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Recent development of polymer nanofibers for biomedical and biotechnological applications

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Research in polymer nanofibers has undergone significant progress in the last one decade. One of the main driving forces for this progress is the increasing use of these polymer nanofibers for biomedical and biotechnological applications. This article presents a review on the latest research advancement made in the use of polymer nanofibers for applications such as tissue engineering, controlled drug release, wound dressings, medical implants, nanocomposites for dental restoration, molecular separation, biosensors, and preservation of bioactive agents.

1. Introduction

The essence of nanotechnology is in the creation and utilization of materials and devices at the level of atoms, molecules, and supramolecular structures, and in the exploitation of unique properties and phenomena of matters with size ranging from 1 to 100 nanometers [1]. Polymer nanofibers, an important class of nanomaterials, have attracted increasing attentions in the last ten years. Within the connotation of nanotechnology and nanostructured materials, a nanofiber generally refers to a fiber having a diameter less than 100 nm. However, one also calls fibers with diameters less than 1000 nanometers (sub-micrometers) produced via certain ultra-fine fiber manufacturing technique such as electrospinning as nanofibers [2]. Fig. 1 shows an SEM image of electrospun nanofibers produced from a gelatin polymer [3].

Research on fabrication methods remains one of the most important topics for polymer nanofibers and has attracted interests from both academia and industry. Several fabrication techniques such as electrospinning [4, 5], melt-blown [6, 7], phase separation [8, 9], self-assembly [10–12], and template synthesis [13, 14] have been employed to produce suitable polymer nanofibers for different purposes. Amongst, electrospinning is the most popular and preferred technique to use. It is simple, cost-effective and able to produce continuous nanofibers of various materials from polymers to ceramics. In addition, electrospinning seems to be the only method which can be further developed for large scale production of continuous nanofibers for industrial applications. A general comparison of electrospinning with the other fabrication methods is presented in Table I.

As the diameter of polymer fibers shrinks from micrometers (e.g., 10–100 μm) to submicrometers or nanometers (e.g. 10–100 nm), a much larger specific surface area can result. Relationship of the surface area versus fiber fineness is shown in Fig. 2. This intrinsic feature makes polymer nanofibers attractive for many practical applications where high specific surface areas are required. Furthermore, it provides an opportunity to effectively modify the fiber surface with specific functions such as enhanced aqueous solubility, biocompatibility, and bio-recognition. The percentage of polymer molecular chains (or functional groups) which are exposed on the fiber surface can be roughly estimated as $100\,\pi d/D$, where d and D represent the diameters of a polymer chain and the fiber, respectively. This means that reducing the fiber diameter can proportionally increase the ratio of the exposed polymer chains together with its functional groups. As an example, if a polymer molecule chain has a diameter of about 0.3 nm, a microfiber of 30 μm in diameter will have an exposed molecular chain percentage of about 0.003% only,

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TABLE I Comparison of various processing methods for producing polymer nanofiber or nanofibrous structure

Processing methods	Descriptions	Fiber dimensions	Features	References
• Electrospinning	To produce nanofibers by electrically charging a polymer solution or melt. The simplest setup consists of only a syringe or pipette to hold polymer solution, two electrodes and a DC high voltage power generator.	Diameter: 3 nm to several micrometers; Length: continuous	– a 'top-down' approach; – simple and cost effective – can be applied to many materials; – fibers produced are continuous and typically distributed randomly; – applicable for industrial production.	[4, 5]
• Melt-blown	Based on traditional melt blowing fiber spinning technology, but using smaller orifices (with diameter as small as few tens of micrometers) made by microfabrication techniques and in combination with high velocity streams of heated air gas applied on extruded molten polymers.	Diameter: 150 to 1000 nm; Length: continuous	– a 'top-down' approach; – fiber size is dependent on the orifice size of extrusion mould, hence, difficult to get fiber diameter smaller than 100 nm; – still under development.	[6, 7]
• Phase separation	Typically consisting of five steps, i.e., raw material dissolution, phase separation and gelation, solvent extraction, freezing, and freeze-drying.	Diameter: 50 to 500 nm; Length: few micrometers	– resulting in nanofibrous foam directly after the freeze-drying; – relatively long time to obtain a batch of nanofibrous foam; – applicable to certain special (able to gel) polymers such as PLLA and its blend.	[8, 9]
• Self-assembly	A process whereby atoms, molecules and molecular aggregates organize and arrange themselves through weak and non-covalent forces such as hydrogen bonding, electrostatic interactions, and hydrophobic forces into stable and structurally well-defined functional entities at the meso- and nanoscale dimensions.	Diameter: well below 100 nm; Length: up to few micrometers.	– a 'bottom-up' approach; – self-assembled materials through purposeful manipulation potentially offering novel property and functionality that cannot be achieved by conventional organic synthesis; – longer preparation time in certain circumstances.	[10–12, 15, 16]
• Template synthesis	To use nanoporous membranes available commercially as template to synthesize or extrude nanoscale fibers.	Diameter: a few to hundreds nm; Length: micrometers	– nanoscale tubules and fibrils from polymers, metals, carbons, etc available; – resultant fiber diameters are monodispersed.	[13, 14]

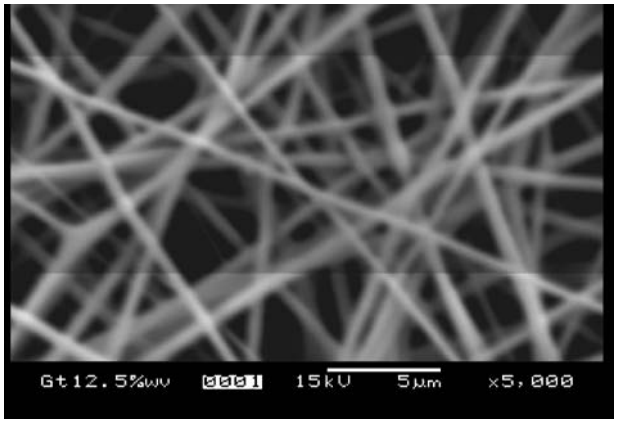


Figure 1 A typical SEM photograph of electrospun gelatin nanofibers.

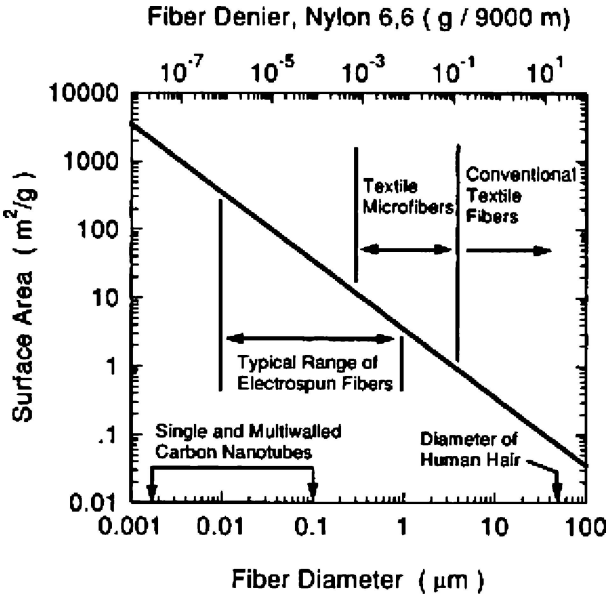


Figure 2 Relationship of fiber surface area versus diameter [86].

this percentage can be 3% for a nanofiber with diameter of 30 nm. Some other properties such as wetting behavior [14, 17] are also related to fiber surface. Polymer nanofibrous structures usually provide superior hydrophobic property because an effective contact angle increases with decrease in fiber diameter.

The use of polymer nanofibers for biomedical and biotechnological applications has some intrinsic advantages [18]. From a biological point of view, a great variety of natural biomaterials are deposited in fibrous forms or structures. Examples include silk, keratin, collagen, viral spike proteins, tubulin and actin, polysaccharide cellulose and chitin. All of these are characterized by well organized hierarchical fibrous structures down to a nanometer scale. With this understanding, polymer nanofibers can provide a proper route to emulate or duplicate biosystems—a biomimetic approach. On the other hand, many researches [19–22] have shown evidences that apart from surface chemistry, the nanometer scale surface features and topography also have important effect on regulating cell behavior in terms of cell adhesion, activation, proliferation, alignment and orientation. This is because cells live in a nano- or micro-featured environment, such as the 66 nm

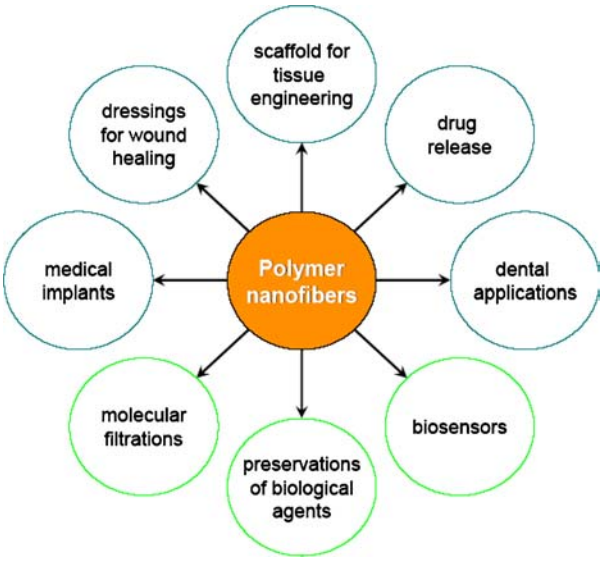


Figure 3 Use of polymer nanofibers for biomedical and biotechnological applications.

banding on collagen fibers with which many cells live [21]. Cells attach and organize well around fibers with diameters smaller than those of the cells [23]. Cells can react to objects as small as 5 nm, which are some 1,000–5,000 times smaller than the sizes of themselves [21].

In this paper, an overview is given on the recent uses of polymer nanofibers for biomedical and biotechnological applications. These include tissue engineering, controlled drug release, dressings for wound healing, medical implants, nanocomposites for dental applications, molecular separation, biosensors and preservation of bioactive agents (Fig. 3).

2. Biomedical and biotechnological applications of nanofibers

2.1. Biomedical applications

2.1.1. Scaffolds for tissue engineering

Biodegradable scaffold is generally recognized as an indispensable element in engineering living tissues. They are used as temporary templates for cell seeding, invasion, proliferation and differentiation prior to the regeneration of biologically functional tissue or natural extracellular matrix (ECM). To better engineer an artificial tissue and to fulfill the desired biological functions, morphological similarity with the native tissue is important. Fibrous scaffolds with fiber diameters down to nanometers are suitable for replicating the physical structure of natural ECM. For example, researchers at the University of Delaware created biomimetic webs of nanofibers (5–25 nm) from natural biopolymers such as collagen spider silk and denatured collagen (Fig. 4) using the electrospinning method [24]. Apart from being able to mimic the structures of natural tissues, the nanoscale size of the biodegradable fibers may also offer an advantage in terms of inducing a desired degradation rate. It has been reported that microcale fibers can affect the degradation feature and related mechanical properties of the bulk material from which the fibers were made [25]. This would be applicable to nanofibers

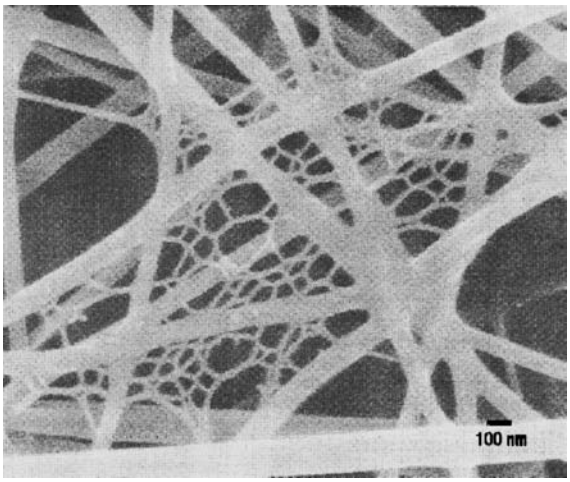


Figure 4 Denatured collagen nanoweb: the fibers of the electrospun membrane are 100–150 nm in diameter and the fibrils, which make up the nanoweb, are 5–15 nm in diameter [24].

either, although a similar report seemed to have not been found in the literature.

Polymer nanofibers have been considered for use as scaffolds for engineering tissues such as cartilages [26–28], bones [29], arterial blood vessels [30–33], heart [34], nerves [9, 35], etc. To prepare these scaffolds, electrospinning has now been the most extensively used fabrication method. Advantages of using electrospinning to prepare tissue scaffolds are as follows: (1) it is capable of producing ultra-thin fibers with diameters ranging from several micrometers down to a few nanometers, which are able to mimic the structure of ECM; and (2) it is versatile in a sense that various monopolymers, blends of polymers, and compositions of polymers with other materials or additives such as inorganics, growth factors, other cell regulatory biomolecules, and even living cells [36] can be used to develop functionally active nanofibrous structures. The scaffolds thus produced provide a highly porous microstructure with interconnected pores and extremely large surface area to volume ratio which is conducive to tissue growth. A summary of various tissue scaffolds electrospun from different biodegradable polymers is given in Table II. Some interesting and representative results of using polymer nanofibers to engineer different tissues are briefly highlighted in the following examples.

The nanofibrous scaffolds for engineering cartilage tissues have been explored by several research groups [26–28, 38, 39]. Favorable biological responses of seeded cells such as enhanced cell attachment and *in vitro* proliferation were demonstrated. Recognizing the importance of electrical and mechanical properties for cartilage reconstruction, conductive nanofibrous scaffolds were fabricated by electrospinning of biodegradable poly(lactic acid) (PLA) mixed with single wall carbon nanotubes (SWNT) [38]. An *in vitro* test showed that the SWNT incorporated nanofiber scaffold still allows cells to grow with no hostile influence on cell proliferation.

In Vacanti's group, mesenchymal stem cells derived from bone marrow of neonatal rats were cultured onto poly(ε-caprolactone) (PCL) nanofibrous scaffolds prepared with electrospinning [29]. Scanning electron microscopy and histological and immunohistochemical examinations showed penetration of cells into the cell-polymer structures and exhibited formation of multilayer cells on the surface of the structures at different culturing periods of up to four weeks. Mineralization and presence of type I collagen were also observed within the period of four weeks.

Fibers produced by electrospinning are mostly in the form of a non-woven structure. It is generally difficult to obtain nanofiber alignment similar to that achieved by conventional textile fibers. Nevertheless, there have been a few attempts to produce grossly aligned nanofibers [5]. In tissue engineering, distribution and arrangement of the extracellular matrix plays a critical role in controlling cell shape, regulating physiological function, and defining organ architecture. For prosthetic vascular grafts, directional bias of fibers with respect to the tubular axis is required to mimetically obtain anisotropic vascular grafts so as to improve burst strength. Very recently, aligned poly(L-lactide-co-caprolactone) (PLLA-CL) copolymer nanofibrous scaffold towards engineering blood vessel application has been successfully produced in our laboratory [32], using a technique proposed by Theron *et al.* [44]. Cell culture results of smooth muscle cells (SMCs) on the nanofibrous scaffolds indicated that SMCs attached and migrated along the direction of aligned nanofibers, and expressed themselves as a spindle-like contractile phenotype. The distribution and organization of smooth cytoskeleton protein inside SMCs were parallel to the direction of nanofibers (Fig. 5). Furthermore, the adhesion and proliferation rate of SMCs on the aligned nanofibrous scaffold were significantly improved as compared to using polymer films.

Biodegradable polymer nanofibers of PLA, PLGA, and PEG-PLA were attempted for use in heart or cardiac tissue constructs [34]. Cultured for seven days on the nanofibrous scaffolds, cardiac myocytes (CMs) were found to remodel an electrospun matrix by pulling on fibers and crawling into the scaffolds to form dense multilayers. Interestingly, it was also reported that the CMs seemed to prefer growing on a relatively hydrophobic surface (or polymers).

Recently, poly (L-lactic acid) (PLLA) polymer nanofibers were used as a scaffold onto which nerve stem cells (NSCs) were cultured in our group [9]. The nanofibers were produced following a liquid-liquid phase separation method [8]. Our preliminary results revealed that NSCs could differentiate on the scaffold and the scaffold acted as a positive cue to support neurite outgrowth. Very recently, neural progenitor cells were encapsulated *in vitro* within a three-dimensional network of epitope containing peptide amphiphile nanofibers prepared by self-assembly [35]. Due to the presence of neurite-promoting pentapetide epitope isolucine-lysine-valine-alanine-valine (IKVAV), it was found that the artificial nanofibers induced very rapid differentiation of cells into neurons and, at the same time, discouraged the development of astrocytes. This selective differentiation phenomenon is interesting.

TABLE II Polymer nanofibers for tissue engineering applications

Scaffold material	Fabrication method	Fiber diameter	Cultured cells	Potential applications in tissue engineering	References
PLGA	Electrospinning	500–800 nm	Fibroblasts, bone-marrow-derived mesenchymal stem cells (MSCs)	Skin, cartilage	[27]
Collagen	Electrospinning	100–730 nm	Aortic smooth muscle cells	/	[37]
PCL	Electrospinning	~700 nm	Fetal bovine chondrocytes (FBCs)	Cartilage	[28]
PCL	Electrospinning	400 nm ± 200 nm	Mesenchymal stem cells (MSCs)	Bone	[29]
SWNT/PLA	Electrospinning	/	Chondrocytes	Bone, cartilage	[38]
PLLA, PLGA, PEG-PLA	Electrospinning	~1000 nm	Primary cardiac myocytes (CMs)	Heart	[34]
Collagen Type II	Electrospinning	110 nm–1.8 μm	Chondrocytes	Cartilage	[39]
DNA in PLGA, PEG-PLA	Electrospinning	250 nm −5 μm	Preosteoblastic cell line MC3T3-E1	Skin, cartilage	[40]
B. mori Silk/PEO	Electrospinning	700 ± 50 nm	human bone marrow stem cells (hBMSCs)	/	[41]
P(LLA-CL)	Electrospinning	~500 nm	Human coronary artery smooth muscle cells (SMCs)	Blood vessel	[32]
P(LLA-CL)	Electrospinning	500 nm–1.5 μm	Endothelial cells (ECs) and SMCs	Blood vessel	[33]
Silk fibroin	Electrospinning	30–120 nm	Human keratinocytes and fibroblasts	Skin	[42]
PLLA	Phase separation	50–350 nm	Neonatal mouse cerebellum C17-2 stem cells	Nerve	[9]
IKVAV-PA	Self-assembly	5–8 nm	Murine neural progenitor cells (NPCs)	Nerve	[35]
Gelatin/PCL	Electrospinning	/	Bone marrow stromal cells (BMSCs)	/	[43]

Nomenclatures: PLGA: Poly(D,L-lactide-co-glycolide); PCL: Poly(ε-caprolactone); SWNT/PLA: Single wall carbon nanotubes/poly(lactic acid) biocomposite; PEG-PLA: Poly(ethylene glycol)-poly(D,L-lactide) diblock copolymer; PEO: Poly(ethylene oxide); P(LLA-CL): Poly(L-lactid-co-ε-caprolactone); IKVAV-PA: peptide-amphiphile (PA) containing epitope isolucine-lysine-valine-alanine-valine (IKVAV).

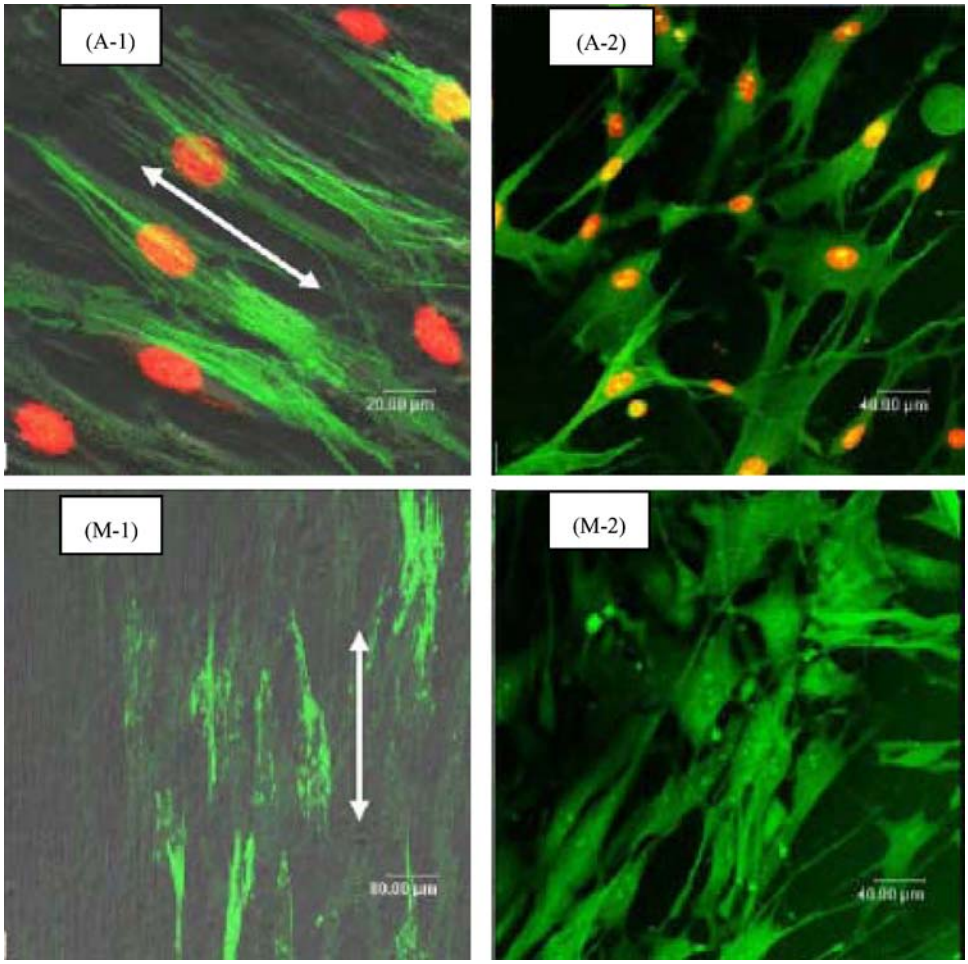


Figure 5 Laser scanning confocal microscope images of immunostained α-actin filaments (A-1, A-2) and myosin filaments (M-1, M-2) in smooth muscle cells on aligned nanofibrous scaffold (A-1, M-1) and tissue culture polystyrene (A-2, M-2) after one day of cell culture [32].

This work suggested that: (1) employing structured polymer nanofibers has an important advantage of being able to physically biomimic the natural ECM for tissue engineering applications; (2) surface functionalization to make the nanofibers bioactive is critical; and (3) cell ingrowth and cell encapsulation in nanofibrous scaffolds are equally important.

Tissue regeneration involves complex sequences of events with cells differentially expressing a vast number of genes [45]. A pioneer work employing a composite nanofibrous scaffold for therapeutic application in gene delivery was recently reported by Luu *et al.* [40]. Such a scaffold was electrospun by incorporating plasmid DNA into synthetic biodegradable polymers of PLGA and PLA-PEG. Their results indicated that the DNA released from the scaffold was not only intact, but also capable of cellular transfection, and had even successfully encoded a protein β-galactosidase.

The architecture of a scaffold and the material used play an important role in modulating tissue growth and response behavior of the cells which have been cultured onto the scaffold. In this regard, the scaffold should not only work as a substrate for cell attachment, growth and proliferation, but also facilitate cell migration, ingrowth and assembly into a stereo-structure. This is because the success in tissue engineering is dependent on the ability to assemble cultured cells into a three-dimensional structure. For example in a clinical application, especially in regeneration of some specific tissue defects, sufficient cell penetration is one of the key requirements [29]. As demonstrated in Silva's work, a nanofibrous artificial extracellular matrix not only provides a mechanical support for cells, but also serves as a medium through which diffusion of soluble factors and migration of cells can occur [35]. Nanofiber membranes are very porous, but the "pores" (it is less appropriate to use the term "pore size" to quantify the porous feature of a nanofibrous structure) formed in the electrospun fibrous structure are much smaller than the size of a living cell which is a few to tens of micrometers in diameter. These small "pores" would inhibit cell migrations. This inhibition phenomenon is similar to that of filtration by nanosized fibers in a filter [46, 47]. To make cell "infiltrate" into such a nanofibrous scaffold, surface functionalization to improve biocompatibility and modify mechanical properties of the scaffold is needed. It has been demonstrated that the strength and deformability of nanofibers do influence *in vitro* migration and morphology of some cells [48]. For this reason, we have recently developed a Gelatin/PCL composite nanofibrous scaffold using the electrospinning technique [43]. The scaffold was later cultured with bone marrow stromal cells. Our results indicated that the cells could not only attach and grow well on the scaffold, but also penetrate into the scaffold up to a depth of 114 μm (Fig. 6). On the other hand, a penetration of only 48 μm in depth

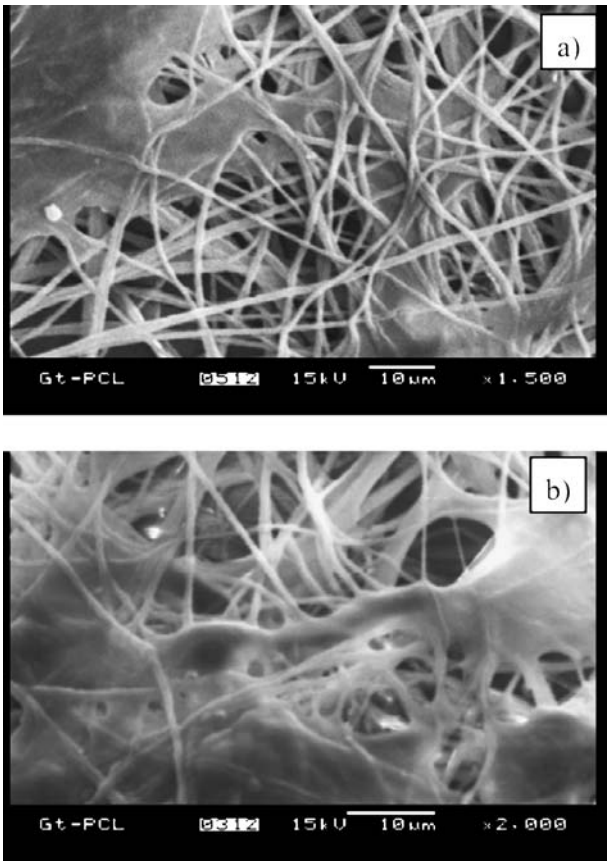


Figure 6 Interaction of bone marrow stromal cells with gelatin/PCL composite scaffolds after 7 days of cell culture: (a) cell ingrowth, and (b) layered cells [43].

was achieved using a pure PCL scaffold cultured with the same cells.

2.1.2. Controlled drug release

Delivery of drugs or pharmaceutical agents to patients in a most physiologically acceptable manner has always been an important concern. New technologies and materials will have a profound impact on drug delivery. Drug delivery with polymer nanofibers is based on the principle that dissolution rate of a drug particulate increases with increased surface area of both the drug and the corresponding carrier if necessary. For controlled drug delivery, in addition to their large surface area to volume ratio, polymer nanofibers also have other additional advantages. For example, unlike common encapsulation involving some complicated preparation process, therapeutic compounds can be conveniently incorporated into the carrier polymers using electrospinning. The resulting nanofibrous membrane containing drugs can be applied topically for skin and wound healing, or post-processed for other kinds of drug release. However thus far, the study on drugs encapsulated by polymer nanofibers is still limited.

Ignatious [49] attempted making nanofibrous polymer carriers by electrospinning for pharmaceutical application. The release of pharmaceutical dosage can be designed as rapid, immediate, delayed, or modified dissolution depending on the polymer carrier used. In addition, delivery of a model drug such as tetracycline hydrochloride from nanofibrous membrane, made by electrospining a blend of poly(ethylene-co-vinylacetate), poly(lactic acid) and the drug, was reported by researchers at the Virginia Commonwealth University [50]. It was found that the electrospun nanofibrous mats gave relatively smooth release of drug over a period of five days. In a different report [51], bioabsorbable nanofiber membranes of poly(lactic acid) was used for loading an antibiotic drug Mefoxin. The efficiency of this nanofiber membrane compared to bulk film was demonstrated. For potential use in topical drug administration and wound healing, poorly water-soluble drugs loaded in water-soluble and water-insoluble nanofibrous polymer carriers were investigated [52, 53]. It was shown that drug loaded polymer nanofibers by electrospinning were able to make the drugs dispersed in an amorphous state which would facilitate the drug dissolution.

All of the aforementioned reports adopted the route of simply mixing drugs and carrier polymers before electrospinning. As drugs and carriers are mixed together to generate nanofibers, depending on interactions of the drugs-polymer carriers, likely interaction modes of the drugs in the resulting nano-structured products are as follows: (1) drugs as tiny particles are merely attached onto the surface of the nanofiber carriers; (2) both the drugs and carriers are electrospinnable, resulting in two kinds of nanofibers interlaced together; and (3) a blend of the drugs and carriers is integrated into one kind of composite nanofibers in different forms, e.g., one (drugs) wrapped with another (carriers), or mixed and/or entangled evenly at molecular level because of good compatibility of both. Modes (1) and (2) tend to give rise to a problem of burst release in the initial stage, and therefore mode (3) is preferred. Recently, it was reported [54] that drugs such as rifampin (a drug for tuberculosis) and paclitaxel (an anti-cancer drug) introduced into poly(L-lactic acid) (PLLA) not only improved electrospun fiber quality (e.g., reduced diameter, enhanced surface uniformity), but also led to capsulation of the drugs within the PLLA nanofibers. With the presence of proteinase *K* (a degradation enzyme), the drug release behavior (see Fig. 7) was that of nearly zero-order kinetics without burst release. Such a result was attributed mainly to the degradation of PLLA in the presence of proteinase *K* but not to the diffusion or permeation of drug through the PLLA carrier [54]. However, a longer period of time, such as tens of hours of observation on the performance of the controlled drug release was not reported. From this work, one can conclude that the drug-polymer interaction mode is important in determining the drug release behavior. The resulting nanofibrous drug-carrier complex is closely associated with the solubility and compatibility of the drug in the blend solution as well as processing conditions used in electrospinning.

Rather than using the approaches mentioned above, another way to develop drug loaded polymer nanofibers for controlled drug release is by using coaxial electrospinning [55] (Fig. 8). Two or more components can be coaxially electrospun through different capillary channels and are integrated into a core-shell structured

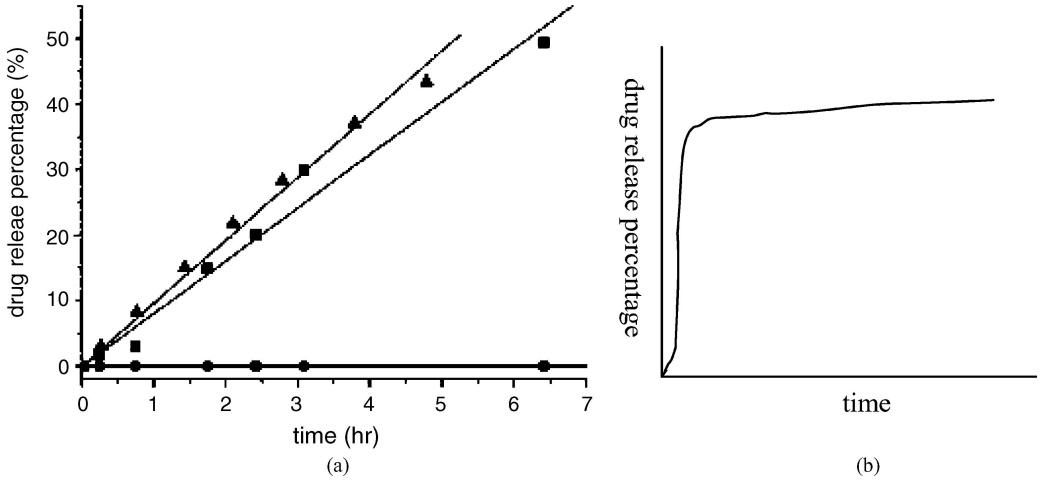


Figure 7 (a) Release percentage of rifampin from electrospun fibers vs. time. (●) No proteinase *K* was added, 15 wt.% rifampin; (■) proteinase *K* concentration 3 μg/ml, 15 wt.% rifampin; (▲) proteinase *K* concentration 3 μg/ml, 25 wt.% rifampin [54]; (b) Schematic of typical drug burst release profile of electrospun drug-loaded polymer nanofibers.

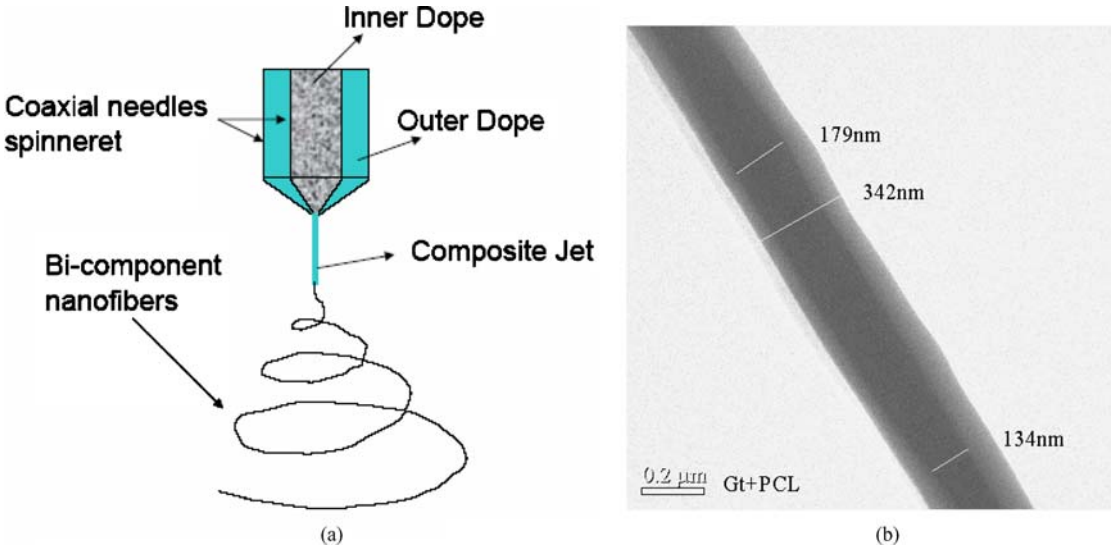


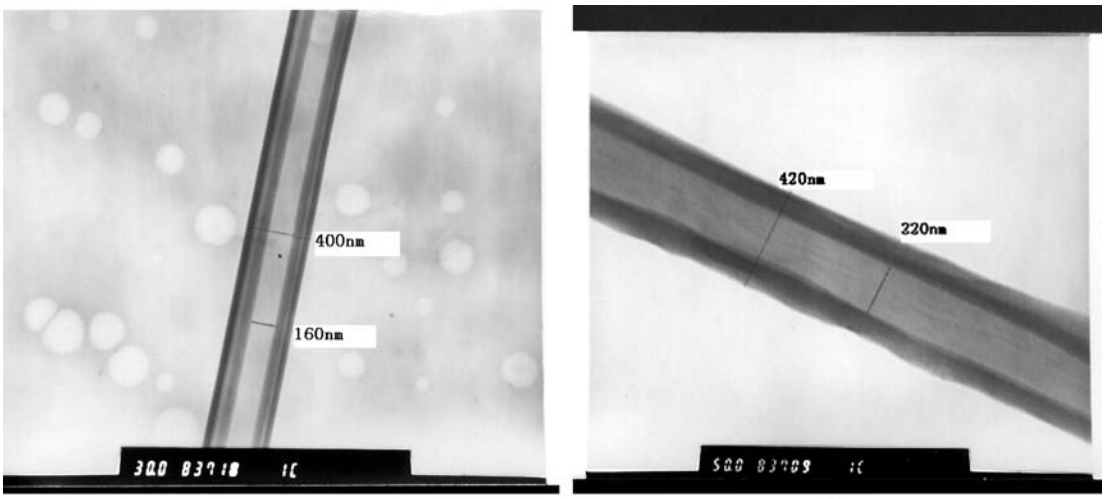
Figure 8 (a) Schematics of coaxial electrospinning, and (b) TEM image of a core-shell structured composite nanofiber with gelatin wrapped by poly(ε-caprolactone) produced in our research group.

composite fiber. With this, drugs or biological active molecules can be released through the skin of the bi-component nanofiber if the carrier polymer is permeable to the drugs wrapped, or can be released over a certain period of time while a biological degradation of the carrier polymer is taking place. Another advantage of the coaxially electrospun nanofibers is that it provides temporal protection for certain bioactive substances such as growth factors which need to be protected for a certain period of time prior to playing their role in the early stage of wound healing. Very recently, we have successfully encapsulated two kinds of medically pure drugs into the cores of bioabsorbable PCL (polycaprolactone) polymer nanofibers through co-axial electrospinning [56] (Fig. 9). One of the drugs used was Gentamycin Sulfate, an antibiotic which can inhibit or kill bacteria and is water-soluble, and another is Resveratrol, a natural antioxidant found in a wide variety of plants dissolvable in alcohol and can be used to keep blood vessels open and pliable as well as to prevent blood platelets from aggregation or clumping together. No other carrying agent, such as a high molecular weight polymer, except for the proper solvents was mixed with the drugs in making the cores. It is noted that the pure drug solutions alone cannot be formed into a fiber.

2.1.3. Dressings for wound healing

Driven by some major factors such as an increasing aging population with more chronic wounds and unexpected sufferings of civilians from terrorist attacks, warfare conflicts, and frequent casualties from traffic accidents, there is an increasing demand for advanced wound care products. In this regard, new technology will dramatically accelerate the development of innovative dressing materials for wound healing.

An ideal dressing is one that can provide an environment at the surface of the wound in which healing can take place at the maximum rate consistent with the reproduction of the healing wound with an acceptable cosmetic appearance [57]. Modern dressings are developed to serve the purpose of facilitating wound healing apart from the basic function of covering wounds from further infection. It has been recognized that ideal dressings should have the characteristics of (1) haemostatic,



(a) (b)

Figure 9 TEM images of core-shell composite nanofiber segments with 7 wt% PCL as shell and (a) 10 wt% Resveratrol and (b) 30 wt% Gentamycin Sulfate as cores.

(2) efficiency as bacterial barrier, (3) absorption of excess exudates (wound fluid/pus), (4) provision and maintenance of a moist environment, or appropriate water vapor transmission rate, and provision of adequate gaseous exchange, (5) ability to conform to the contour of the wound area, (6) functional adhesion, i.e., adherent to healthy tissue but non-adherent to wound tissue, (7) painless to patient and ease of removal, and (8) low cost.

Current efforts using polymer nanofibrous membranes as medical dressings are still in its early stage [36, 42, 58–63]. Bowlin's research group [60–62] attempted to generate fibrinogen nanofiber mats for potential use as a wound dressing or haemostatic bandage. Fibrinogen, which is in blood plasma and plays a key role in wound healing, was dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) and electrospun into nanofibers of diameters ranging from 80 to 700 nm. As regular cotton gauze tourniquets are not so efficient for the case of hemorrhagic shock which remains a problem in very healthy individuals [64], a BioHemostat dressing based on electrospinning is considered and under development [62]. Very recently, cytocompatibility and cell behavior of normal human keratinocytes and fibroblasts cultured onto silk fibroin nanofibrous membranes prepared from electrospinning were reported [42]. The nanofibers used had an averaged diameter of 80 nm. In another report, the performance of electrospun nanofibrous polyurethane membranes as dressings with fiber diameters ranging from 250 to 300 nm was examined *in vivo* using a pig model [63]. All of these efforts are based on a particular nanofiber manufacturing technique—electrospinning. The benefits of using electrospun nanofibrous mat for wound dressing applications are as follows:

(1) *Hemostasis*. Nanoscale fibers that impart the dressing with small interstices and high effective surface area can promote hemostasis [60, 65]. Such function of hemostasis is activated from the physical feature of the nanofibrous dressings without using a haemostatic agent.

(2) *Absorptibility.* Due to high surface area to volume ratio, nanofibers of the same polymers have exhibited water absorption of between 17.9 to 213% whereas typical film dressings only demonstrated water absorption of 2.3% [36]. Thus, if hydrophilic polymers are employed, the dressings will be able to absorb wound exudates more efficiently than film dressings.

(3) *Semi-permeability.* The nanofiber structured dressing is porous which is good for the respiration of cells and does not lead to wound dessication [63]. This indicates a proper control of a moist environment for the wound. In the meantime, the small pore size can effectively protect the wound from bacteria infection. Electrospun nanofibrous membrane wound dressings can also meet the requirement of high gas permeation apart from providing effective protection of wound from infection and dehydration [63].

(4) *Conformability.* Conformability or the ability to conform to the contour of the wound is one of the parameters that needs to be clinically assessed for the suppleness and resiliency of the medical dressings. In textile industry, it is widely recognized that the conformability of a fabric is closely related to the fiber fineness. Finer fiber fabrics are easier to fit to complicated 3-D contours. Therefore, dressing materials made of ultrafine fibers can provide excellent conformability and thus result in better coverage and protection of the wounds from infection.

(5) *Functionability*. Polymer nanofibrous membranes, which can be made bioactive via the electrospinning process, can enhance their efficacy in applications. Such multi-functional bioactive nanofibrous dressings are achievable because of the ease in incorporating therapeutical compounds into the nanofibers via the electrospinning process. The previous section has illustrated the use of polymer nanofibers for controlled drug release in topical application. Depending on the stage of treatment and the intended functionality of the drugs, active components including pharmaceutical compounds such as antiseptics, antifungals, vasodilators (e.g. minoxidil used to promote wound epithelialization and neovascularization), growth factors

(e.g. fibroblast growth factor (FGF), epithelia growth factor (EGF), and transforming growth factor (TGF)), and even cells (e.g. keratinocytes [36]) can be integrated into the same nanofibrous substrate. Another advantage of using electrospinning is that, unlike commercial dressings using multi-layer configuration to attain desired objectives, different functions desired such as medication, growth factors, and so forth can be achieved by electrospinning various functional materials into one blended layer to achieve an all-in-one wound dressing [36]. This brings an extra benefit of reduced frequency in changing dressings which may disturb the regeneration of neo-tissue.

(6) *Scar-free.* Ultimately, nanofibers also hold a promise of healing wounds without leaving scars. Although this is hard to achieve, researchers and clinicians nevertheless seek to heal a wound with little scar as possible. For example, Coffee, an Oxford University's biochemist and the president of an Oxford-based biotech company, used electrospinning technique to make fibrous dressings on a wound which he believed would encourage normal skin to grow immediately instead of scarring because the biodegradable fibrous scaffolds would give skin cells a better road map for self-repair [66]. From a tissue-engineering point of view, biomimically adopting nanofibrous structure has good cell conductivity and can improve blood and other tissue fluid compatibility, which will facilitate wound healing and skin regeneration.

As an extension of wound dressing, polymer nanofibrous structures can also be employed in skincare as disclosed in a recent patent [67]. Due to the very small interstice and high surface area of the nanofibrous skin care mask, it is believed that far greater utilization and fast transferring rate of additives to the skin will be facilitated.

2.1.4. Medical implants

Since the early 80's, electrospun polymer nanofibers had already been proposed for vascular and breast prostheses applications. A number of US patents were issued on fabrication methods and techniques for these prostheses. US Patents covering vascular prostheses include 4044404, 4552707, 4689186, 4878908, 4965110, and 5866217 [68–73]. Breast prosthesis was disclosed in a US patent 5376117 [74]. Recently, polymer nanofibers have been used in medical prostheses of other forms. For example, in the University of Michigan [75–78], electrospun submicron protein fibers were deposited as a thin porous film onto a prosthetic device which was designed to implant into the central nervous system. This coating film with gradient fibrous structure works as an interphase between the neural system and the prosthetic device, and is expected to efficiently reduce the stiffness mismatch at the tissue/device interphase and hence prevent device failure after implantation. On the other hand, as abdominal procedure is routinely practiced, adhesion has become a common cause of complication after the operation, which includes small bowel obstruction, female infertility, chronic debilitating pain, and difficulty in future operation [79]. Pertaining to this, Zong *et al.*. [80] examined the effect of using electrospun non-woven bioabsorbable poly(lactide-co-glycolide) (PLGA) impregnated with antibiotics (Mefoxin®) as an anti-adhesion membrane based on an *in vivo* rat model. They concluded that the membrane was effective in reducing post-surgery adhesion and could act as a physical barrier as well as a local drug delivery vehicle. Therefore, the combined feature of composition adjustment, drug loading capability and easy handling ability made these nanofibrous membranes ideal candidates for future clinical evaluations.

2.1.5. Nanocomposite for dental application

Polymer nanofibers can be used as reinforcement in dental composite applications. A dental restorative composite is generally made of some dental resin such as 2,2'-bis-[4-(methacryloxypropoxy)-phenyl]- propane (bis-GMA) and tri-ethylene glycol dimethacrylate (TEGDMA) and certain amount (up to 70 wt%) of fillers (e.g. silica or ceramic particles). In practice, it has been found that the fillers which are initially used for the purpose of improving mechanical properties, can eventually play a side-effect in accelerating the damage of the dental composite due to the stress concentration resulted from the introduction of the fillers. Investigation on the composites used for a longer time period was considerably less optimistic [81]. To overcome this problem, Fong [81] incorporated the electrospun Nylon 6 nanofibers prepared from 10 wt% 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) with a diameter less than one micron and a uniform cylindrical shape was introduced into the bis-GMA/TEGDMA resin. Three-point bending test of this modified dental composite indicated that flexural strength, elastic modulus and work of fracture were distinguishably increased after embedding relatively small amount of Nylon 6 nanofibers into the resin. Unfortunately, mass fraction of Nylon 6 nanofibers added beyond 5% did not improve the mechanical properties of the dental composite significantly.

The use of polymer nanofibers as reinforcement for engineering composites have so far provided only marginal enhancement in terms of strength and stiffness properties. Nevertheless, limited research work along this direction has indicated that polymer nanofibers are effective for improving fracture toughness of the composites [82, 83]. Fracture toughness is one of the important considerations in developing polymer composite dental devices such as an orthodontic bracket [84]. An additional merit of using nanofibers as reinforcement is that no adverse effect on the transparency of the developed composite dental device can result when the fiber diameter becomes smaller than the wavelength of a visible light [85]. These advantages pose polymer nanofibers as very promising candidates for the future development of orthodontic composite devices that are lightweight, have optimal mechanical property and possess desired aesthetic feature.

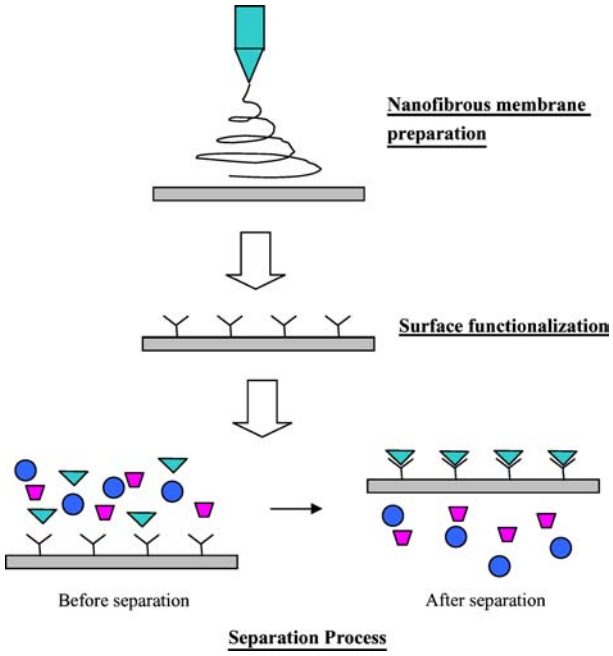


Figure 10 Polymer nanofibers for targeted molecular separation.

2.2. Biotechnological applications

2.2.1. Molecular separation

Recognizing their high filtration efficiency, polymer nanofibers have been used as industrial filters, and as a potential candidate for protective clothing against biochemical attacks [2, 46, 47, 86, 87]. Likewise, electrospun polymer nanofibers with functionalized surface can be extended for use for high efficient biomolecular or protein separation as schematically shown in Fig. 10. The high surface area to weight ratio makes nanofibers an ideal substrate for molecular separation. The principle of separation is similar to that of affinity chromatography. This involves utilizing a specific interaction between one kind of solute molecule and a second molecule or functional group that is immobilized on the nanofibrous membrane (stationary phase). For example, the immobilized molecule may be an antibody to some specific protein. When the solute containing a mixture of proteins is passed by the antibody, only the specific protein is reacted and is bound to the stationary phase. To fulfill this molecular separation, proper surface functionalization of the nanofibrous membrane is important. Structural and material properties of the nanofibrous membranes are also important so that the membranes can withstand the imposed forces acting on them during the filtration process. At present, polymer nanofibrous membrane for molecular separation is still a concept that needs to be realized.

2.2.2. Biosensor

Biosensors, which typically consist of biofunctional membrane and transducer (Fig. 11), have been widely used for environmental, food, and clinical purposes. Parameters affecting the performance of a sensor generally include sensitivity, selectivity, response time, reproducibility, and aging, all of which are dependent directly on the property of the sensing membrane used. Among these, sensitivity is particularly important because there is a strong need for detection of gases and biological substances at low concentration. Improve the sensitivity will require using sensor films with larger surface area to unit mass ratio [88]. This provides an opportunity for polymer nanofibers to be used as biosensors.

Kwoun *et al.* pioneered the work of developing chemical and biochemical sensors by making use of a poly(lactic acid co glycolic acid) (PLAGA) nanofiber film as a new sensing interface [89, 90]. Researchers at the University of Massachusetts Lowell [91–93] have also investigated the use of electrospun polymer nanofibers for sensor application. They demonstrated that sensors made from electrospun nanofiber (100–400 nm in diameter) membranes containing fluorescent poly(acrylic acid)-poly(pyrene methanol) (PAA-PM) for detecting metal ions (Fe^{3+} and Hg^{2+}) and 2,4-dinitrotulene (DNT) exhibited a sensitivity of almost three orders of magnitude higher than that of thin films of the same material. A sensing material of hydrolyzed poly(2-(3-thienyl) ethanol butoxy carbonylmethyl urethane) (H-PURET) was recently assembled onto the surface of electrospun nanofibrous cellulose acetate membrane [92]. High sensitivity in detecting extremely low concentration (ppb) of methyl viologen (MV^{2+}) and cytochrome c (cyt c) was reported. It is believed that the high surface area to volume ratio of the electrospun nanofibrous membrane and efficient interaction between sensing material and detected substance are responsible for this significant improvement.

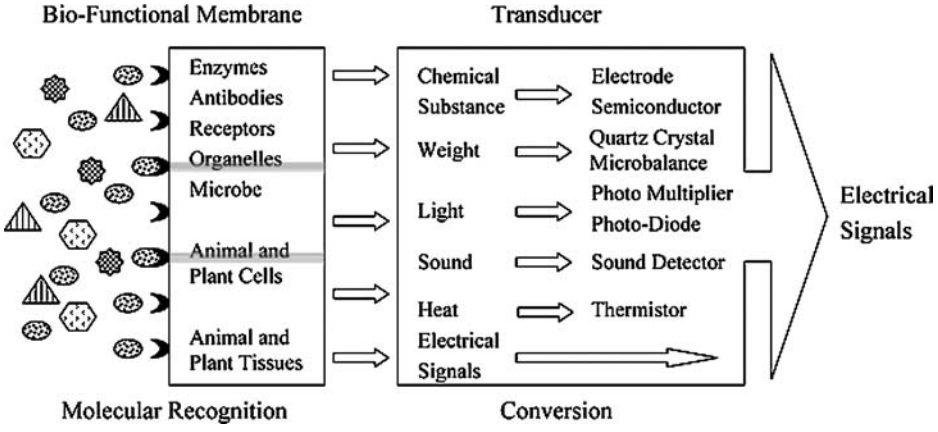


Figure 11 The principles of a biosensor [94].

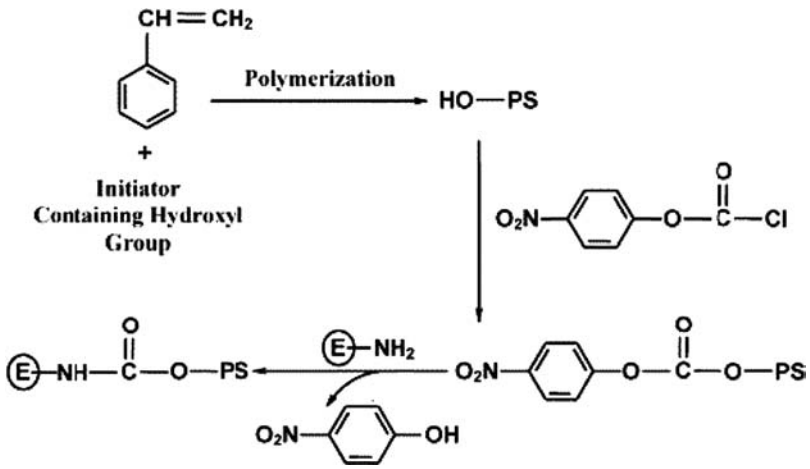


Figure 12 Chemical route for synthesis of functionalized polystyrene and subsequent attachment of enzyme. Nanofibers were prepared by electrospinning of functionalized PS, followed by immobilization of enzyme [95].

2.2.3. Preservation of active biological compounds

In therapeutic compound delivery, polymer nanofibers can also be used to preserve and immobilize a biologically active material (e.g., enzyme) [95, 96]. Fig. 12 outlines the scheme of achieving bioactive polystyrene nanofibers containing α-chymotrypsin (CT) enzyme used in ref. [95]. It was found that the nanofibrous CT possesses high activity in both aqueous and organic media. This is a significant finding because most immobilized enzymes studied previously showed a high activity in an aqueous or organic medium, but few reported having high activity in both [95]. In addition, it was reported that this nanofibrous enzymes can be easily recovered and have a longer half-life.

3. Summary

The past decade has seen considerable efforts in the use of polymer nanofibers for biomedical and biotechnological applications. These include tissue engineering, controlled drug release, wound dressings, medical implants, dental composites, molecular filtration, biosensors and preservation of bioactive agents. An overview of these applications has been presented here. Most of these applications are still being tested in laboratories worldwide with some at the infancy stage. Significant advancements are necessary before clinical usage or commercialization can be realized. Listed below are some of the points that deserve to be taken into account in the future research and development on polymer nanofibers.

The fabrication method of polymer nanofibers can determine the eventual application of these fibers. From this review, it is seen that electrospinning is a suitable method of fabricating polymer nanofibers for different purposes described in this article. However, most of the electrospun fibers obtained are synthetic. More attention should be given to natural biopolymers (e.g., chitin, alginate, etc) so that better biological compatibility and performance can be realized. Additional efforts should be made on controlling processing variables so as to obtain defect-free and uniform diameter nanofibers, as well as on improving throughput of the nanofibers. For effective applications of nanofibers, functionalizing the fiber surface or developing novel functional nanofibers are generally required. Finally, investigation on how to integrate the top-down physical assembly of the fibers with bottom-up chemical and biological assembly of drugs or chemical agents is needed to create fully functional nanostructures at both the meso- and nano- scales.

Effective applications also depend on how good is understanding for the property of used materials. Majority of the current investigations has made use of the advantage of high specific surface area of polymer nanofibers. Other size-dependent features of these materials in terms of mechanical, physical, chemical, and biological properties are needed to explore.

In order to advance the biotechnological and especially biomedical applications of polymer nanofibers from perspective to commercialized stages, collaborative interdisciplinary researches involving surgeons, material scientists, biologists, physiologists, clinicians, and engineers are required. It is believed that continual investments from academia, government, and industry into this field will not only shorten the distance between laboratory and practical utilization stages in any of the above reviewed areas but also open up other new range of opportunities for polymer nanofibers in biomedical and biotechnological applications.

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