a range of polar groups, with little attention to specific functionality. As surface functionalization is regarded as a precursor technique for attachment of a bioactive compound, these techniques must be customized to incorporate a specific functionality. Otherwise, surface functionalization by introducing random, non-specific groups or by coating the surface are less useful in bioconjugation to polymer surfaces. Techniques for surface functionalization of polymeric biomaterials without involving biomolecules are briefly discussed below.

1.6.2.2.1 Wet chemical

This is a classical approach, where a material is treated with liquid reagents to generate reactive functional groups on the surface and can be performed in most of the laboratory condition without any need of specialized equipment. This method allows greater penetration of functionalizing agents in porous three-dimensional substrates for in situ surface functionalization [234]. For example, chromium trioxide, water, and sulphuric acid were submersed in a 29:42:29 weight ratio solution at 72°C for 1 min for introducing 3.3 nmol/cm^2 of carboxylic acid functionalities on polyethylene [235]. Concentrated sodium hydroxide and sulfuric acid have been used to generate carboxylic acid groups by base and acid hydrolysis of polymethylmethacrylate (PMMA) after treatment for 16 h at 40°C [236]. Aminolysis is an another approach to introduce amine groups on various polymers, viz., poly(methyl methacrylate) (PMMA), poly(urethane) (PU), poly(lactic acid) (PLA) and poly(lactic-co-glycolic acid) (PLGA) using range of diamines such as hydrazine hydrate, 1,6-hexanediamine, ethylenediamine, and N-aminoethyl-1,3-propanediamine, as well as lithiated diamines [234]. Various CPs based biomaterials were also surface functionalized using this method for improved function as tissue engineered scaffolds [234]. However, these wet chemical methods are non-specific and produce a range of oxygen-containing functional groups. The efficiency of this method is highly dependent on side chain surface orientation. Therefore, the degree of surface functionalization may not be uniform among polymers of different molecular weight, crystallinity, or tacticity. It may also lead to the generation of hazardous chemical waste and irregular surface etching and it often, need extended treatment in concentrated corrosive solutions. Although this wet chemical approach is suitable in the laboratory environment, it may not be suitable large scale applications.

1.6.2.2.2 Silane monolayers

It is a widely used surface functionalization technique of inorganic substrates and recently, it emerges for polymer surface functionalization as well. The immobilization of organosilanes on inorganic substrate was developed initially to couple an organic polymer to promote adhesion between glass and polymers in the development of glass-reinforced polymers [237]. Whitesides group extensively studied the unique arrangement of silanes on a surface and they named it a self-assembled monolayer (SAM) because they can organize by themselves as ordered single molecular layer on a properly functionalized surface [238]. Organosilanes with different end functionalities with poly(ethylene glycol) (PEG), bromine, and vinyl (-$CHQCH_2$), were investigated for protein and fibroblast adhesion [239]. Similarly, a range of polymers such as polyethylene terephthalate (PET), ethylene vinyl alcohol, and Nylon 6 were coupled with titanium oxide using silane coupling agents were investigated for a bone tissue scaffold [240]. SAMs, due to their crystalline organization, has the potential for better defined surface functionalization than typical wet chemical or ionized gas functionalization techniques. However, hydrolysis of the siloxane linkage at high temperatures or alkaline pH, is the main disadvantage of this method [241].

1.6.2.2.3 Ionized gas treatments

Another approach to introduce polar functionalities, viz., hydroxyl, carboxyl, amino and sulphate groups on polymer surfaces, is plasma treatment using various reaction gases like air, NH_3, SO_2, CO_2 or other organic compounds [234]. Plasma is higher energy state of matter, where a gas is partially ionized into charged particles, electrons, and neutral molecules [242]. Plasma gas treatment provides the option of modification of the top nanometer of a polymer surface without using toxic solvents and creating hazardous chemical waste with much less degradation and roughening of the material than many wet chemical treatments [243]. Hence, it is a potential technique of surface modification of CP based biomaterials. The method also offers the facility to select the desired type of functionalization, which can be chosen by appropriate plasma gas such Ar, N_2, O_2, H_2O, CO_2, and NH_3. O_2 plasma is widely used to incorporate oxygen containing functional groups onto polymer surfaces such as PCL, PE, and PET [234]. CO_2 plasma has been used to impart carboxyl groups on PP, PS, and PE. Inter gas like Ar plasma was used to introduce radical sites on PTFE, PVDF, and PCL for subsequent graft copolymerization of acrylic acid [234]. NH_3 and

N_2 plasmas have been used to impart amine functionality to the surface of PTFE and PS, respectively [234]. However, the plasma generation requires a vacuum to empty the chamber of latent gases, which causes complexities in continuously operating plasma treatment in a large scale industrial setting [234]. The plasma chamber should be cleaned properly so that there are no latent chemicals of the prior user, otherwise, there is a risk of contamination. There are various parameters such as time, temperature, power, gas composition/flow/pressure, orientation of reactor and distance of substrate from plasma source etc, are involved during plasma treatment and needs to be optimized, which makes it difficult to reproduce the results in different laboratories [243].

Corona discharge is another method of ionized gas treatment on polymeric materials, which is a simple, low cost and continuous process. In corona discharge, an electrically induced stream of ionized air is bombarded on the polymer surface to introduce oxidation products. This method is commercially used to improve printability and adhesion of inert polymers such as polyolefins [244]. However, this method produces a broad range of oxygenated groups resulting reduction in effectiveness in introducing specific functionalities for bioconjuagtion. This method, further, creates contamination on the final product and the results are varied depending upon the temperature and humidity [234, 245].

Ionized air treatment is also achieved by means of a non-specific surface functionalization method, known as flame treatment, which generates a broad spectrum of surface oxidation products to the top several monolayers [234, 244, 245]. Flame treatment has been shown to improve printability, wettability, and adhesion of PE through incorporation of hydroxyl, aldehyde, and carboxylic acid functionalities [246]. However, this method can reduce the optical clarity of the polymer and there is always a risk of burning of the polymer.

1.6.2.2.4 UV irradiation

UV irradiation on polymer surfaces can generate reactive sites and can be used for graft polymerization. This method is distinct from the ionized gas treatment in the sense that the surface reactivity can be modulated varying wavelength and consequently, the absorption coefficient [243]. Polymers such as PMMA and polystyrene (PS) were irradiated with UV radiation to impart carboxylic acid functionality for enzyme immobilization and tissue engineering applications [247,

248]. UV radiation induced grafting of PU and PCL grafted with PHEMA, poly(methacrylic acid) (PMAA), poly(acrylamide) or poly(N,N-dimethylaminoethyl methacrylate) to improve the surface hydrophilicity and endothelial cell adhesion [234].

1.7 Electrical stimulation through CP based biomaterials

Some of the tissues in our body exhibit a wide range of electrical activities to maintain cellular physiological processes and to modulate range of molecular events, engaged in the development, adaptation, repair, and regeneration of tissues [120, 165, 196]. The biological tissues, specially, cardiac, neural, bone and muscle tissues utilize the electrical conductivity mechanisms such as accumulation and flow of charges to regulate its physiological behavior and to propagate electrical potentials through their cellular components [120, 165, 196, 249-251]. With the understanding of the bioelectrical phenomenon in the human body and the experimentally demonstrated role of cell-biomaterial interactions to regulate cellular fate processes during implantation, fabrication of CP-based biomaterials is one of the major interests in current biomaterials research due to its outstanding ability to interface with bioelectric fields in cells and tissues to simulate normal electrophysiology of the body. Electrical stimulation was demonstrated as one of the promising therapeutic strategy to expedite regenerative axonal growth of regenerating axons across surgical repair sites and to improve regeneration accuracy especially of motor axons [251]. Under electrical stimulation, the release and uptake of negative/positive ions from/by the polymer, as well as electrophoretic redistribution of cell surface receptors and the increase in ECM molecules adsorption (i.e. fibronectin) onto the surface of the CP based biomaterials, are enhanced remarkably, which, in turn, improves the cellular activities [120, 251].

Electrical stimulation was used to achieve neurite outgrowth in nerve cells through modulation of cell-biomaterial interaction in various CP based biomaterials such as PPy, PAni, and PT derivatives [120, 122, 152, 155, 156, 162, 164-166, 170, 196]. It was reported that electrical stimulation of PC12 cells through NGF-doped PPy films induced 50% more neurites when compared to unstimulated cells [252]. Similarly, ES of PC12 with an electrical field of 10 mv/cm through PPy-PLGA scaffold showed more neurite formation and longer neurite outgrowth than without stimulation [155]. CP based biomaterials PPy-PDLLA [253] and PLLA-PAni were

also fabricated and showed similar results [165]. Weng *et al.* showed electrical stimulation of PC12 cells through inkjet-printed collagen coated PPy tracks, where electrical stimulation was shown to increase and direct neurite outgrowth parallel to the PPy tracks due to enhanced fibronectin adsorption [254]. Electrical stimulation of 250 Hz biphasic current delivered via PPy/PMAS composite films was observed to increase neural differentiation in the presence of NGF [255].

Improved growth of NIH-3T3 fibroblasts was also observed after electrical stimulation was delivered through a PAni-based electrospun scaffold and PPy-PDLLA scaffold [256] as an example of beneficial effect of electrical stimulation on other cells and tissues other than neural cells. Cardiomyocytes cultured on PANI-PLGA composite nanofibers have been shown to synchronize their beating under electrical stimulation [257]. However, the electrical stimulation has to be carried out carefully since long time exposure of biological tissues to currents of above 1 mA can lead to cell death [258]. Furthermore, as electrical stimulation proceeds, the resistivity of CP based biomaterials increases [166].

1.8 Motivation of the thesis

Donor dependent organ transplantation, the major current clinical treatments for damaged tissues or organs, is subject to a major organ shortage crisis worldwide. According to reports published in 2008, the incidence of renal failure 380-430 million/y, whereas the supply of organs from deceased donors (DCD) is 38-53 million/y [259, 260]. In India, 4 lakh people across the nation die each year, while waiting for an organ transplant [261]. The depressing statistics in India as published in 2016 are as: 1,00,000 people die of liver diseases, while 1,000 get a liver transplant; 2,20,000 awaits kidney transplants, while 15,000 get a transplant; while people awaiting corneal transplant 10 lakh, heart transplants 50,000, lung transplants 20,000 [262, 263]. There is a wide gap between the number of transplants awaited and the organs available. While host rejection is one of the main problems, other clinical treatments available such as autografting and allografting have also several disadvantages including loss of function at the donor site and the need for multiple surgeries. Also, when the damaged area is large, these techniques are not suitable. Tissue engineering, as a solution to address the above limitations, aims to create living, functional tissues which can be used in the replacement or repairing of tissues lost due to disease, age, congenital defects, or physical damage by combining

scaffolds, cells and bioactive molecules. Biomaterials provide exciting new opportunities for tissue repair and reconstruction by fabrication of biomimetic, biocomposite scaffolds upon which new cells can regenerate *in vivo.*

The global regenerative medicine market with a worth $18.9 billion globally at present, is growing at rapid pace and is expected to grow tremendously at the CAGR (Compound Annual Growth Rate) of 23.6% during 2016-2021 [264]. But, most of the tissue engineered products concentrated in developed regions such as Europe and North America, India is yet to witness widespread penetration of tissue repair biomaterial products.

Thus, future development of regenerative medicine is focused to address the worldwide crisis of organs and the consequent rise of the risk of organ trafficking along with the shortcomings of the current clinical treatments for damaged tissue or organ. Although there are considerable evidences of efficient working of tissue engineered products such as biomaterials with biological tissues, the key challenges are to ensure its safety to use *in vivo* and to meet the Food and Drug Administration (FDA) approved quality standards. Efforts to resolve these issues are in progress, and if successful, tissue engineering will have the major impact on future healthcare practices.

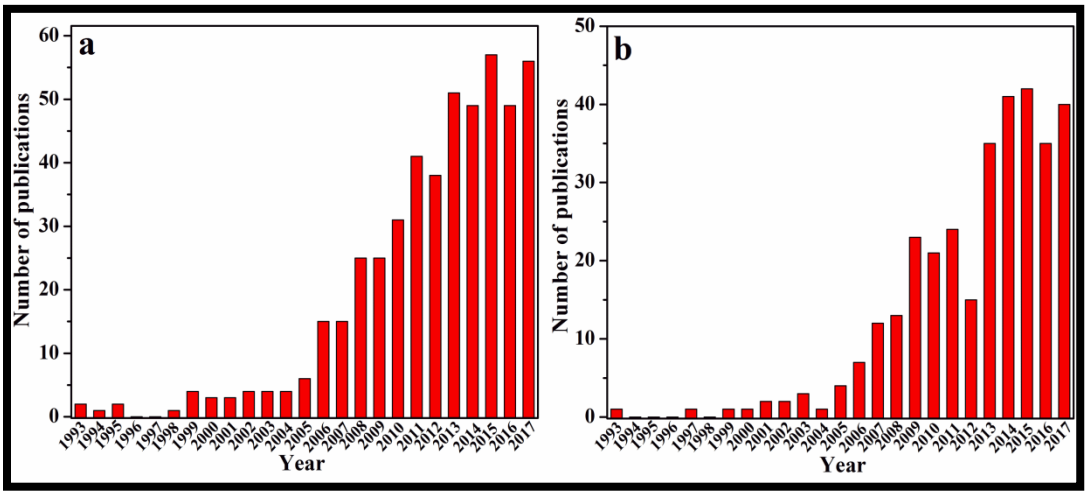

Figure 1.15.Statistics on the literature published on (a) CPs in tissue engineering applications and (b) CP based biomaterials for electrical stimulation during the period 1993-2017(search made using the Web of Science using as keywords: (a) Conducting polymers + Tissue engineering or (b) Electrical stimulation + Conducting polymers).

Additionally, it is well known that electricity plays a vital role in normal functioning of our body. In fact, without electricity our brain cannot work, muscles cannot contract, we cannot visualize things with our eyes, we cannot sense, the rhythm of our heartbeat and the blood transportation through our circulatory system could not be controlled. All the biological processes in the body are controlled by charge transport phenomena such as transport of ions across the plasma membranes and of electrons along biomolecules. Electric potentials exist inside and outside cells and continuous electrical signals through neurons are responsible for the internal body function. Therefore, due to the ability to regulate cell growth and function, there is a growing interest of utilizing the beneficial effect of electrical stimulation for wound healing, stem cell differentiation and nerve repairing purposes. In the context of cellular activities, the beneficial effects of electrical stimulation have been demonstrated in more than 50,000 articles in the form of scientific journals, patents, books etc [Source: Web of Science]. Consequently, during past 25 years, especially from 2006 onwards, research on CP in tissue engineering**[Figure 1.15 (a)]** and CP based biomaterials for electrical stimulation has been increasing significantly **[Figure 1.15 (b)]**.

Unlike natural biodegradable polymers, electroactive biomaterials are a part of a new generation of ‘‘smart’’ biomaterials that delivers the electrical, electrochemical and electromechanical stimulation to cells directly [196]. There are other electroactive biomaterials such as electrets (materials with a quasi-permanent surface charge provided by trapped charge carriers) and piezoelectric materials (materials which generate transient electrical charges under mechanical deformation) that can deliver electrical stimulation without the need for an external power source but with limited control over the stimulus [196].

However, CPs exhibit many advantages over these materials in terms of excellent control of the electrical stimulus over level and duration, good electrical and optical properties, a high conductivity/weight ratio, ability to entrap and controllably release biological molecules via reversible doping, ability to transfer charge from a biochemical reaction, and the potential to easily alter its electrical, chemical, physical, and other properties needed for desired specific application. Furthermore, CPs can be made biocompatible, biodegradable and porous and additionally, their properties can further be altered and controlled through stimulation (e.g. electricity, light, pH) even after synthesis [196]. Thus, CPs become an important and attractive class of materials in many biomedical applications, such as biosensors, tissue-engineering scaffolds,

neural probes, drug-delivery devices, and bioactuators as discussed in **Section 1.3**. Interestingly, electrical signal has been shown beneficial effects on cellular activities. Potential clinical applications of electrical stimulation range from wound healing, bone regeneration and nerve repairing to the treatment of ulcers and pressure sores on diabetic and bedridden patients. Therefore, there is a tremendous potential of development of conductive tissue engineered scaffold for modifying the regeneration, differentiation, or function of cells both *in vivo* and *in vitro* at a faster rate than conventional non conductive scaffolds. Research in this direction using conductive polymers has been started in the past decade enabling to exercise control over the level and duration of the electrical stimulation for improved nerve regeneration, bone and cartilage repairing. In combination with the properties of novel scaffold and options for electrical stimulation, CPs lend themselves as one of the most promising biomaterials in the future development of regenerative medicine to address the worldwide crisis of organs and the consequent rise of the risk of organ trafficking along with the shortcomings of the current clinical treatments for damaged tissue or organ.

1.9 Scope of the thesis and statement of the problem

The development of CPs for biomedical applications gained momentum after the discovery in the 1980s that these materials were compatible with many biological molecules such as those used in biosensors. In the mid 1990s, CPs were found to modulate cellular activities, including cell adhesion, migration, DNA synthesis, and protein secretion via electrical stimulation. Due to the unique characteristics of CPs as discussed in the previous section, CPs had been studied with different cell types ranges from nerve, bone, muscle, keratinocytes, fibro-blasts, cardiac cells, and mesenchymal stem cells, with or without electrical stimulation. Thus, the fact that variety of tissues respond to electrical or electromagnetic fields and stimuli has made CPs attractive for a number of biological and medical applications giving it an edge over other non-conductive scaffolds. It is worthy to be noted that currently, a number of FDA approved devices are available for electrical stimulation, viz., pacemakers (bladder, cardiac, diaphragmatic and gastric), electrodes for deep-brain stimulation (for the treatment of dystonia, essential tremor and Parkinson's disease), spinal cord stimulators for pain management, vagal nerve stimulators for seizure/hiccup management, devices to improve surgical outcomes for cervical fusion surgery for

patients at a high risk of non-fusion, and non-invasive devices to stimulate bone growth. Even after that, the role of CPs in absolute tissue regeneration cannot be ruled out and it continues to gain importance due to its cost-effectiveness, simple synthesis and modification process along with other unique properties as discussed in the previous sections.

However, there exist practical problems in the preparation of clinically relevant CP based tissue scaffolds with biomimetic chemical, mechanical, and topological properties. The ideal tissue engineered scaffold as a temporary support should be able to control the interplay between cells, materials, and delivery of growth factors to create environments that promote the regeneration of functional tissues and organs [196, 265]. It should, therefore, be biocompatible, biodegradable with non-toxic degradation products, bioactive, highly porous for the transportation of small molecules with a large surface area to volume ratio and able to provide optimal mechanical strength and controllability during cell growth, implantation, and sterilization and capable of being formed into desired shapes. Moreover, the scaffold degradation should also coincide with the rate of tissue formation. The tissue engineered scaffold analogous to both the nanofibrillar structure and the complex function of the native ECM along with the conductive properties is still challenging.

The main drawbacks with the CPs are toxicity, poor cell-biomaterial interactions, the absence of cell interaction sites, poor hydrophilicity, poor solubility and processability, as well as uncontrollable mechanical properties [196, 265]. Non-biodegradability of CPs is one of the greatest challenges, which may trigger an inflammatory response inside the body [196, 265]. **Table 1.1** summarizes the key properties of widely studied CPs in various biomedical applications along with their limitations.

Table 1.1. Key properties of some commonly used CPs along with MEH-PPV and their limitations including their major biomedical applications.

CPs	Key properties	Demerits	Biomedical applications
PPy	High conductivity, High stability in air, Electroactivity pH [4-11], Biocompatibility	Poor solubility, Poor processability, No biodegradability	Biosensors, Drug delivery, Tissue engineering, Neural probes, Bioactuators
PAni	High conductivity, Enviromental stability, Electroactivity pH < 4, Suitable redox properties, Biocompatibility	Poor solubility, Poor processability, No biodegradability	Biosensors, Drug delivery, Tissue engineering,Neural probes, Bioactuators
PT	High conductivity, Biocompatibility, High doping levels	Instability in air, Poor solubility, Poor processability, No biodegradability	Biosensors, Tissue engineering
PEDOT	High transparency, High stability in its oxidation state *vs* biological reducing agent, Low oxidation potential, High compatibility with aqueous Electrolytes	Poor solubility, Poor processability, No biodegradability	Biosensors, Drug delivery, Tissue engineering, Neural probes
MEH-PPV	Excellent solubility, Easy processing, High density of holes-traps	Low conductivity Complex synthesis	Biosensors, Bioimaging

The present doctoral research strives to overcome the above drawbacks of CP based biomaterials for tissue engineering applications. It also tries to gain deeper insight into the synthesis and optimization of ECM analogue nanostructured CP based biomaterials with considerable focus on the improvement of cell-biomaterial interactions. After incorporating adequate optimization, the present thesis work

further emphasizes on the potential of CPs in neuronal stimulation upon application of an external electrical field for nerve repairing applications.

In the present research work, the following measures have been taken into account to address the aforesaid drawbacks associated with CP based biomaterials:

- To overcome the poor surface hydrophilicity of CPs, which is the foremost condition for cell-biomaterial interactions, efforts have been made to impart bioactivity introducing polar functional groups like aldehyde (-CHO), carboxyl (-COOH), amine ($-NH_2$), hydroxyl (-OH) etc. by surface functionalization technique.
- The poor biodegradability, poor processability and poor surface hydrophilicity of CPs have been overcome by blending CPs with natural biodegradable biopolymers like chitosan and polycaprolactone (PCL), in addition to surface functionalization technique.
- To confer the analogous the nanofibrillar morphology of native ECM, one dimensional nanostructures of CPs have been explored. Nanofibers of CPs and CP based composites have been synthesized chemically and by electrospinning. In fact, electrospinning provides the best option to achieve mechanically strong and highly porous nanofibrous arrangement mimicking native arterial ECM. The mechanical properties of the electrospun membranes are significant to be considered for specific tissue engineering applications as it determines the integrity and stability of implanted graft at the site of injury. The shape, structure, and orientation of fibers are few factors that contribute to the mechanical properties of the scaffold. A high level of porosity is required for an efficient influx of anabolic nutrients and outflow of catabolic waste (approx 10-1000 nm).

In order to fulfill the aforementioned objectives of the doctoral research, three systems have been selected for investigation:

- Pure polyaniline (PAni) nanofibers synthesized by using dilute polymerization method because of ease in synthesis, processability, environmental stability and the unique and exciting properties that PAni offers as a CP. PAni nanofibers (PNFs) have been surface functionalized by one step wet chemical method using glutaraldehyde to confer bioactivity through the incorporation of aldehyde (-CHO) and hydroxyl functionality (-OH) for monitoring interaction with amino

acids and for enhanced cell-biomaterial interactions with a view to potential biomedical applications such as biosensing and tissue engineering.

- Polyaniline nanofibers:Chitosan (PAni:Ch) nanocomposites synthesized chemically to overcome poor biodegradability and surface functionalized by wet chemical method using glutaraldehyde to introduce polar functionalities such as aldehyde (-CHO) and hydroxyl functionality (-OH) and using glycine N-hydroxysuccininmide (NHS) ester to incorporate polar functionalities such as carboxylic (-COOH) and amine ($-NH_2$) functionality on the surface for enzyme immobilization and for growth of cell types such as MDA-MB-231, NIH 3T3 fibroblasts and neuronal model rat pheochromocytoma 12 (PC12) cells with a view to potential biomedical applications such as biosensors and tissue engineering. Electrical stimulation of neuronal model PC12 cells has been performed to check the applicability in neural tissue engineering.
- Electrospun nanofibers of poly[2-methoxy-5-(2-ethylhexyloxy)-1,4-phenylenevinylene] (MEH-PPV) blended with polycaprolactone (PCL) have been investigated for the first time to grow cell types NIH 3T3 fibroblasts and neuronal model PC12 cells on it. This system has also been surface functionalized by simple one step wet chemical method using APTES and 1,6-Hexanediamine by wet chemical method through the incorporation of amine ($-NH_2$) functionality for effective control of cell behaviour. This system has been further used for stimulation of neuronal model PC12 cells under the application of external electric field for its potential application in nerve repairing.

Effect of surface functionalization through the incorporation of different polar functionality of conductive polymer based nanocomposites on its physicochemical properties and its consequences on cell-biomaterial interactions have not been studied extensively. Moreover, only a handful of strategies have been explored for the development of PAni and PAni based composites with good biocompatibility, conductivity, and mechanical properties. In the present work, efforts have been made to achieve a better understanding in the fabrication and modification strategies of nanostructured CP based biomaterials and to explore subsequently, the potential of these materials towards biomedical applications such as biosensors and tissue engineered scaffolds.

In the present work, PAni nanofibers and PAni:Ch nanocomposites have been synthesized chemically rather by electrospinning due to poor solubility of PAni. PAni

related systems have been investigated with biomolecules such as amino acids and enzyme in order to predict the bioactivity of the surface of the materials for favorable interactions with cell surface receptor integrin proteins like fibronectin, vitronectin, collagen and laminin that facilitate cell-extracellular matrix (ECM) adhesion. Moreover, MEH-PPV has not been tested with biological tissue until now, even though it has great potential in terms of ease in scaffold designing owing to its easy processability. MEH-PPV with PCL has been electrospun in two different formulations: one in blended from and the other is in core-sheath form and subsequently, physicochemical and biological characterization has been performed with and without conjunction with surface functionalization. Out of the three systems, the best one with adequate porosity, mechanical stability and nanofibrous morphology analogues to native ECM, has been chosen for electrical stimulation of neuronal model PC12 cells.

All the systems, before and after surface functionalization, have been investigated using different sophisticated analytical tools. Electron microscopy has been used to study the morphology and structural details of the nanomaterials. X-ray diffraction studies have been carried out to investigate the degree of crystallinity, domain length and strain in the nanomaterials. The thermal stability and electrical properties of the materials have been investigated using Thermogravimetric analyzer and current-voltage (*I-V*) characteristics, respectively. Mechanical strength test has been carried out to study the mechanical properties of the materials. The optical properties of the nanomaterials (only PAni nanofibers) have been explored using UV-vis and Photoluminescence spectroscopy. The surface chemistry of the materials has been explored using Fourier Transform Infrared (FTIR) and X-ray photoelectron (XPS) spectroscopy. Wettability and surface energy measurements of PAni and its composites have been carried out by using contact angle measurements. Nuclear Magnetic Resonance (NMR) spectroscopy has been performed to study the chemical structure of PAni nanofibers before and after surface functionalization.

The degradation of the materials has been investigated using Scanning Electron Microscopy (SEM) and *I-V* characteristics after keeping them in physiological solutions such as Phosphate Buffer Saline (PBS, pH=7.4) at room temperature for 15-45 days. Urease activity test after immobilization on PAni:Ch nanocomposites has been accomplished with Nessler's method. The biocompatibility/cytotoxicity of the materials has been assessed using Hemolysis

assays and MTS proliferation assays. Live-dead assays of MDA MB 231 cells have been performed using acridine orange/ethidium bromide (AO/EB) staining, while calcein AM, ethidium homodimer-1, and DAPI staining has been done for NIH 3T3 fibroblasts. PC12 cell differentiation on electrospun MEH-PPV:PCL nanofibers has been studied by beta (III) tubulin immunochemistry. Electrical stimulation of PC12 cells has been carried out to study the effect of electrical signals on neuronal growth as compared to unstimulated cells. Cell adhesion tests have been accomplished using SEM.