

Feature Article

Use of electrospinning technique for biomedical applications

Seema Agarwal*, Joachim H. Wendorff, Andreas Greiner*

Philipps-Universität Marburg, Department of Chemistry and Scientific Center for Materials Science, Hans Meerwein Strasse, 35032 Marburg, Germany

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ABSTRACT

The electrospinning technique provides non-wovens to the order of few nanometers with large surface areas, ease of functionalisation for various purposes and superior mechanical properties. Also, the possibility of large scale productions combined with the simplicity of the process makes this technique very attractive for many different applications. Biomedical field is one of the important application areas among others utilising the technique of electrospinning like filtration and protective material, electrical and optical applications, sensors, nanofiber reinforced composites etc. Electrospinning assembly can be modified in different ways for combining materials properties with different morphological structures for these applications. The importance of electrospinning, in general, for biomedical applications like tissue engineering drug release, wound dressing, enzyme immobilization etc. is highlighted in this feature article. The focus is also on the types of materials that have been electrospun and the modifications that have been carried out in conventional electrospinning apparatus keeping in view the specific needs for various biomedical applications.

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1. Introduction

The combined use of two techniques namely electrospray and spinning is made use in a highly versatile technique called electrospinning (electro + spinning). A high electric field is applied to the droplet of a fluid which may be a melt or solution coming out from the tip of a die, which acts as one of the electrodes. This leads to the droplet deformation and finally to the ejection of a charged jet from the tip of the cone accelerating towards the counter electrode leading to the formation of continuous fibers. There is an ever increasing interest in this field of electrospinning shown by both researchers in the academic institutions and industries as evident from the increased number of publications and review articles every year. Some of the review articles mentioning the fundamentals of electrospinning, its historical development, modified electrospinning techniques and their application areas are recently published by us and others [1,2]. In fact, Polymer International volume 56 (11), 2007 has a sequence of IN FOCUS mini review articles about different aspects of electrospinning. The important advantages of electrospinning technique are the production of very thin fibers to the order of few nanometers with large surface areas, ease of functionalisation for various purposes, superior mechanical properties and ease of process as suggested by many experts in this field. The possibility of large scale productions combined with the simplicity of the process makes this technique

very attractive for many different applications. Biomedical field is one of the important application areas among others utilising the technique of electrospinning like filtration and protective material, electrical and optical applications, sensors, nanofiber reinforced composites etc. There are some specific review articles on this subject also [3]. Laurencin et al. have reviewed recently the patents on electrospun biomedical nanostructures [4]. The current available techniques for nanofiber synthesis and the use of nanofibers in tissue engineering and drug-delivery applications are reviewed by Vasita et al. [5]. A brief overview about electrospinning approaches towards scaffold engineering is given by us [6].

Here we would like to highlight the importance of electrospinning, in general, for biomedical applications like tissue engineering, drug release, wound dressing, enzyme immobilization etc. We make no attempt to give a complete collection of related literature but provide a progress in the field of electrospinning for biomedical applications including electrospinning of man-made synthetic, natural and blend of synthetic and natural polymers, chemically modified and designed polymers and modified electrospinning techniques suitable for biomedical applications.

2. Tissue engineering applications

Tissue engineering, also called regenerative medicine is an interdisciplinary field involving knowledge from medicine, biology, engineering and materials science fields. Tissue engineering makes use of scaffolds to provide support for cells to regenerate new extra cellular matrix which has been destroyed by disease, injury or congenital defects without stimulating any immune response.

* Corresponding authors.

E-mail addresses: seema@chemie.uni-marburg.de (S. Agarwal), greiner@staff.uni-marburg.de (A. Greiner).

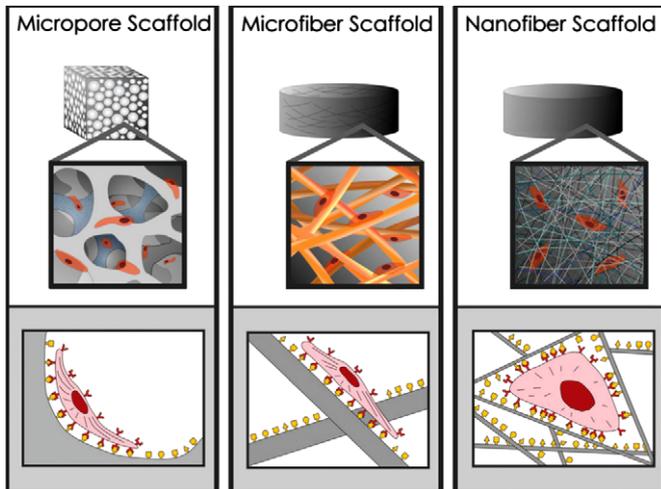


Fig. 1. Scaffold architecture affects cell binding and spreading [7].

Natural extra cellular matrix (ECM) separates different tissues, forms a supportive meshwork around cells, and provides anchorage to the cells. It is made up of proteins and glycosaminoglycans (GAGs) which are carbohydrate polymers.

Electrospinning generates loosely connected 3D porous mats with high porosity and high surface area which can mimic extra cellular matrix structure and therefore makes itself an excellent candidate for use in tissue engineering. The requirements for a material to be used for tissue engineering purposes are biocompatibility, and biodegradability, as the scaffold should degrade with time and be replaced with newly regenerated tissues. Also, the scaffold architecture is very important and affects cell binding (Fig. 1) [7]. The cells binding to scaffolds with microscale architectures flatten and spread as if cultured on flat surfaces. The scaffolds

with nanoscale architectures have bigger surface area for absorbing proteins and present more binding sites to cell membrane receptors. The adsorbed proteins further can change the conformations, exposing additional binding sites, expected to provide an edge over microscale architectures for tissue generation applications.

The use of electrospinning in the field of tissue engineering till date is mainly concentrated towards the following:

1. Formation of non-woven mats of different biomaterials to biomimic physical dimensions of native ECMs, i.e. geometry and morphology with nanodimensions. Proper choice of biomaterials is required in terms of mechanical properties and degradation time which depends upon the type of scaffold required, type of the tissues to be regenerated and their regeneration time. This includes electrospinning of known and commercially available synthetic and natural biomaterials like polylactide (PLA), polycaprolactone (PCL), poly(glycolic acid) (PGA), and their copolymers etc. and especially synthesised novel biomaterials that are designed to direct the organization, growth, and differentiation of cells in the process of forming functional tissues.
2. Modification of the electrospinning process for the mimicking of extra cellular matrix providing enhanced proliferation and differentiation of cells.

3. Formation of non-woven mats for biomedical applications

Various synthetic biopolymers have been electrospun to satisfy different clinical requirements. Chemical structures of some of the representative synthetic polymers electrospun are shown in Fig. 2.

Poly(α -hydroxy acids), especially lactic acids, glycolic acids and their copolymers with ϵ -caprolactone, are the most commonly known and used among all biodegradable polymers for fabrication

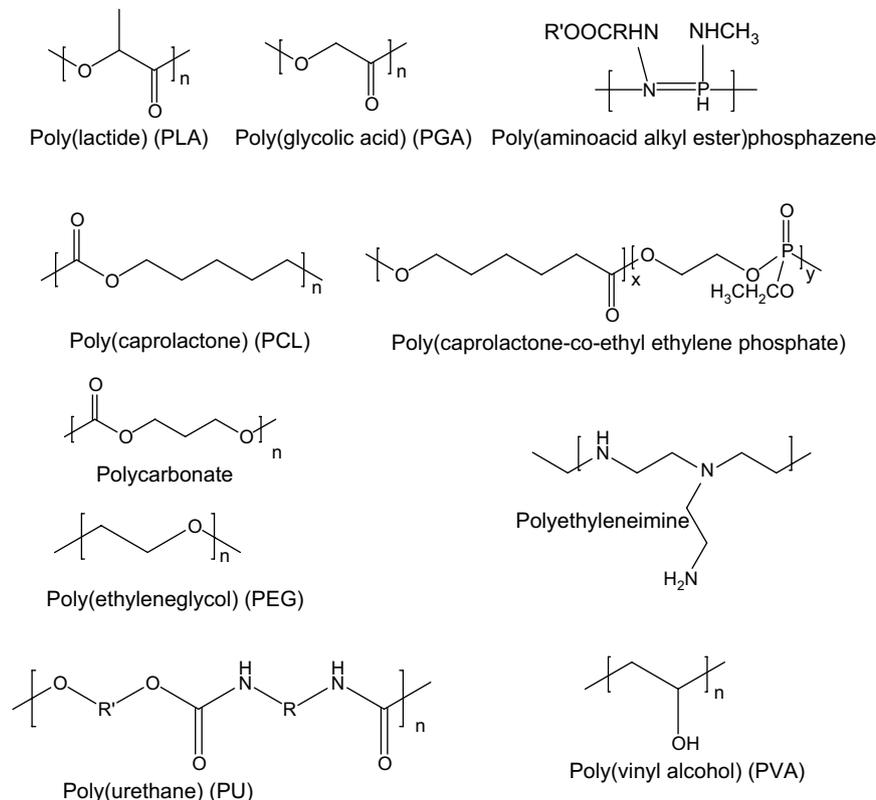


Fig. 2. Chemical structures of some of the synthetic polymers electrospun for biomedical applications.

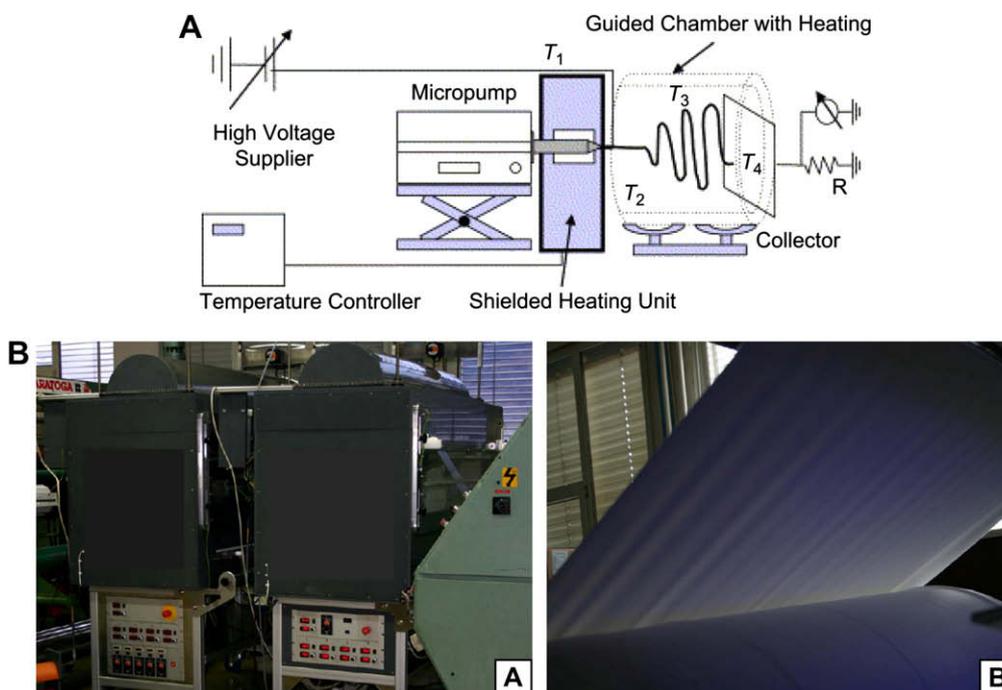


Fig. 3. (A) Melt electrospinning setup [9]. (B) Technical electrospinning setup with large scale width (A) and technical textile sheets coated by continuous electrospinning. Photos TransMIT Marburg (www.transmit.de).

of novel materials for medical use and for tissue engineering applications. Piskin et al. [8] have reviewed the electrospinning process of these polymers and give selected recent applications of electrospun matrices made from these polymers. They degrade *in vivo* into nontoxic end products mainly by hydrolysis in a few weeks to several months, depending on molecular structure/morphology, average molecular weight etc. Also, melt electrospinning of different polymers like poly(lactide) [9] (Fig. 3A), poly(ethyleneglycol-caprolactone) [10] is known to generate sub-micron scale fibers. This solvent-free approach to produce sub-micron scale fibers is more advantageous in terms of environmentally benign process than common solution electrospinning processes, and has a potential to increase the production rate significantly. Technical scale electrospinning of biodegradable polymers is also available now-a-days using specially designed electrospinning setup (Fig. 3B).

Ashammakhi et al. [11] and Teo et al. [12] have briefly overviewed the formation of biodegradable nanomats by electrospinning and their potential use for tissue engineering applications. Chew et al. [13] have reviewed the analysis and control of electrospinning process, and describes the types of fibers fabricated for biomedical applications such as drug delivery, tissue engineering. Most recently a review article providing insight to the generation of smart scaffolds by different technologies including electrospinning is given by Moroni et al. [14A]. An overview about functional electrospun nanofibrous scaffolds for biomedical applications is provided by Chu et al. [14B]. It is evident from this vast literature that electrospinning of any biodegradable and biocompatible polymer is no more a problem. By adjusting electrospinning conditions like voltage, distance between the electrodes, flow rate of solution during electrospinning and polymer solution properties like viscosity, conductivity etc. any polymer can be electrospun.

Further to mimic ECM and to propose electrospun biodegradable mats for any tissue engineering application, various research groups have studied growth of different kinds of cells (Table 1). In a study by Ramakrishna et al., human vascular endothelial cells cultured on smooth solvent-cast PLA surface were shown to be

more than on the rough electrospun surface [15]. On contrary, Boudriot et al. [16] have shown the preference of human osteosarcoma MG-63 cells growth on electrospun poly(L-lactide) nanostructured scaffold (average fiber diameter between 200 nm and 5 μm ; electrospun from dichloromethane with microporous surface structure) along the nanofibers covering them, and showed no sign of degeneration or apoptosis. Bini et al. [17] have shown the growth of C17.2 nerve stem cells on poly(lactide-co-glycolide) (PLGA) nanomats, microbraided and aligned microfiber scaffolds. They also showed attachment and differentiation of these cells along the direction of the fibers.

Table 1

Cell studies on electrospun fibers made of man-made materials

Electrospun polymer fibers	Cells or scaffolds	Reference
Poly(lactide) PLA	Endothelial	[15]
	Human osteosarcoma MG-63 cells	[16]
Poly(lactide-co-glycolide) PLA-PGA	C17.2 nerve stem cells	[17]
Polyurethane (PU)	Smooth muscle cells	[18]
Modified polyethyleneimine (PEI)	Normal human fibroblast (NHF)	[19]
Poly(propylene carbonate), poly(cyclohexyl carbonate)	L929 fibroblasts, primary rat hepatocytes	[20]
Poly(propylene carbonate)	Bone marrow mesenchymal stem cells (MSCs)	[21]
Silicate	Human osteoblastic MG63 cells	[24]
Polyphosphazenes	Endothelial cells	[25]
Plasma modified and acrylic acid grafted PGA, PLA and PLGA	Fibroblasts	[27]
Polyester urethane	Fibroblasts	[28]
Poly(L-lactide)-hydroxyapatite composite (PLLA/HA)	Osteoblast cell MG-63	[29]
Poly(L-lactide)-multiwalled carbon nanotubes-hydroxyapatite (polyPLLA/MWNTs/HA)	Periodontal ligament cells	[30]
Poly(lactide-co-glycolide) (PLGA)/amorphous tricalcium phosphate	Human mesenchymal stem cells	[31]

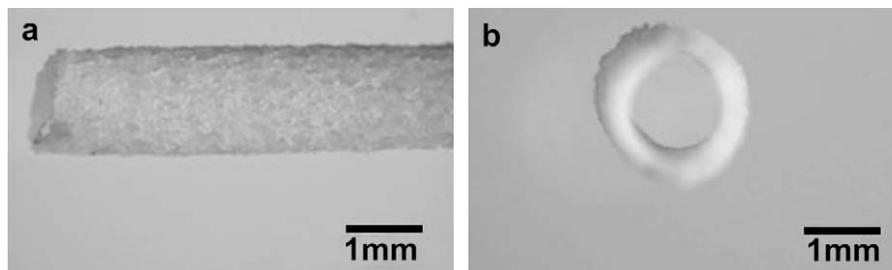


Fig. 4. Electrospun PPC grafts under stereoscopic microscope: (a) Lateral view and (b) transverse view [21].

PU is a biodegradable material which degrades by hydrolysis and enzyme based chain cleavage and has been used by Rockwood et al. [18] for use as a temporary scaffold in tissue engineering. The phase segregated morphology of the polyurethane imparts elastomeric properties that are attractive for soft tissue engineering. Non-woven mats of polyethyleneimine (PEI) cross-linked with succinic anhydride and 1,4-butanediol diglycidyl ether were evaluated as interaction scaffolds for normal human fibroblast (NHf) cells [19]. Non-woven mats of PEI in the range 1600–687 nm were evaluated as interaction scaffolds for normal human fibroblast cells. Cell viability was evaluated by staining with propidium iodide for dead cells and fluorescein diacetate for live cells. Fluorescence studies confirmed that NHf cells were attached and spread throughout the cross-linked linear polyethyleneimine scaffold. The attachment and spreading of cells suggests that electrospun linear polyethyleneimine scaffolds support growth of normal human fibroblasts cells.

Aliphatic polycarbonates like poly(propylene carbonate) (PPC) and poly(cyclohexyl carbonate) were also electrospun with an intend to be utilized for tissue engineering [20]. They were used for cell culture of L929 fibroblasts and primary rat hepatocytes. The cells showed good adhesion to the scaffolds and high viability. Tissue engineering of vascular grafts with genetically modified bone marrow mesenchymal stem cells (MSCs) on poly(propylene carbonate) graft is studied by Zhang et al. [21]. The MSCs were seeded onto the electrospun fibrous grafts and cultured. The authors showed the integration of seeded cells with the microfiber of the scaffold to form a three-dimensional cellular network, indicating a favourable interaction between the synthetic PPC scaffolds with MSCs. The use of MSCs and therapeutic genes in tissue engineering of blood vessels could be helpful in improving regeneration. A tubular scaffold (2 mm in diameter) with a microstructure of non-woven fibers was produced by electrospinning of poly(propylene carbonate) (Fig. 4). A recent feature article by Bowlin et al. [22] highlights the importance of electrospinning for vascular grafts. According to the authors the laboratory research performed on electrospun bioresorbable vascular prosthetics has proven the potential to be transferred to clinical use in the future. Electrospinning offers the ability to use a wide range of polymers, thereby allowing control over graft's mechanical and bioactive properties that can closely mimic the behaviour of a native artery.

Electrospun mats of tyrosine-derived polycarbonate fibers are provided by Meechaisue et al. for potential use as tissue scaffolding material [23]. The modified polycarbonates were produced from

Desaminotyrosyl-tyrosine ethyl ester and desaminotyrosyl-tyrosine monomers to tune the degradability rates. Silicate fibers prepared via electrospinning and sol-gel process were evaluated as scaffolds for bone tissue engineering by Sakai et al. [24]. They found that human osteoblastic MG63 cells successfully adhered on individual silicate fibers, and proliferated on them. Carampin et al. showed [25] the use of biocompatible polyphosphazenes (i.e. poly(aminoacid alkyl ester) phosphazenes) to construct human tissues such as vessels or cardiac valves. The flat or tubular matrices from poly(ethyl phenylalanato)_{1.4}(ethyl glycinato)_{0.6}phosphazene by electrospinning was evaluated for the growth of rat neuromicrovascular endothelial cells cultured on sheets and tubes with an average fiber diameter of 850 ± 150 nm. Microscopic examination of the seeded tubes showed the formation of monolayer of endothelial cells after 16 days incubation.

4. Electrospinning of modified degradable polymers

Further, modified biodegradable polymers were electrospun in order to either improve cell proliferation or growth on the scaffolds or for modifying the biodegradability rate or mechanical properties of the scaffolds. To improve the hydrophilicity, liability, and degradability of biodegradable polymer like PLA, a triblock copolymer of PEG and lactide (PLA-PEG-PLA) is made and electrospun into fibrous membranes in the fiber sizes 7.5 μm –250 nm [26]. Surface modification of biodegradable electrospun nanofiber scaffolds and their interaction with fibroblasts is studied by Park et al. [27]. The surfaces of electrospun PGA, PLA and PLGA nanofibers were chemically modified using oxygen plasma treatment and in situ grafting of hydrophilic acrylic acid (AA). The fiber thickness, pore size and porosity were estimated to be 200–800 nm, 2–30 micron and 94–96 %, respectively. Fibroblasts once seeded on the scaffolds were spreading over large surface area on the AA-grafted surface as compared to the unmodified PGA, PLA and PGLA nanofibrous scaffolds. Another modification of polyesters, i.e. polyester urethane (Fig. 5) with slowed degradation was electrospun and tested for fibroblasts viability and suggested as potential biodegradable and biocompatible material for tissue engineering by Jerome et al. [28].

Naturally, bones are generally strengthened by the nucleation of hydroxyapatite (HA) into nano sized gaps between collagen molecules. In order to mimic and to have strengthened scaffolds for bone tissue engineering, PLA/hydroxyapatite (HA) hybrid membranes were fabricated by electrospinning of PLA/HA dispersion [29A,29B].

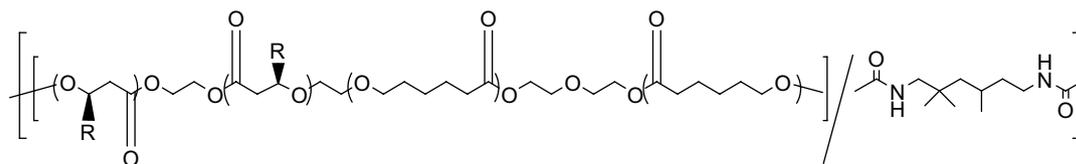


Fig. 5. Chemical structures of the polyesterurethane.

Table 2
Mechanical properties of PLLA and PLLA/HA hybrid membrane

Sample	Thickness (mm)	Modulus (MPa)	Ultimate strength (MPa)	Ultimate strain (%)
PLLA	0.040 ± 0.005	12 ± 2	1.40 ± 0.11	90.0 ± 6
PLLA/HA	0.0406 ± 0.005	118 ± 10	2.86 ± 0.24	5.6 ± 0.5

The osteoblast cell (MG-63) was cultured in PLLA/HA hybrid membrane. The cell adhesion and growth capability were investigated by SEM observation and MTT assay. HA nanoparticles were not only dispersed in PLLA but also reacted with the functional group of PLLA, resulting in strong surface bonding and high tensile strength of hybrid membrane (Table 2). The cell adhesion and growth on the PLLA/HA hybrid membrane were far better than those on the pure PLLA membrane, which proves that the PLLA/HA hybrid membrane can be one of the promising biomaterials for bone tissue regeneration.

However, in order to produce electrospun fibers with homogeneous structure, it is essential for the ceramic powder to be fine and to remain stable in suspension. Kim et al. [29C] have provided a modified method of making nanocomposite of PLA/HA by electrospinning. They have introduced a surfactant hydroxyl stearic acid between hydrophilic HA powder and hydrophobic PLA in a chloroform solution.

A new type of guided tissue regeneration (GTR) membrane is developed by Mei et al. [30] by electrospinning a suspension consisting of poly(L-lactic acid), multiwalled carbon nanotubes, and hydroxyapatite (PLLA/MWNTs/HA). Authors have shown the enhanced adhesion and proliferation of periodontal ligament cells (PDCLs) by 30%. After PDCL cells were seeded onto the electrospun membranes, cell/membrane composites were implanted into the leg muscle pouches of immune deficient mice and functioned well in vivo. Authors are of the opinion that compared to other GTR membranes on sale or research, this system can simplify the manufacturing process, reduce the fabrication cost and avoid possible mistakes in clinical applications. Schneider et al. [31] have evaluated the in vitro biomedical performance of an electrospun poly(lactide-co-glycolide) (PLGA)/amorphous tricalcium phosphate (ATCP) nanocomposites. Experiments on in vitro biomineralization, applicability in model defects and a cell culture study with human mesenchymal stem cells (hMSC) allowed assessing the application of the material for potential use as a bone graft. A novel kind of poly(butylene succinate) (PBSU)/wollastonite/apatite composite scaffold was fabricated by electrospinning by Zhang et al. [32] for bone tissue engineering. Wollastonite (β -CaSiO₃) nanowires were first prepared by a hydrothermal route and then surface modified using dodecyl alcohol to enhance the dispersion of Wollastonite and promote the interfacial adhesion with PBSU. SEM and XRD data showed the deposition of apatite on the surface of composite fibers and the microstructure of the scaffolds could be controlled by the amount of Wollastonite content and the incubation time.

Layer-by-layer electrospinning (LBL) was shown first by Kidoaki et al. [33]. In LBL electrospinning, each polymer solution is electrospun to form its individual layer and is collected on the same target collector. A new type of guided bone regeneration (GBR) membrane was fabricated by polycaprolactone (PCL)/CaCO₃ composite nanofibers using layer-by-layer electrospinning method [34]. In order to achieve mechanical stability of GBR membranes, composite nanofibers were spun on PCL nanofibrous membranes which has high tensile strength, i.e. the membranes consist of two layers of functional layer (PCL/CaCO₃) and mechanical support layer (PCL). Also, Layman et al. [35] have utilized LBL electrospinning for the development of the biohemostat, a treatment for high pressure bleeding based on super-absorbent polymers and electrospun membranes.

5. Electrospinning of natural polymers

Electrospinning method has been utilized to generate non-woven mats for tissue engineering using different natural biopolymers including proteins (gelatine, collagen and silk fibrinogen) and polysaccharides (chitosan, hyaluronic acid and cellulose) (Table 3). These materials have their own advantages and disadvantages for biomedical applications. On one side unlike synthetic biomaterials, these natural polymers provide many of the instructive cues required by the cells attachment and proliferation and on the other side have the problem of batch-to-batch property variation. Cellulose acetate (CA) (Fig. 6) is a biomaterial of choice for building the urinary bladder matrix (UBM) mimicking scaffolds and its electrospinning for this purpose is shown by Han et al. [36].

Ohkawa et al. showed the electrospinning of natural polymer chitosan [37] (N-deacetylated derivative of chitin), a natural cationic polysaccharide having hydroxyl and amino groups regularly arranged at the equatorial positions in the β (1,4)-linked D-glucosamine repeating units. It is environmentally friendly, nontoxic, biodegradable and antibacterial. Water-insoluble chitosan nanofiber sheets and tubes are prepared by Matsuda et al. and 28% ammonium aqueous solution is used for bringing water insolubility [38]. Schiffman et al. [39] have provided Schiff base cross-linked chitosan mats using a one-step electrospinning process that is claimed to be 25 times faster and, therefore, more economical than a previously reported two-step vapour-cross-linking method. Combination of synthetic and natural polymers could combine the advantage of easy properties modification of synthetic polymers with biofunctionalities of natural polymers.

Table 3

Cell studies on electrospun fibers made of bio-based polymers and mixtures with man-made materials

Electrospun fibers	Proposed biomedical use	Reference
Cellulose acetate (CA)	Urinary bladder matrix	[36]
Chitosan and derivatives	Antibacterial material	[38–40]
Silk fibroin (SF)	Tissue engineering (Growth of keratinocytes and fibroblasts)	[3]
<i>B. mori</i> silk fibroin	Vascular grafts (Tubular scaffolds) (growth of human endothelial cells and smooth muscle cells)	[41]
Fibrinogen	Tissue engineering (Growth of neonatal rat cardiac fibroblasts)	[42]
Alginate	Tissue engineering	[43]
Collagen	Tissue Engineering Aortic smooth muscle cells	[44–48]
Blend of polyaniline/gelatin	Vascular grafts (Growth of H9C2 rat cardiac myoblast cells)	[51]
Blends of collagen, elastin with biodegradable polymers	Soft tissue engineering (Heart, lung and blood vessels) (Growth of H9c2 cardiac myoblasts and bone marrow stromal cells (BMSCs))	[52,53]
Composite of gelatine/hydroxyapatite (HA)	Osteoblast MG63	[54]
Blends of collagen/poly(caprolactone) PCL	Artificial implant	[55,117]
	Schwann cell growth, dermal fibroblast cells	
Blends of chitosan/polycaprolactone (PCL)/poly(vinyl alcohol) PVA	Tissue engineering	[56]
Blends of PLGA/chitosan/PVA	Mesenchymal stem cells	
Blend of collagen/poly(3-hydroxybutyrate-co-3-hydroxyvalerate) PHBV	Tissue engineering	[57]
	Scaffolds	[58]
Plasma treated and grafted polycaprolactone (PCL) by collagen	NH3T3 cells	
Collagen/PCL/tricalcium phosphate	Tissue engineering	[59]
	Orecurson based osteochondral repair	[60]

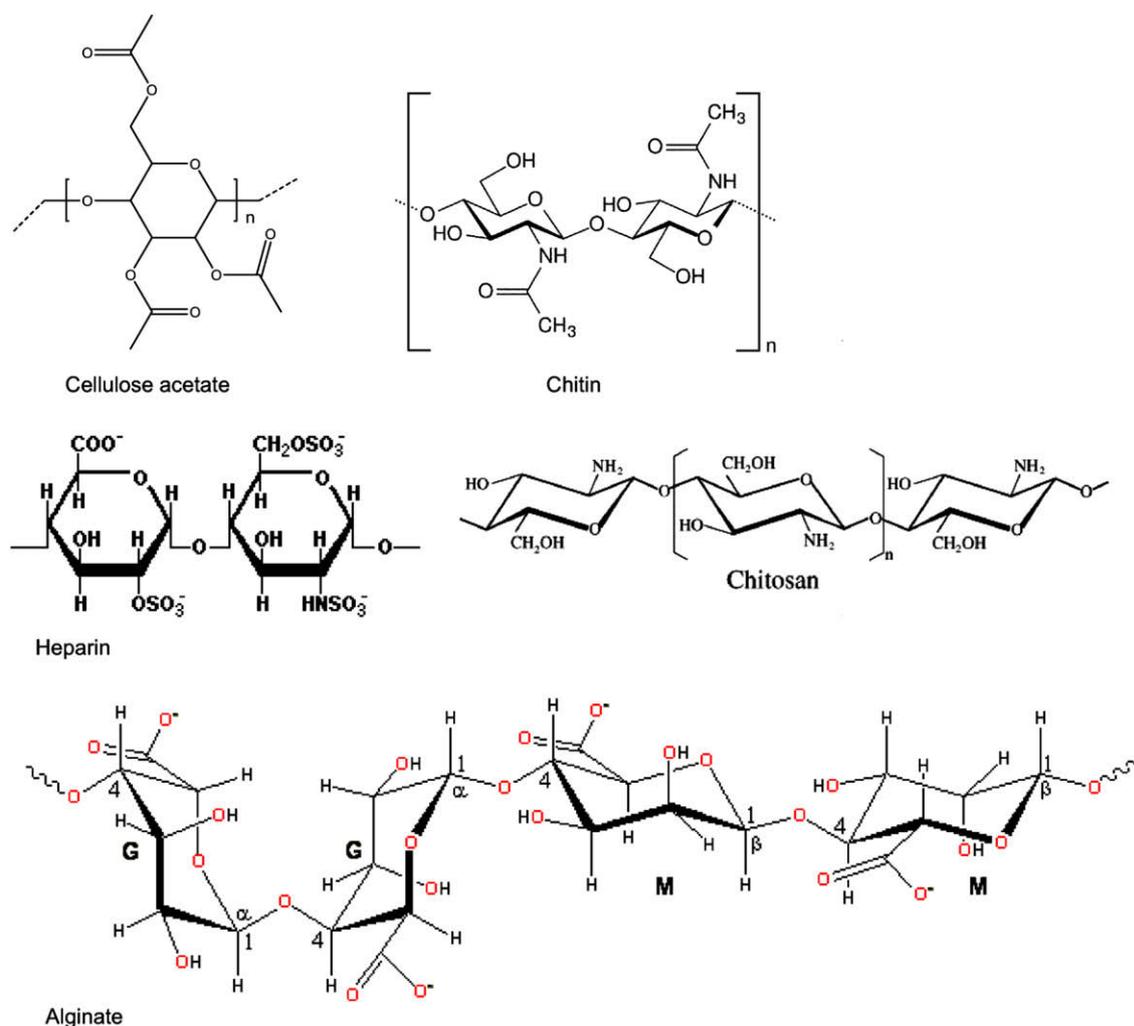


Fig. 6. Chemical structures of some natural polymers.

Chitosan–poly(L-lactic acid-co-ε-caprolactone) (50:50) CS–P(LLA–CL) blends were electrospun into nanofibers using 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) and trifluoroacetic acid (TFA) as solvents by Chen et al. [40]. Chitosan, which is otherwise difficult to electrospin into nanofibers, could be easily electrospun into nanofibers with addition of a small portion of P(LLA–CL). The fiber diameter depended on both the polymer concentration and the blend ratio of chitosan to P(LLA–CL). The average fiber diameter increased with increasing polymer concentration and decreasing the blend ratio of chitosan to P(LLA–CL). The porosity of CS/P(LLA–CL) nanofiber mats increased with increasing the weight ratio of chitosan to P(LLA–CL), while both the tensile strength and the ultimate strain increased with increasing P(LLA–CL) ratio. Fibroblast cell growth on nanofiber mats was investigated with MTT assay and scanning electron microscope (SEM) observation. The highest cell proliferation was observed on the nanofiber mats when the weight ratio of chitosan to P(LLA–CL) was 1:2.

Min et al. [3] have electrospun regenerated silk fibroin (SF) in formic acid. Fibroin consists of approximately 42% glycine and 25% alanine as the major amino acids. The remaining components are mostly glutamine, serine, leucine, valine, proline, tyrosine and arginine. To assay the cytocompatibility and cell behaviour onto the electrospun SF nanofibers, cell attachment and spreading of normal human keratinocytes and fibroblasts were seeded and the results indicated SF to be a good candidate for tissue engineering scaffolds. Electrospinning was also used to fabricate non-woven

nanofibrous tubular scaffolds from *Bombyx mori* silk fibroin using an all aqueous process [41]. Cell studies and mechanical characterization tests were performed on the electrospun silk tubes to assess the viability of their usage in bioengineering small-diameter vascular grafts. Human endothelial cells and smooth muscle cells were successfully cultured on the electrospun silk. Mechanical characterization tests demonstrated burst strength sufficient to withstand arterial pressures and tensile properties comparable to native vessels. These cellular and mechanic outcomes demonstrate potential utility of these electrospun silk scaffolds for small-diameter vascular grafts.

McManus et al. [42] have demonstrated based on cellular interaction (neonatal rat cardiac fibroblasts) and scaffold remodeling that electrospun fibrinogen can be used successfully as a tissue engineering scaffold. Zhang et al. [43] have fabricated alginate based nanofibers and also investigated many issues related to the spinnability and reproducibility. Alginate is a biodegradable polymer derived from sea weed. It is a linear polysaccharide copolymer consisting of two sterically different repeating units, (1,4)-α-L-guluronate (G unit) and (1,4)-β-D-mannuronate (M unit) in different proportions (Fig. 6). In the initial stage, the gelation of alginate solution at very low polymer concentrations (e.g. around 2 wt% for alginate in water) made electrospinning difficult. Their study showed that polymer solution viscosity is the key factor that regulates the spinnability and structure of electrospun product. Solution viscosity can be changed in a number of ways including

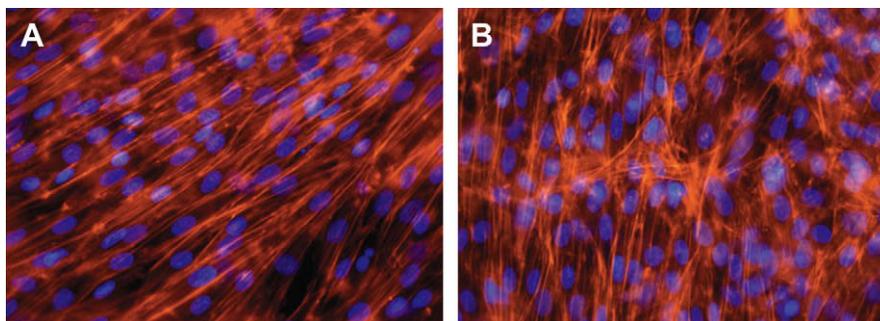


Fig. 7. Fluorescence graphs of confluent H9C2 myoblast cells on (A) PLGA (B) PLGA-gelatin-elastin (PGE) [53].

addition of a second hydrophilic polymer and/or surfactants or alternation of polymer molecular weight. The sustained structural integrity of nanofibers in aqueous environment as well as simulated blood fluid, which is essential for tissue engineering applications, was improved by cross-linking using CaCl_2 , epichlorohydrin, gluteraldehyde, hexamethylenediisocyanate and adipic acid hydrazide.

Many authors have reported the electrospinning of one of the most commonly used proteins, i.e. collagen, a natural component of ECM in solvents like 1,1,1,3,3,3-hexafluoro-2-propanol and showed cell growth and proliferation both in vivo and in vitro [44–46]. Very recently Zeugolis et al. [47] showed for the first time that the very properties of collagen that established it as natural biomaterial are lost when it is electrospun from 1,1,1,3,3,3-hexafluoro-2-propanol or 2,2,2-trifluoroethanol. These solvents led to denaturation of collagen and the resulting electrospun scaffolds lack the unique ultra-structural axial periodicity that confirms quarter-staggered supramolecular assemblies and the capacity to generate second harmonic signals. Yang et al. [48] have also reported the denaturation of collagen Type I fibers electrospun from 1,1,3,3,3-hexafluoro-2-propanol.

Since natural polymers like chitosan, gelatine etc. have limited solubility in water, toxic and highly acidic solvents like 1,1,1,3,3,3-hexafluoro-2-propanol and trifluoroacetic acid are used for their electrospinning. Song et al. [49] electrospun gelatine from a newly developed water-based co-solvent composed of ethyl acetate and acetic acid in water. The optimal composition of the co-solvent was found to correspond to a ratio of ethyl acetate to acetic acid of 2:3. Under this solvent condition, the gelatine could be dissolved at concentrations of upto ~ 11 wt% and produced fibers in the diameter range 47–145 nm.

Comparative electrospinning behaviour of different proteins like collagen, gelatine (denatured collagen), solubilised α -elastin and recombinant human tropoelastin is given by Li et al. [50]. Also, a blend of polyaniline, a conductive polymer with a natural protein, gelatine, was electrospun by Li et al. [51] to investigate the potential of such a blend as conductive scaffold for tissue engineering purposes. The blend fibers supported the growth of H9C2 rat cardiac myoblast cells. Due to diversity in size, mechanical and biochemical properties, cellular content and ultra-structural organization of blood vessels, it is required to control the fabrication of vascular grafts for obtaining desirable characteristics of blood vessel substitutes. Lee et al. [52] have fabricated various scaffolds with blends of collagen, elastin and several biodegradable polymers using electrospinning. Materials were blended at a relative concentration by weight of 45% collagen, 15% elastin, and 40% synthetic polymer to mimic the ratio of collagen and elastin in native blood vessels. The introduction of synthetic biodegradable polymers enabled tailoring of mechanical properties of vascular substitutes and improving compliance matching for vascular tissue engineering. Also, Li et al. [53] have studied composite scaffolds composed of natural materials (gelatin and elastin) and synthetic polymer (PLGA). The resulting PLGA-gelatin-elastin (PGE) fibers were homogenous with an average diameter of 380 ± 80 nm. Cultured H9C2 rat cardiac myoblasts grew slightly better on the scaffolds (Fig. 7) and are proposed for engineering soft tissues, such as heart, lung and blood vessels.

Nanofibers of gelatine-hydroxyapatite mimicking human bone matrix were also generated for guided tissue engineering by Kim et al. [54]. The HA precipitate/gelatin matrix are lyophilized and dissolved in an organic solvent, and then electrospun (Fig. 8). These nanocomposites are shown to have improved bone-derived cellular

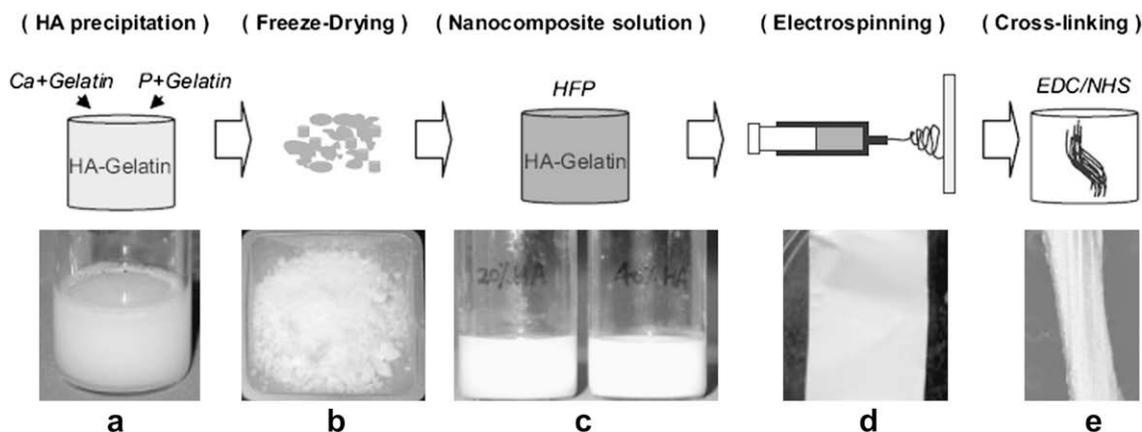


Fig. 8. Schematic illustration of the process used to produce the gelatin-HA nanocomposite fiber. The HA precipitate/gelatin matrix nanocomposite (a) was lyophilized (b) and dissolved in an organic solvent HFP (c) for the electrospinning process. The electrospinning fibers were subsequently cross-linked with EDC (1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride/NHS) (*N*-hydroxysuccinimide) [54].

activity when compared to the pure gelatine equivalent. The biocompatibility of the nanocomposite fibers was assessed by measuring their bone-derived cell (MG63) responses.

Guidance of cell migration and axonal growth on electrospun nanofibers of PCL and Collagen/PCL blend was studied by Schnell et al. [55] with an aim to develop an artificial implant as a conduit for axonal regeneration after peripheral nerve injury. Schwann cell migration, neurite orientation, and process formation of Schwann cell, fibroblasts cells were improved on collagen/PCL fibers, when compared to pure PCL and showed this to be a good material for artificial nerve implants.

3D nanofibrous hybrid scaffolds consisting of PCL, poly(vinyl alcohol) (PVA) and chitosan were prepared via a multijet electrospinning method [56]. The influence of chemical, physical, and structural properties of the scaffolds on the differentiation of mesenchymal stem cells into osteoblasts, and the proliferation of the differentiated cells were investigated. Similarly, Duan et al. [57] have studied electrospun PLGA–chitosan/PVA membranes and their cytocompatibility *in vitro*. They have made hybrid membranes by simultaneous electrospinning of PLGA and chitosan/PVA from two different syringes. The fibrous composite membranes were investigated as a promising scaffold for human embryo skin fibroblast culture. The introduction of chitosan/PVA changed the hydrophilic/hydrophobic balance and, thus influenced the mechanical properties, degradation behaviour and cell proliferation and attachment to the membranes. Also, Meng et al. [58] studied biodegradable hybrids of poly(3-hydroxybutyrate-co-3-hydroxyvalerate)(PHBV) and type I collagen in 1,1,1,3,3,3-hexafluoro-2-isopropanol (HIFP) as nanofibrous (300–600 nm) scaffolds. The cell culture experiments indicated an accelerated adhesion and growth of NH3T3 cells on hybrid scaffolds instead of PHBV scaffolds. In another study, non-woven PCL nanofibers were prepared by electrospinning and type I collagen was then immobilised on the nanofibers after surface modification by remote plasma treatment [59] to be a potential scaffold material for tissue engineering. Schumann et al. [60] have combined electrospinning with fused deposition modelling (FDM), to produce bioactive poly-(caprolactone)/collagen type I and type II PCL-tricalcium phosphate composites for *in vivo* repair of osteochondral repair. The application of these two design techniques (electrospinning and FDM) specifically produced a suitable three-dimensional (3D) environment for the cells to grow into a particular tissue (bone and cartilage) *in vitro* prior to *in vivo* implantation. Zhang et al. [61] provides an overview on the current development and application status of employing electrospun composite nanofibers for constructing biomimetic and bioactive tissue scaffolds. Also, tubular scaffold of marine source collagen and PLGA fibers were fabricated by Jeong et al. [62]. The hybrid scaffolds, composed of a porous collagen matrix and a fibrous PLGA layer, had an average pore size of 150+/-50 μm . The electrospun PLGA layer on the surface of a porous tubular collagen scaffold improved the mechanical strength of the collagen scaffolds in both dry and wet states. A significant cell alignment in a directional radial to the distending direction was observed in tissues exposed to radial distention, which is similar to the phenomenon of native vessel tissues *in vivo*. On the contrary, cells which were tissue engineered in the static condition were randomly aligned.

6. Biofunctionalisation for biomedical applications

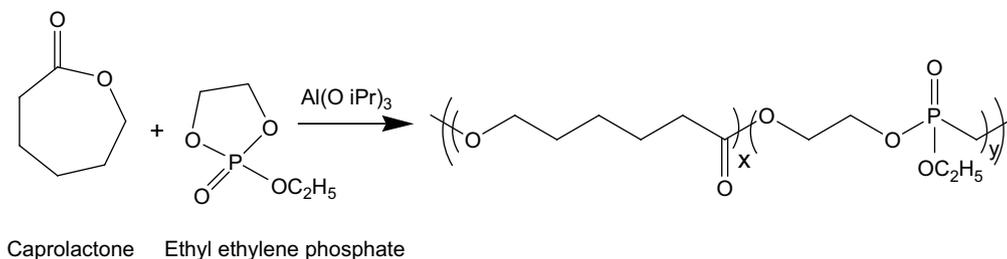
Mimicking ECM by electrospinning of various materials is now easily possible. The efficiency of using the process for any useful tissue engineering application will be further decided by bioactive groups incorporated into artificial ECM structures. Incorporation of bioactive agents into electrospun fibers could lead to a biofunctional tissue engineering scaffold and biofunctionalization of

electrospun fibers will determine the efficiency of these fibers for regenerating biological functional tissues. Bioactive agents could be either incorporated via encapsulation or could be covalently conjugated to the matrix polymer. Zhang et al. [61] have reviewed composite nanofibers for constructing biomimetic and bioactive tissue scaffolds. ECM-derived scaffolds contain bioactive molecules that exert *in vivo* mimicking effects as applied for soft tissue engineering, yet do not possess the same flexibility in mechanical property control as some synthetics. Stankus et al. [63] tried to combine the controllable properties of a synthetic, biodegradable elastomer with the inherent bioactivity of an ECM-derived scaffold. A hybrid electrospun scaffold composed of a biodegradable poly(ester urethane)urea (PEUU) and a porcine ECM scaffold (urinary bladder matrix, UBM) was fabricated and characterized for its bioactivity and physical properties both *in vitro* and *in vivo*. Increasing amounts of PEUU led to linear increase in both tensile strength and breaking strain while UBM incorporation led to increased *in vitro* smooth muscle cell adhesion and proliferation and *in vitro* mass loss. Subcutaneous implantation of the hybrid scaffolds resulted in increased scaffold degradation and a large cellular infiltrate when compared with electrospun PEUU alone. Electrospun UBM/PEUU combined the attractive bioactivity and mechanical features of its individual components to result in scaffolds with considerable potential for soft tissue engineering applications. Rabolt et al. [64] fabricated poly(ethylene glycol) PEG functionalized with low molecular weight heparin (PEG-LMWH) using electrospinning technique for possible use in drug delivery, tissue engineering or wound repairing applications. The incorporation of heparin into the electrospun PEG and PLGA (poly(lactide-co-glycolide)) fibers did not affect the surface morphology or fiber diameters. Improvements in the binding of basic fibroblast growth factor to the electrospun fibers were also observed for fibres functionalised with PEG-LMWH. A brief overview on the development and application of electrospun composite nanofibers (blend composite nanofibers, core-shell structured and nanofibrous mingled) for constructing biomimetic and bioactive tissue scaffolds is written by Zhang et al. [65].

Electrospun natural polymer membranes were fabricated from collagen or gelatine coated with a bioactive recombinant fragment of perlecan, (PInDI) a natural heparin sulphate proteoglycan by Rabolt et al. [66]. Glutaraldehyde was used as cross-linking agent. It cross-links collagen and gelatin fibers via the reaction between the carboxyl groups on the glutaraldehyde and the amide groups of the collagen or gelatin to form cross-links between polymer chains. Laser scanning confocal microscopy revealed that osteoblast-like MG63 cells infiltrated the depth of the electrospun membrane evenly without visible apoptosis. PInDI coated electrospun collagen fibers were 10 times more effective than heparin-BSA collagen fibers at binding growth factors.

A non-woven fabric made up of electrospun polyamide nanofibers [67] supported axonal regeneration in injured rat spinal cord. It was shown by the authors that covalent modification of the nanofibers with a bioactive peptide derived from the neuro-regulatory extra cellular matrix molecule tenascin-C enhanced the ability of the nanofibers to facilitate axonal growth.

Leong et al. [68] have investigated the encapsulation of protein human β -nerve growth factor (NGF) in a copolymer of caprolactone and ethyl ethylene phosphate by electrospinning (Scheme 1; Fig. 9). The authors have taken a copolymer of PCL to fine tune the degradation rate of PCL by introducing more hydrophilic poly(phosphoesters) in the polymer. Bioactivity of NGF was retained partially for at least three months, thereby demonstrating the feasibility of encapsulating proteins via electrospinning to produce biofunctional tissue scaffolds. Continuous nanostructure embedded with proteins present topographical and biochemical signals to cells for tissue engineering applications.



Scheme 1. Synthetic route to poly(caprolactone-co-ethyl ethylene phosphate) (PCLEEP) [68].

We reported for the first time the formation of core-shell nanofibers by coaxial electrospinning [69]. This technique proved to be very versatile for encapsulation of biorelevant molecules and nanocomposites. Liao et al. [70] have shown coaxial electrospinning of aligned PCL nanofibers encapsulated with bovine and platelet-derived growth factor-bb for demonstration of controlled release and bioactivity retention. Controllable release kinetics is achieved by incorporation of PEG as a porogen in the shell of nanofibers. Zhang et al. [71] have also demonstrated the successful encapsulation of a model protein, fluorescein isothiocyanate-conjugated bovine serum albumin (fitcBSA), along with PEG, within the biodegradable poly(caprolactone) PCL nanofibers using a coaxial electrospinning technique (Fig. 10). Simply, by varying the inner flow rate with a constant outer flow rate, fitcBSA loadings could be varied. Composite nanofiber PCL/fitcBSA/PEG blend was prepared from normal electrospinning method, as a negative control. The core-sheath nanofibers compared to that of blend gave better sustainability.

Living membranes containing biological moieties like bacteria, viruses, cells etc. carrying specific functions, offer many unique opportunities for materials and life sciences. Immobilization of biological objects with intact functions in living membranes is of considerable technical interest. Jaysinghe et al. [72,73] have explored the possibility of using coaxial electrospinning technology for the generation of scaffolds comprised of living organisms in the form of threads and scaffolds. The method of coaxial electrospinning has also shown its advantage in depositing active biological threads and scaffolds. This has been achieved by use of a coaxial needle arrangement where a concentrated living bio-suspension flows through the inner needle and a medical grade poly(dimethylsiloxane) (PDMS) medium with high viscosity (12 500 mPa s) and low electrical conductivity ($10\text{--}15\text{ S m}^{-1}$) flows through the outer needle. First they demonstrated the process with immortalized human brain astrocytoma (1321N1, European collection of cell cultures) cell line at a cell concentration of $10(6)$ cells/mL. Later, they employed primary porcine vascular and rabbit aorta smooth muscle cells prepared as cellular suspensions at cell concentrations of $10(7)$ cells/mL which seems to be the highest ever cell concentration threaded by any threading methodology. The cell

electrospinning device uses a coaxial needle arrangement with the flow of highly concentrated cellular suspension in the inner needle and medical grade polydimethylsiloxane in the outer needle. The post-cell electrospun organisms are viable over long periods of time. Collected cells that have been cultured, postelectrospinning, have been found to be viable and show no evidence of having incurred any cellular damage during the bionanofabrication process (Fig. 11).

Bacteria and viruses [74] were also encapsulated in electrospun polymer nanofibers and were shown to survive electrospinning process and remained viable for three months at lower temperatures like -20 and -55°C . The bacteria (*Escherichia coli*, *Staphylococcus*) and viruses (T7, T4, λ) were suspended in a solution of poly(vinyl alcohol) PVA in water and subjected to electrostatic field of the order 1 kV cm^{-1} for encapsulation [74A]. In our group we have shown the survival of *Micrococcus luteus*, an airborne non-pathogenic Gram-positive bacteria under electrospinning conditions [74B] (Fig. 12).

Also, dispersions of M13 viruses in water soluble polyvinylpyrrolidone PVP were processed to nanofibers by electrospinning [74C]. Resulting virus-PVP electrospun fibers maintained their ability to infect bacterial hosts after resuspending in buffer solution. These remarkable findings clearly showed that non-wovens composed of polymer nanofibers and complex biological objects can be prepared directly without total loss of biological functionality.

The effect of nanofiber surface coatings on the cell's proliferation behaviour was studied by Zhang et al. [75]. Coaxial electrospinning technique was used for producing collagen coated polycaprolactone (PCL) nanofibers (Fig. 13). Coatings of collagen on PCL were shown to favour proliferation of human dermal fibroblasts and also encouraged cell migration inside the scaffolds. Using a similar approach, biodegradable fibrous scaffolds composed of gelatine coated PCL were prepared by Zhao et al. by coaxial electrospinning [76].

In order to mimic normal epithelial regeneration on synthetic scaffold in vitro, biodegradable elastic poly(lactide-co-caprolactone) (PLLC) was processed into a nanofibrous scaffold using electrospinning technology [77]. An adhesive protein, fibronectin

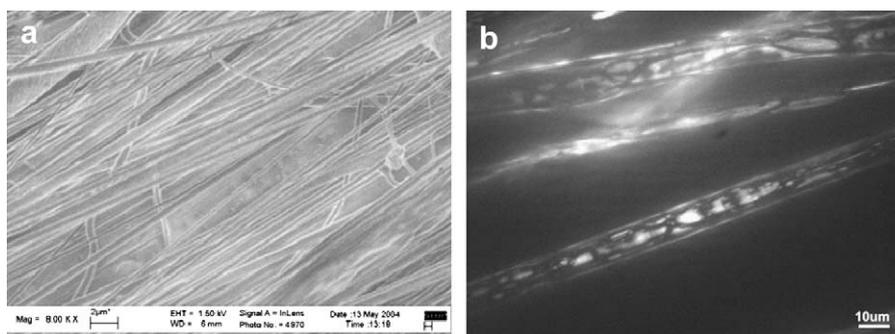


Fig. 9. (a) Aligned BSA encapsulated PCLEEP fibers electrospun at 1 mL/h. (b) Fluorescein isothiocyanate-conjugated bovine encapsulated electrospun fibers of PCLEEP [68].

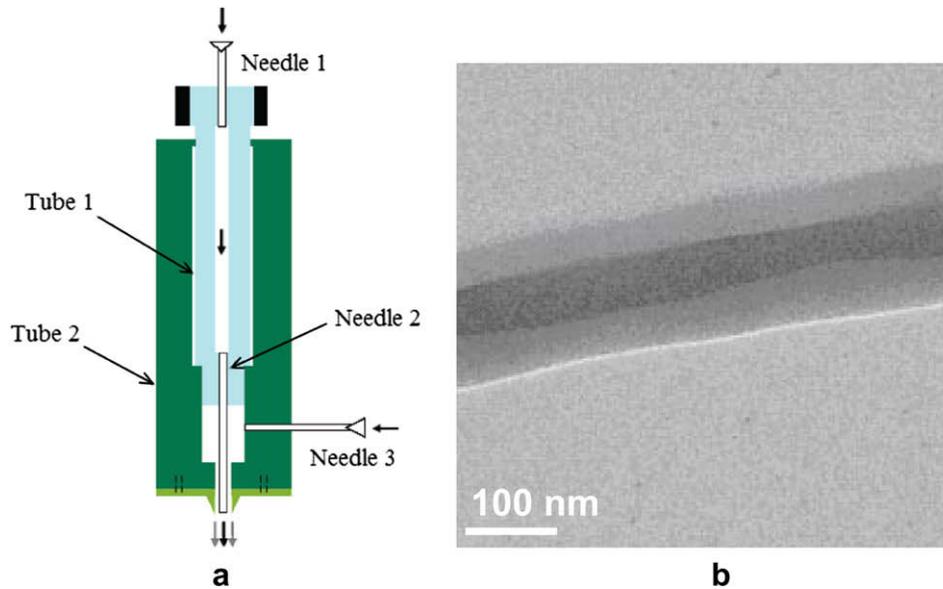


Fig. 10. (a) Schematic illustration of a coaxial electrospinning spinneret used in preparing PCL-r-fitcBSA/PEG core-sheath nanofibers (PCL-r-fitcBSA/PEG denotes the core fitcBSA/PEG is wrapped inside the PCL sheath); (b) TEM image of coaxially electrospun PCL-r-fitcBSA/PEG nanofiber [71].

(Fn), was grafted onto the scaffold surface via a two-step reaction: polyester aminolysis followed by Fn coupling via glutaldehyde. Porcine esophageal epithelial cells were seeded on the Fn bonded scaffold to test the cell growth promotion against the control unmodified PLLC nanofiber scaffold using tissue culture polystyrene plate as reference. Fn grafted on PLLC scaffold greatly promotes epithelium regeneration and is expected a good candidate for functional esophagus substitutes.

Wang et al. [78] have shown improved catalytic efficiency of immobilized enzymes in bioactive electrospun membranes. Bioactive polystyrene nanofibers with a typical diameter of 120 nm were prepared by electrospinning of functionalized PS followed by chemical attachment of a model enzyme, α -chymotrypsin and examined for catalytic efficiency for biotransformations (Scheme 2). Nanofibrous α -chymotrypsin exhibited over 3 orders of magnitude

higher non-aqueous activity than that of its native counterpart suspended in organic solvents.

Biodegradable synthetic matrices that resemble the size, scale, architecture and mechanical properties of the native extra cellular matrix (ECM) can be fabricated through electrospinning. Tubular conduits may also be fabricated with properties appropriate for vascular tissue engineering. Achieving substantial cellular infiltration within the electrospun matrix in vitro remains time consuming and challenging. This difficulty was overcome by electrospaying smooth muscle cells (SMCs) concurrently with electrospinning of a biodegradable, elastomeric poly(ester urethane) urea (PEUU) small-diameter conduit [79]. Hematoxylin and eosin (H&E) staining demonstrated qualitatively uniform SMCs integration radially and circumferentially within the conduit after initial static culture. Conduits were strong and flexible with mechanical behaviors that mimicked those of native arteries, including static compliance of $1.6 \pm 0.5 \times 10^{-3} \text{ mmHg}^{-1}$, dynamic compliance of $8.7 \pm 1.8 \times 10^{-4} \text{ mmHg}^{-1}$, burst strengths of $1750 \pm 220 \text{ mmHg}$, and suture retention. This method to rapidly and efficiently integrate cells into a strong, compliant biodegradable tubular matrix represents a significant achievement as a tissue engineering approach for blood vessel replacement.

7. Modified electrospinning processes for biomedical applications

Electrospinning procedure is further modified to accommodate needs of materials for biomedical applications. Dual syringe reactive electrospinning is one of such modifications (Fig. 14) [80].

A facile fabrication of cross-linked hyaluronic acid hydrogel nanofibers for tissue engineering by a reactive electrospinning method is described by Ji et al. [80]. A thiolated HA derivative, 3,3'-dithiobis(propanoic dihydrazide)-modified HA (HA-DTPH), and poly(ethyleneglycol) diacrylate (PEGDA) are selected as the cross-linking system (Scheme 3). The cross-linking reaction occurs simultaneously during the electrospinning process using a dual-syringe mixing technique. A cell morphology study on fibronectin (Fn) adsorbed HA nanofibers scaffolds shows that the NIH3T3 fibroblasts migrate into the scaffold through the nanofibrous network, and demonstrate an elaborate three-dimensional dendritic morphology within the scaffold.

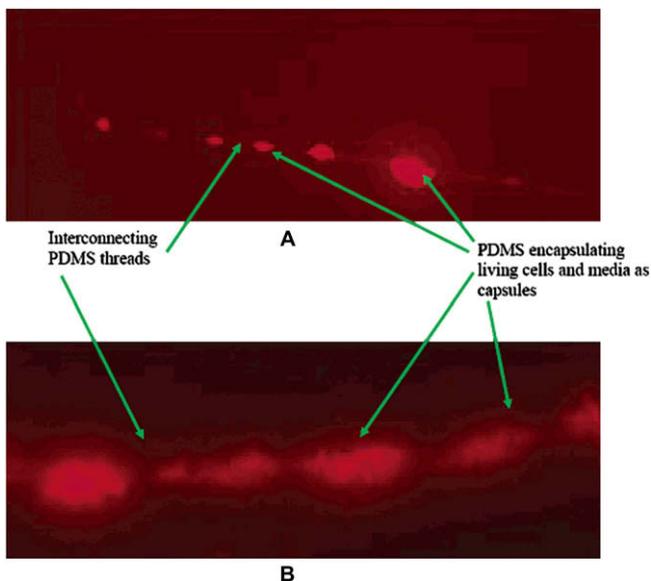


Fig. 11. Characteristic fluorescent micrographs showing the variation in fiber diameter that results from cell encapsulations. Experiments were conducted at an applied voltage of 9.5 kV under flow rate conditions E6 (A) and A2 (B) [72].



Fig. 12. SEM image of composite fibers consisting of PEO and *M. luteus* (a) and optical fluorescence microscopy of electrospun PEO fibers containing *M. luteus* functionalized by a fluorescent dye under normal illumination (b) and under UV illumination (c).

Electrospun fiber orientation could influence cell proliferation besides controlling cell orientation and tissue growth. For example, many musculoskeletal tissues exhibit significant anisotropic mechanical properties which shows highly oriented underneath extra cellular matrix [81]. Latest developments in the field of electrospinning have made it possible to generate nanofibers based scaffolds with controlled fiber orientations [82]. A good reference to this aspect is a review article by Murugan et al. [83]. Ayres et al. [84] have shown how incremental changes in fiber alignment modulate the material properties of a model scaffold taking gelatin as an example. Nanofiber of poly(L-lactide-co-caprolactone) (75:25) having diameters around 500 nm with aligned topology mimicking the circumferential orientation of cells and fibrils found in the medial layer of a native artery were produced by Xu et al. [85] This scaffold showed to have a very favourable interaction with human coronary artery smooth muscle cells (SMCs). SMCs migrated along the axis of the aligned nanofibers and expressed a spindle like contractile phenotype. The adhesion and proliferation rate of SMCs on the aligned nanofibrous scaffold was shown to be more in comparison to the plane polymer films. Fibrous membranes of aligned poly(hydroxybutyrate-co-hydroxyvalerate) (PHBV) fibers have been made through electrospinning [86]. A high drum

rotating speed of 3000 rpm could lead to a nearly perfect alignment of PHBV fibers during electrospinning. Huber et al. [87] have projected the problem of mechanical destabilisation of the spontaneously contractile neo tissues, resulting in the complete loss of differentiating myotubes. They have therefore, tried to use parallel aligned nylon 6/6 arrays for the culture of C2C12 myoblasts and their differentiation to form mechanically stable, oriented myotubes in vitro. The myogenic potential of these cells was shown not impaired and resulted in the formation of elongated myotubes expressing alpha-actinin, adult myosin heavy chain and nicotinic acetylcholine receptors as muscle specific marker proteins. Newly formed C2C12 myotubes were oriented in parallel to the direction of the underlying fibrous substratum and exhibited a high level of structural integration with the surrounding cells. In contrast, non-woven, non-oriented nylon 6/6 meshes, produced by conventional electrospinning, exhibited greatly reduced levels of C2C12 myoblast attachment and adherent myoblasts did not differentiate into myotubes.

The fabrication of aligned collagen nanofibrous scaffolds is described by Zhong et al. [88]. The electrospinning apparatus used by them is shown in Fig. 15. The structure and in vitro properties of these scaffolds were compared with a random collagen scaffold.

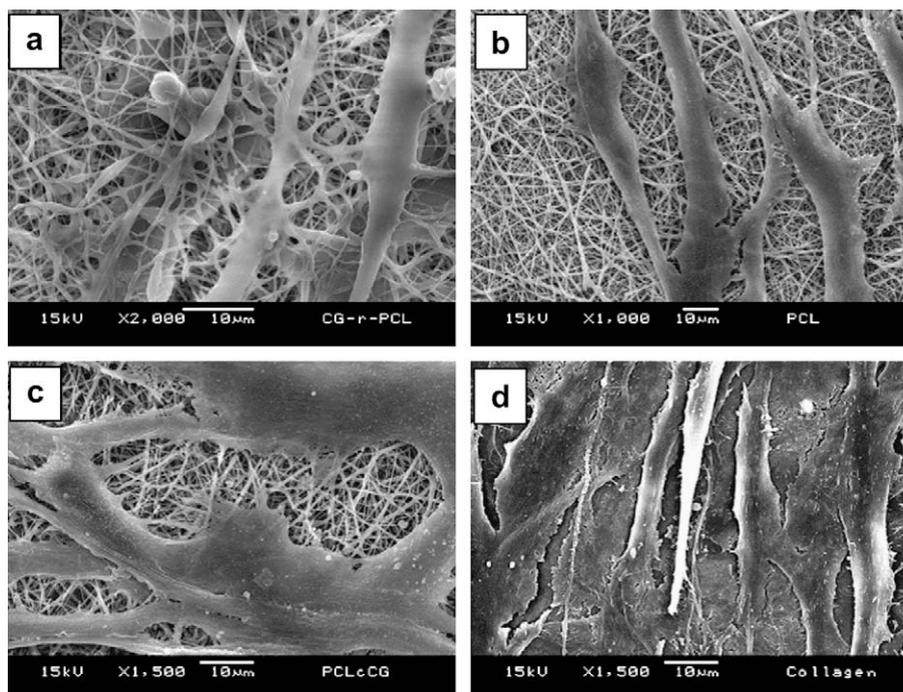
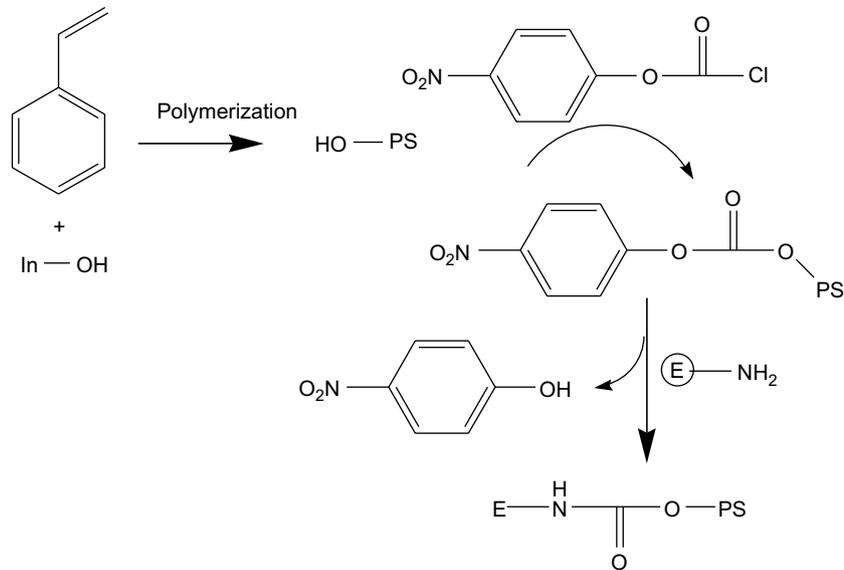


Fig. 13. A comparison of cells (human dermal fibroblasts) in growth behaviour on different nanofibrous scaffolds: (a) individually collagen-coated PCL (core-shell) (Col-r-PCL); (b) pure PCL; (c) PCL with surface roughly collagen coated; and (d) pure collagen nanofibers [75].



Scheme 2. Chemical route for synthesis of functionalised polystyrene and subsequent attachment of enzyme [78].

From, the *in vitro* culture of rabbit conjugation fibroblast, the aligned scaffold exhibited lower cell adhesion but higher cell proliferation.

Kim et al. [89] have made a 3D nanofibrous fibroin scaffold (NFS) with very high porosity (94%) and examined its feasibility in bone regeneration and showed its superior performance over a 2-D scaffold. A modified electrospinning method has been used. As-spun nanofibers are collected in a coagulation bath and formed in a dispersed state. The dispersion is solidified by freeze drying, resulting in a sponge like 3D nanofibrous structure. 3D NFS with high porosity is shown superior to 2-D NFS in terms of both cell adhesion and proliferation and could be promising candidate scaffold for large bone defects. Highly porous 3D nanofibrous scaffold of PCL is made by electrospinning using auxiliary electrode and chemical blowing agent (BA) by Kim et al. [90]. The detailed shape and geometry of the auxiliary electrode are shown in Fig. 16.

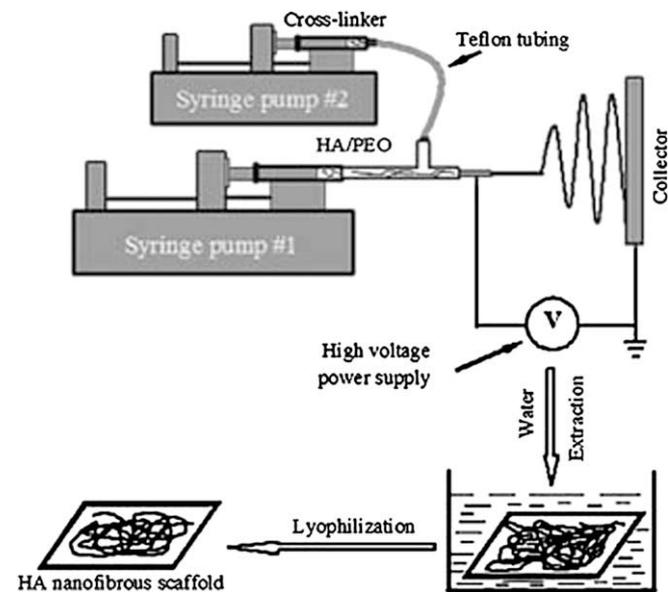


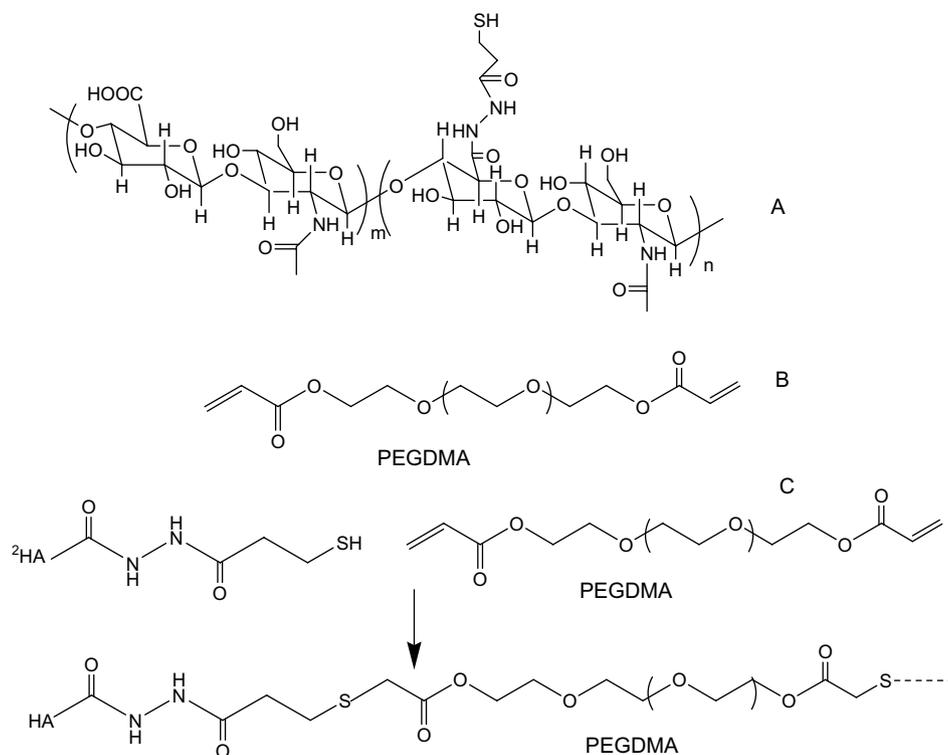
Fig. 14. Schematic illustration of the electrospinning setup and the procedure for the fabrication of HA-DTPH nanofibrous scaffolds [80].

High porosity and optimally designed pore size provide structural space for cell accommodation and migration and enable the exchange of nutrients between the scaffold and environment. The growth characteristic of human dermal fibroblast cells cultured in the webs showed the good adhesion with the blown web relative to a normal electrospun mat. The nanofiber web had good tensile properties and high porosity compared to a typical electrospun nanofiber scaffold.

Different fiber meshes were prepared by Neves et al. [91] from PEO and PCL using various patterned collectors with specific dimensions and designs with an aim to evaluate the resulting mesh properties relevant to biomedical applications.

The published literature clearly shows an edge of electrospinning technique for tissue engineering over other conventional methods in terms of its simplicity and providing high porosity, variable fiber diameters and fiber textures combined with suitable biocompatible and bioerodable materials. Electrospinning assembly can be varied in different ways for combining materials properties with different morphological structures for tissue engineering. Cell-fiber interaction studies for tissue engineering purposes are challenging. Due to the three-dimensional nature of the matrix, there could be lack of nutrients and oxygen in the inner core of the matrix that could lead to misleading results regarding cell-fiber interactions. In spite of these and other problems although these scaffolds at present are far from being optimized for the individual target tissue, there is no doubt that electrospun scaffolds are going to capture major place in future for tissue engineering.

A novel structure combining polymeric micro and nanofibers in the same construct for bone tissue engineering is provided by Tuzlakoglu et al. [92]. Macro support is sometimes required by the cells to grow. Though there are many promising materials and studies for tissue engineering, they have a limitation for 3D applications due to their small pore size, smaller than a cellular diameter and cannot allow cell migration within the structure. Furthermore, the small size of the fibers tends not to maximize the points of cell attachment which is a negative effect on the expression of several factors and on cell spreading and differentiation according to Tuzlakoglu et al. [92]. They have developed a novel Starch based scaffold with 70% porosity from a blend of starch/polycaprolactone (SPCL) (30/70 wt%) by a fiber bonding process. Later electrospinning was used to obtain nanofibers onto SPCL fiber mesh



Scheme 3. (A) Structure of HA-DTPH. (B) Structure of PEGDA. (C) Cross-linking reaction to form HA-PEGDA-HA linkages [80].

scaffolds. The results showed that cell (SaOs-2 human osteoblast-like cell line and rat bone marrow stromal cells) viability and alkaline phosphatase (ALP) activity for both cell types were found to be higher in nano/micro combined scaffolds than in control scaffolds based on fiber meshes without nanofibers (Fig. 17).

8. Drug release and implants

Drug release and tissue engineering are closely related areas. Sometimes release of therapeutic factors can increase the efficiency of tissue engineering. Various nanostructured materials for applications in tissue engineering and in drug delivery have been reviewed by Goldberg et al. [93]. Electrospun fiber mats provide the advantage of increased drug release as compared to cast-films due to the increased surface area. Kanawung et al. [94A] have studied the release of model drugs like diclofenac sodium (DS) and

tetracycline hydrochloride (TH) from electrospun PCL and poly(vinyl alcohol) (PVA) fiber mats. The cumulative release of the model drugs increased monotonically with increasing immersion time and became practically constant at long immersion times. The release of antibiotic tetracycline hydrochloride and mefloxin is also studied using electrospun poly(lactic acid) PLA and poly(ethylene-co-vinylacetate) [94B] and PLA, respectively [94C]. Release studies of diclofenac sodium from electrospun poly(maleic anhydride-alt-2-methoxyethyl vinyl ether) mats [95] and poly(caprolactone-D,L-lactide) [96] are also known.

Electrospun PLGA-based micro and nanofibers as implants for the sustained delivery of anticancer drug (paclitaxel) to treat C6 glioma (brain tumor) *in vitro* has been developed by Xie et al. [97A]. The sustained release of drug with this system was achieved for

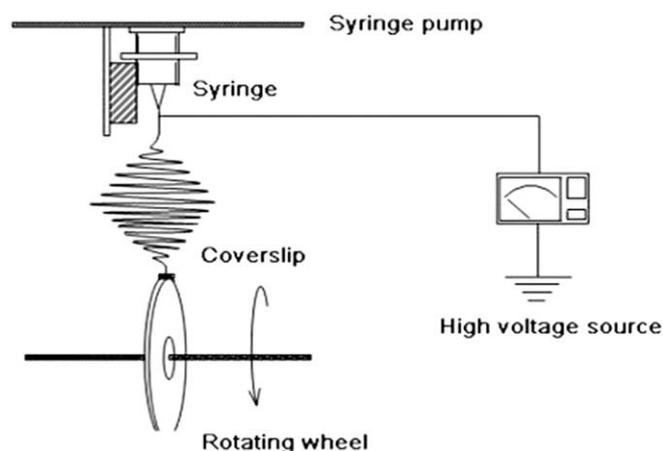


Fig. 15. Fiber alignment during electrospinning [88].

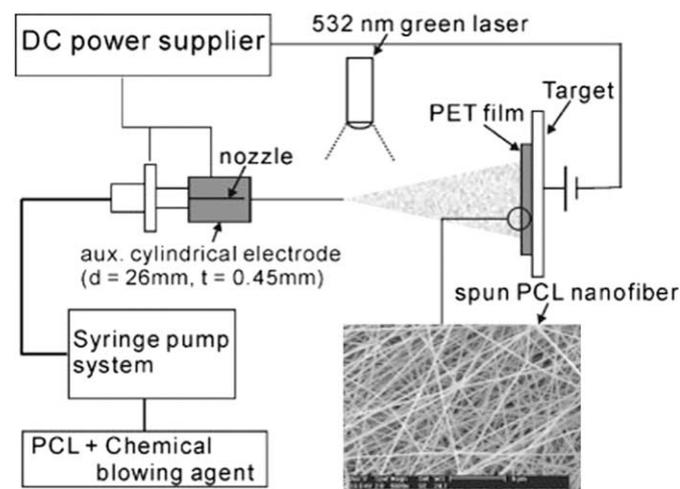


Fig. 16. Schematic illustration of the electrospinning setup with an auxiliary cylindrical electrode. The cylindrical electrode has a radius of 13 mm and is 0.45 mm thick [90].

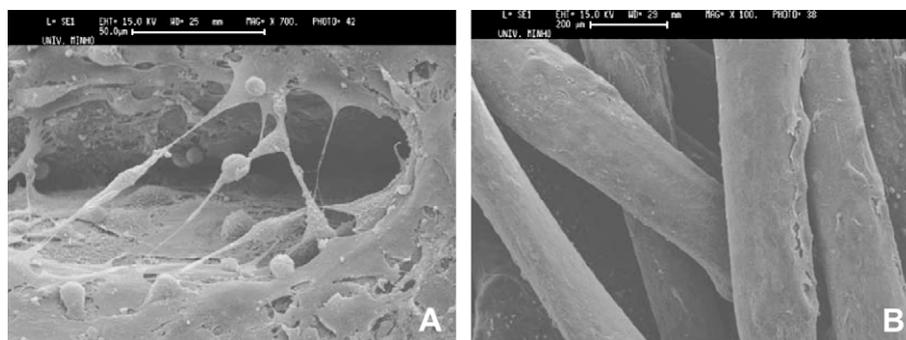


Fig. 17. Rat bone marrow cells seeded on nano and microfiber combined scaffolds (A) and only microfibers scaffolds (B) after 14 days of culture [92].

more than 60 days. Mats of PVA nanofibers were developed as carriers of drugs for transdermal drug-delivery system by Supaphol et al. [97B]. Four types of non-steroidal anti-inflammatory drug with varying water solubility, i.e. sodium salicylate, diclofenac sodium, naproxen, and indomethacin were tried as model drugs.

Nie et al. [98] have also constructed a PLGA/HA composite scaffold for delivery of BMP-2 plasmid DNA. This work was carried out to improve one of the present methods of gene delivery using DNA loaded chitosan particles. Poly(lactide-co-glycolide) (PLGA)/hydroxyapatite (HA) composite scaffolds with different amounts of HA (0–10 %) are fabricated by electrospinning and DNA is incorporated into the scaffold in 3 different ways, i.e. naked DNA, encapsulation of DNA/chitosan nanoparticles into scaffolds after fiber fabrication by dipping, and encapsulation of DNA/chitosan nanoparticles into scaffolds by mixing with PLGA/HA solution before fiber fabrication. In this study it was shown that the addition of HA nanoparticles increased the release rate of DNA for both naked and encapsulated DNA. Cell culture experiments with human marrow stem cells (hMSCs) show that the scaffolds with encapsulated DNA/chitosan nanoparticles have higher cell attachment, higher cell viability and desirable transfection efficiency of DNA. Table 4 gives a small summary of some of the representative electrospun systems studied for drug-release applications.

A protein-loaded three-dimensional scaffold can be used for protein delivery and bone tissue regeneration. The recombinant human bone morphogenetic protein-2 (rhBMP-2) loaded poly(D,L -lactide-co-glycolide)/hydroxyapatite (PLGA/HAp) composite fibrous scaffold by electrospinning were fabricated by Nie et al. [99A]. It was also shown that BMP-2 protein maintained its integrity and natural conformations after electrospinning and scaffold allowed sustained release of BMP-2 whose release rate can be controlled with the amount of HAp. Cell culture experiments also showed that the encapsulation of HAp could enhance cell attachment to scaffolds and lower cytotoxicity.

Recently Supaphol et al. [99B] have shown the release characteristic of *Centella asiatica*-herbal extract from electrospun gelatin fibers. *Centella asiatica*-herbal extract is widely known for its traditional medical applications including its wound healing ability. Vitamin-loaded electrospun cellulose acetate nanofiber mats as transdermal and dermal therapeutic agents of vitamin A acid and Vitamin E are made by Supaphol et al. [99C].

Weak electrolytes like poly(acrylic acid) (PAA) and poly(allylamine hydrochloride) (PAH) were also electrospun with methylene blue as model drug to evaluate the electrospun fibers for drug-delivery applications [100]. Sustained release of the model drug was achieved by using perfluorosilane networks on the fiber surfaces as capping layers. The authors have also showed temperature controlled release of model drug by depositing temperature sensitive poly(*N*-isopropylacrylamide) (PNIPAAm) onto the surfaces. The antibiotic (Biteral) embedded PCL membranes were formed by electrospinning by Bolgen et al. [101] with an aim to

prevent post surgery induced abdominal adhesions. The antibiotic embedded PCL membranes were implanted on the abdominal wall of rats and macroscopical and histological evaluations showed the reduced extent, type, and tenacity of adhesion.

Further, Hu et al. [102] prepared composite fibers with beads-in-string structures via electrospinning from either W/O or O/W emulsion and thus proved this technique (Fig. 18) as an effective method for microencapsulation. To immobilize hydrophilic protein in hydrophobic polymer, Ca-alginate microspheres were prepared in W/O emulsion and served as drug reservoirs for bovine serum albumin (BSA). After dissolving with poly(L-lactic acid) (PLLA) to its continuous phase, the emulsion was spun into fibers by the electrospinning technique. In the *in vitro* release test, BSA was retained in composite fibers for 120 h, in contrast to in naked microspheres, which were almost completely released in the first 10 h.

In general, the drug release profile is dependent on how good the drug is dispersed in the matrix polymer. In all the representative examples given above, the drugs are mixed (dissolved/dispersed in the polymer solution before electrospinning). There is always a probability of finding drug on nanostructured surfaces besides being encapsulated inside thereby leading to burst release in the initial stages.

An alternative method to encapsulate drugs, proteins, growth factors, genes etc. inside polymeric nanofibers is by coaxial

Table 4

Some of the representative electrospun systems studied for drug-release applications

Electrospun mat	Drug	Reference
Poly(caprolactone) PCL	Diclofenac sodium	[94A]
	Tetracycline hydrochloride	[94A]
	Resveratrol	[100]
	Gentamycin Sulfate	[100]
	Biteral	[104]
Poly(lactic acid) PLA	tetracycline hydrochloride and mefoxin	[94B,94C,100]
Poly(caprolactone- D,L -lactide)	Diclofenac sodium	[96]
Poly(vinyl alcohol) PVA	Diclofenac sodium	[94,97B]
	Tetracycline hydrochloride	[94A]
	Sodium salicylate, naproxen, indomethacin	[97B]
Poly(maleic anhydride-alt-2-methoxyethyl vinyl ether)	Diclofenac sodium	[95]
Poly(lactide-glycolide) (PLGA)	Paclitaxel (anticancer)	[97A]
	Tetracycline hydrochloride	[106]
Poly(ethylene-co-vinylacetate)	tetracycline hydrochloride	[94B]
Gelatin	<i>Centella asiatica</i> -herbal extract	[99B]
Cellulose acetate	Vitamin A and E	[99C]

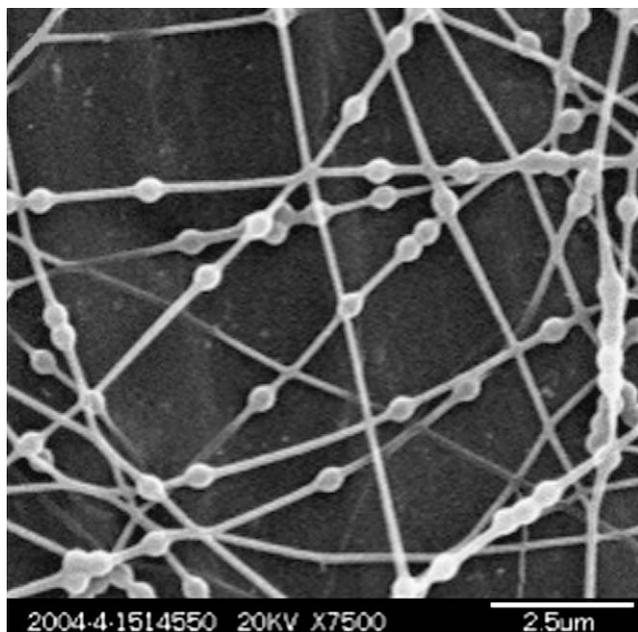


Fig. 18. SEM micrograph of electrospun fibers by emulsion [102].

electrospinning. A coaxial electrospinning technique to fabricate core-shell ultrafine fiber mats for drug-delivery application is described by He et al. [103A]. PLA and tetracycline hydrochloride were employed as shell and core materials, respectively. Their results showed that a reservoir type drug release device can be conveniently obtained through encapsulation. The electrospun ultrafine fiber mats containing drugs may be used as drug release carriers or made into biomedical devices such as sutures and wound dressings. Huang et al. [103B] have encapsulated drugs like Resveratrol (antioxidant) and Gentamycin Sulfate (an antibiotic) in biodegradable poly(caprolactone) PCL ultrafine fibers through coaxial electrospinning. The release profiles of the two drugs exhibited a sustained release characteristic, with no burst-release phenomenon. Also, biodegradable core-shell structured fibers with poly(caprolactone) as shell and bovine serum albumin (BSA)-containing dextran as core were prepared by Hongliang et al. [104] by coaxial electrospinning technique for incorporation and controlled release of proteins. Protein loading percent in the fibers and its release rate can easily be controlled by either the feed rate of the inner dope during electrospinning or by making the shell more hydrophilic. Coaxial electrospinning is shown to have the advantages of being fast, high loading efficiency, and controllable release behaviour over conventional methods of encapsulating proteins, growth factors and DNA like water-in-oil-in-water emulsion, gas foaming/particulate leaching methods. Zhang et al. [71] have shown better sustainability of release of biomolecules from core-shell fibers of PCL and fitcBSA/PEG fibers. Burst-release effect could be suppressed but could not be eliminated completely.

In our group [105] we have tried to control the burst-release effect of biomolecules, a very common problem, from electrospun reservoirs by providing an extra protective coating on the drug reservoirs. The concept was shown using poly(vinyl alcohol) (PVA) based protein delivery system. Protein-loaded (bovine serum albumin (BSA) or luciferase) PVA nanofibers were obtained by electrospinning. Poly(*p*-xylylene) (PPX, also coined as parylene) coated PVA/BSA nanofibers were prepared by chemical vapor deposition (CVD). The release of BSA from PVA nanofibers under physiological conditions was monitored by absorption spectroscopy. Burst release of BSA was noted with uncoated PVA nanofibers. In contrast, PPX-coated nanofibers exhibited a significantly retarded release of BSA depending on the coating thickness

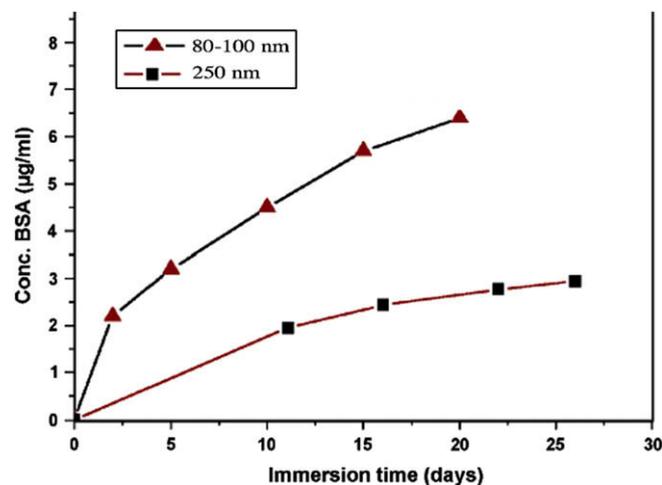


Fig. 19. Release of fitcBSA from PPX-coated PVA/BSA nanofibers which were immersed in water at 37 °C for different periods with different layers thickness of PPX [105].

of PPX (ranging from 40 to 300 nm) (Fig. 19). Luciferase was used here as model enzyme, which after electrospinning retained its enzyme activity. This preservation of enzyme activity and the continuous release of the intact enzyme from the immersed fibers meet a fundamental prerequisite for the application of enzymes or other sensitive agents released from electrospun nanofibers.

Further, a modified electrospinning method has been provided by Wagner et al. [106] for the creation of an elastomeric, fibrous sheet (fibrous composite sheet with two distinct submicrometer fiber populations: biodegradable poly(ester urethane) urea (PEUU) and poly(lactide-co-glycolide) (PLGA)), where the PLGA was loaded with the antibiotic tetracycline hydrochloride (PLGA-tet) capable of sustained antibacterial activity in vitro. Composite sheets were flexible with breaking strains exceeding 200%, tensile strengths of 5–7 MPa, and high suture retention capacity. The blending of PEUU fibers markedly reduced the shrinkage ratio observed for PLGA-tet sheets in buffer from 50% to 15%, while imparting elastomeric properties to the composites. In development of this material, a new approach to two-stream electrospinning (Fig. 20) was used wherein one component stream provided for antibiotic release while the other provided mechanical properties deemed essential for the desired application. This material may find applicability in the treatment of temporary abdominal wall closure.

9. Wound dressing

Electrospinning could generate scaffold with more homogeneity besides meeting other requirements like oxygen permeation and protection of wound from infection and dehydration for use as wound-dressing materials. The conventional skin substitutes are made up of fibroblasts and/or keratinocytes on collagen scaffolds, mainly generated by freeze drying (FD) which generates structural heterogeneity. ES could provide wound-dressing materials in a simple way. Powell et al. [107] have compared FD and ES skin substitutes based on natural polymer, collagen. They are compared for cell distribution, proliferation, organization, and maturation engraftment and healing of full thickness wounds in a thymic mice. Although no significant difference in cell proliferation, surface hydration or cellular organization between FD and ES scaffolds were seen, wound contraction was potentially reduced with ES scaffold. This provides the advantage of reduced morbidity in patients treated with skin substitutes from ES collagen. Many other synthetic and natural polymers like carboxyethyl chitosan/PVA [19], collagen/chitosan [108], Silk fibroin [3], ABA type poly(dioxanone-co-L-lactide)-block-poly(ethylene glycol) (PPDO/PLLA-b-PEG) block

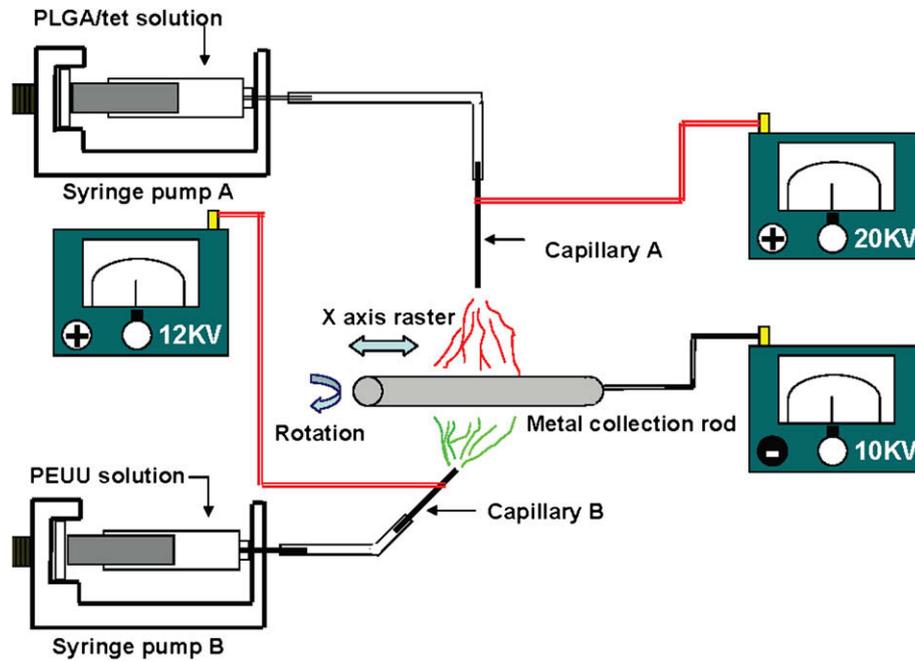


Fig. 20. Two-stream electrospinning setup [106].

copolymer [109], have been electrospun to suggest them for wound-dressing applications.

Further, wound-dressing material was prepared by electrospinning of (PVA)/AgNO₃ aqueous solution into non-woven webs and then treating the webs by heat or UV radiation [110]. Through SEM, TEM, and XPS analyses, it was observed that the silver (Ag) nanoparticles were generated and existed in the near surface of the electrospun nanofibers (Fig. 21). Heat treatment as well as UV radiation reduced the Ag ions in the electrospun PVA/AgNO₃ fiber web into the Ag nanoparticles. Also the heat treatment improved the crystallinity of the electrospun PVA fiber web and so it made the web insoluble in moisture environment. As we know, silver has long been recognized as a broad-spectrum and highly effective antimicrobial agent for treating wounds and burns. Silver ion works

by denaturing the proteins and nucleic acids of the bacteria by binding to their negatively charged components. Besides, silver acts in generating oxygen which in turn destroys the cell wall membranes of bacteria [111]. As pointed out by Hong et al. [110] metallic silver is also used commercially in wound dressings such as Acticoat (Smith & Nephew Health), in which silver is applied to the polymer mesh by a vapor deposition process. It surely shows an excellent antibacterial activity above 99.9%. However, there are some demerits; for instance, the metallic silver of the wound dressing leads to gray-blue discoloration on the skin and moisture supplies are regularly necessary to dissolve the metallic silver and also the manufacturing cost is nevertheless relatively high. Therefore, the electrospun PVA/AgNO₃ fiber webs prepared are promising materials as wound dressings.

Duan et al. [111] have also produced antimicrobial nanofibers of poly(ϵ -caprolactone) (PCL) by electrospinning of a PCL solution with small amounts of silver-loaded zirconium phosphate nanoparticles (nano AgZr) for potential use in wound-dressing applications. The results of the antimicrobial tests showed that these fibers have maintained the strong killing abilities of Ag existed in the nano AgZr against the tested bacteria strains (Gram-positive *Staphylococcus aureus* (ATCC 6538) and Gram-negative *E. coli* (ATCC 25922)). Discoloration has not been observed in the nanofibers. To test the biocompatibility of nanofibers as potential wound dressings, primary human dermal fibroblasts (HDFs) were cultured on the nanofibrous mats. The cultured cells were evaluated in terms of cell proliferation and morphology. The results indicated that the cells attached and proliferated as continuous layers on the nano AgZr-containing nanofibers and maintained the healthy morphology of HDFs. Electrospinning of poly(vinyl pyrrolidone)-iodine complex and poly(ethylene oxide)/poly(vinyl pyrrolidone)-iodine complex as prospective route to antimicrobial wound dressing materials was also shown by Ignatova et al. [112].

Effects on the early stage wound healing of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) nanofiber matrices cultured with hair follicular cells were tested by Han et al. [113]. PHBV only, PHBV/Colagen and PHBV/gelatin at a 7:3 weight ratio were produced by electrospinning. Their in vitro cell culture and in vivo wound healing as biological dressings were also examined. In

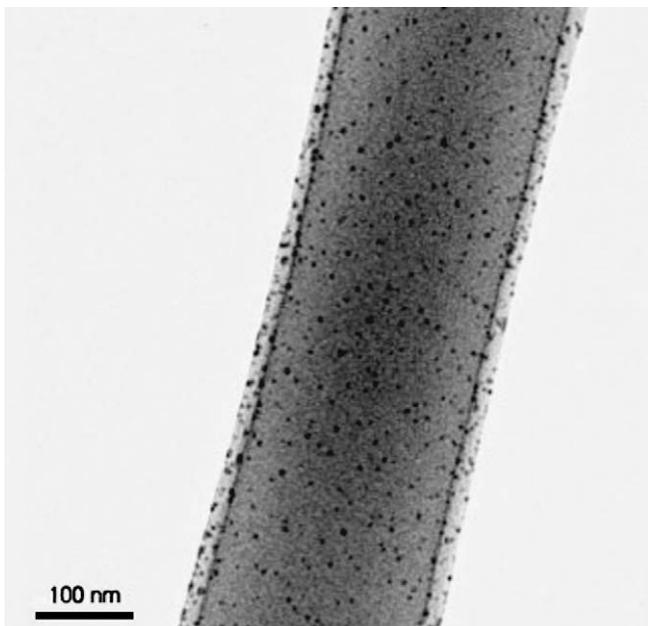


Fig. 21. TEM image of post-UV treated electrospun PVA/AgNO₃ fiber web [110].

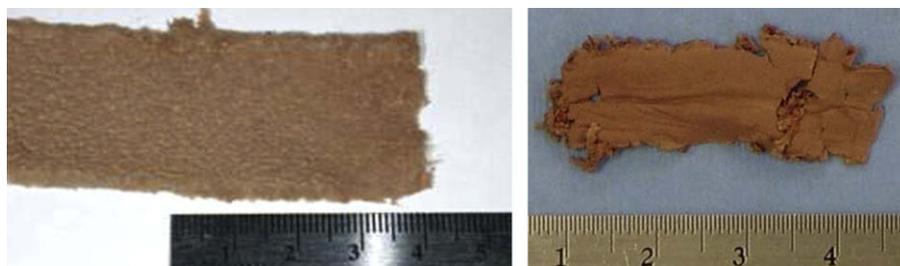


Fig. 22. Electrospun Hemoglobin and myoglobin mats using 2,2,3-trifluoroethanol solvent [115B].

cell attachment and growth on matrices, dermal sheath (DS) cells attached to hydrophilic PHBV/collagen and PHBV/ gelatin faster than hydrophobic PHBV. Also if cocultured for 3–5 days with DS and epithelial outer root sheath (ORS) cells expressed more extra cellular materials, such as type I collagen, elastin and alpha smooth muscle actin. In vitro culture studies of human dermal fibroblasts on electrospun PCL collagen blend nanofibrous membranes are shown to be promising for dermal substitute for the treatment of skin defects and burn wounds by Ramakrishna et al. [114]. Also, Barnes et al. [115A] have provided an overview of tissue engineering via electrospun biomimetic nanofibers and also electrospun hemoglobin and myoglobin [115B] with an aim that their inclusion in wound dressings may help in delivering oxygen directly to the healing tissues (Fig. 22).

Further, Kim et al. [116] have introduced a direct-electrospinning apparatus that uses a guiding electrode and an air-blowing system to enable the fabrication of wound-dressing membranes of PCL micro/nano fibers. Stable, steady deposition of electrospun fibers on any substrate occurred without interrupting the charges on the substrate with sufficient removal of solvent. The guiding electrode was used to eliminate static charges in the electrospun fibers (Fig. 23).

10. Miscellaneous

Polymeric electrospun nanofiber web-based artificial renal microfluidic chip was developed by Lee et al. [117]. They have produced polyethersulfone and polyurethane based nanofiber webs by electrospinning and combined this with the poly(dimethylsiloxane) (PDMS) based microfluidic platform top create a chip-based portable hemodialysis system. The filtration capability of this dialyzing chip was measured for molecules in broad ranges of sizes. Blood cells were not mechanically affected during the filtration and their transportation through chip. This demonstrated the feasibility of chip-based hemodialysis realization of portable hemodialysis systems.

The nanofiber deposition method, by electrospinning, was employed to introduce antibacterial activity and biocompatibility to the surface of poly(ethylene terephthalate) (PET) textiles [118]. The polymer blends of PET and chitosan were electrospun on the PET micro-non-woven mats for biomedical applications. Antibacterial activity is reported against *S. aureus* and *Klebsiella pneumoniae*.

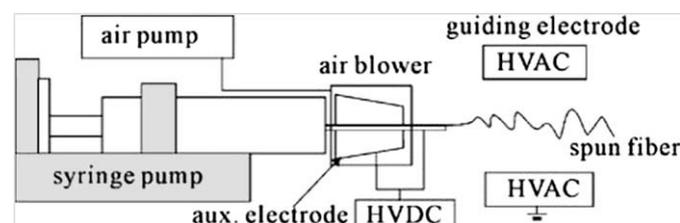


Fig. 23. Schematic illustration of the wound dresser using an electrospinning process, that includes supporting electrodes (a conical electrode and guiding electrodes) and an air-streaming system [116].

11. Conclusions

The important advantages of electrospinning technique are the production of very thin fibers with large surface areas, ease of functionalisation for various purposes, superior mechanical properties and ease of process. These advantages provide a wide range of opportunities for their use in many different biomedical applications. These applications range from tissue engineering, drug release, implants, biotransformations to wound healing. Electrospinning assembly can be varied in different ways for combining materials properties with different morphological structures for these applications. Also, although there is ever increasing literature on use of electrospinning for various biomedical applications but still the field is in its infancy. Although a series of new and commercially available polymers have been proposed as matrices for cell regeneration and proliferation for tissue engineering applications but lot of questions like interaction of scaffolds with biological systems, toxicity, in vivo studies etc. have to be thoroughly investigated before the technology can be used for any real practical biomedical application.

Drug release and tissue engineering are closely related areas. Large number of biodegradable and biocompatible polymers have also been electrospun by conventional electrospinning or modified electrospinning methods like co-axial electrospinning etc. loaded with different drugs as model studies. These studies show the advantages of electrospinning technique for example in reducing the burst release of drugs in vitro. There are only few studies which have been really carried out in animals in vivo. There is a need for making joint efforts by material scientists and biologists to make serious in vivo drug release studies under physiological conditions. This is the time to transfer laboratory research on use of electrospinning for biomedical applications to clinical use. There is no doubt that electrospun materials are going to take major place in future for biomedical applications, but methods and materials have to be provided which can be used on a technical scale as well.

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Seema Agarwal PD, PhD, is working in the department of chemistry at Philipps-University, Marburg Germany since the year 2000. She did double masters in chemistry and polymers science and technology and later obtained her PhD from Indian Institute of Technology, Delhi, India under the supervision of Prof. I.K. Varma. Her scientific professional career started in India by working as Scientist for about 9 years and then after she shifted to Germany. She was a Humboldt Fellow from 1997–1999 in the laboratory of Prof. Andreas Greiner, Germany. She accomplished her habilitation for macromolecular chemistry in 2007 at the University of Marburg. She has more than 50 scientific publications in different journals and her research interests are: biodegradable and biopolymers, structure–property-relationship studies of polymers, NMR of polymers, controlled polymerizations and electrospinning.



Joachim H. Wendorff received his doctorate from the University of Marburg in 1969 for work in the field of polymer physics with F.H. Müller. After a postdoctoral stay with F.P. Price in Amherst, he became a research assistant with E.W. Fischer at the University of Mainz. In 1982, he completed his habilitation in physical chemistry there. From 1976 to 1991, he headed in Physics Department at the German Polymer Institute (DKI) in Darmstadt. In 1990, he took up a professorship in physics at the TH Darmstadt, and in 1991, he moved to a professorship in physical chemistry at the University of Marburg. His research interests include functional polymers, liquid crystals, polymer alloys, molecular reinforcement and nanostructuring techniques including electrospinning as well as applications of the nanostructured systems in Material and Life Science.



Andreas Greiner received his PhD degree in Chemistry from Philipps-University of Marburg in 1988. In 1989 he joined as postdoc Prof. H.-W. Schmidt at the Materials Department, University of California, Santa Barbara, CA (USA). In 1990 he returned as research associate to the Department of Chemistry, Philipps-University of Marburg and accomplished his habilitation in polymer chemistry in 1995. In 1999 he joined Johannes-Gutenberg University as associate professor for organic chemistry and macromolecular chemistry and became head of the TransMIT Center for Plastic Science. Since 2000 he holds a position as full professor for polymer chemistry and technology (successor of Prof. Dr. W. Heitz). Present research topics of his group are general monomer and polymer synthesis, electrospinning of polymer nanofibers, functional polymers, functionalized nanoparticles, poly(*p*-xylylene)s, polymers for filtration, textiles, agriculture, pharmacy, and agriculture.