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Applications of Polymer Nanofibers in Biomedicine and Biotechnology

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Abstract

Recent advancements in the electrospinning method enable the production of ultrafine solid and continuous fibers with diameters ranging from a few nanometers to a few hundred nanometers with controlled surface and internal molecular structures. A wide range of biodegradable biopolymers can be electrospun into mats with specific fiber arrangement and structural integrity. Through secondary processing, the nanofiber surface can be functionalized to display specific biochemical characteristics. It is hypothesized that the large surface area of nanofibers with specific surface chemistry facilitates attachment of cells and control of their cellular functions. These features of nanofiber mats are morphologically and chemically similar to the extracellular matrix of natural tissue, which is characterized by a wide range of pore diameter distribution, high porosity, effective mechanical properties, and specific biochemical properties. The current emphasis of research is on exploiting such properties and focusing on determining appropriate conditions for electrospinning various polymers and biopolymers for eventual applications including multifunctional membranes, biomedical structural elements (scaffolds used in tissue engineering, wound dressing, drug delivery, artificial organs, vascular grafts), protective shields in specialty fabrics, and filter media for submicron particles in the separation industry. This has resulted in the recent applications for polymer nanofibers in the field of biomedicine and biotechnology.

Index Entries: Electrospinning; nanofibers; tissue engineering; biotechnology; scaffolds.

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Introduction

Nanofibers are biocompatible and biodegradable and are used for the replacement of structurally or physiologically deficient tissues and organs in humans. The use of nanofibers in tissue restoration is expected to result in an efficient, compact organ and a rapid recovery process owing to the large surface area offered by nanofibers made from polymer and protein used for wound healing; the epithelialization of implants and the construction of biocompatible prostheses, cosmetics, face masks, bone substitutes, artificial blood vessels, and valves; and drug delivery applications *(1)*. Scaffold materials produced from nanofibers offer large surface areas that can support cell growth. Nanotechnology has the potential to revolutionize many areas such as surface microscopy, silicon fabrication, biochemistry, molecular biology, physical chemistry, and computational engineering. Nanofibrous scaffolds designed to elicit specific cellular responses through the incorporation of signaling ligands (e.g., growth factors, adhesion peptides) or DNA fragments are viewed as particularly promising in near-term strategies. Nanoparticles and nanospheres enable the controlled release of therapeutic agents, antibodies, genes, and vaccines into target cells.

Polymers such as polyglycolide (PGA), polylactide (PLA), and their random copolymer poly(glycolide-*co*-lactide) are often used as the base materials for implant devices, such as suture fibers and scaffolds, for tissue engineering *(2,3)*. These materials meet several controlled-release criteria: they are biocompatible and biodegradable and they can provide high efficiency in drug loading. Many different techniques have been developed to produce nanostructured biodegradable materials such as microspheres, foams, and films. It has been demonstrated that the molecular structure and morphology of PLA, PGA, and their copolymers can play a major role in the degradation and mechanical properties of the final products *(4,5)*. The electrospinning technology is well suited to process natural biomaterials and synthetic biocompatible or bioabsorbable polymers for biomedical applications. Polycaprolactone (PCL) has been investigated mainly for long-term implants for drug release and support of mineralized tissue formation and may be a suitable substrate for the treatment of bone defects. An improvement in the mechanical properties of PCL has been achieved by copolymerization with PLA, enabling its use for orthopedic applications, such as the repair of bone defects.

Biological functioning of the organs is regulated by biologic signals from growth factors, extracellular matrix (ECM), and the surrounding cells. ECM molecules surround the cells to provide mechanical support and regulate cellular activities. The ultimate goal of the novel modified nanofibrous scaffold design is the production of an ideal structure that can replace the natural ECM until host cells can repopulate and resynthesize a new natural matrix. Collagen in its native state is a natural substrate for cell attachment, growth, and differentiation. The use of these modified nanofibers in tissue restoration is expected to result in an efficient, compact organ and a rapid recovery process owing to the large surface area offered by nanofibers made from protein used for wound healing; the epithelialization of implants and the construction of biocompatible prostheses, cosmetics, face masks, cartilage, bone substitutes, artificial blood vessels, and valves; stem cell expansion; and drug delivery applications.

Nanofibers provide a connection between the nanoscale world and the macroscale world, because the diameters are in the nanometer range and the lengths are in kilometers. Therefore, the current emphasis of research is on exploiting such properties and focusing on determining appropriate conditions for electrospinning various polymers and biopolymers for eventual applications including multifunctional membranes, biomedical structural elements (scaffolds used in tissue engineering, wound dressing, drug delivery, artificial organs, vascular grafts), protective shields in specialty fabrics, filter media for submicron particles in the separation industry, composite reinforcement, and structures for nanoelectronic machines *(6)*.

Applications in Biotechnology

The variety of nanomaterials have a wide range of properties and possible applications appear to be enormous, from extraordinarily tiny electronic devices, including miniature batteries, to biomedical uses and as packaging films, superabsorbants, biosensors, components of armor, and parts of automobiles *(7)*. Nanomaterials can be metals, ceramics, polymeric materials, or composite materials. Their defining characteristic is a very small feature size in the range of 1–100 nm. The most energetic research probably concerns carbon nanotubes *(8)*. The high surface area-to-weight ratio of nanofibers makes an ideal substrate for molecular filtration and potentially are ideal scaffold for protective clothing applications against biochemical attacks and, thus, the membrane can be fabricated using electrospinning technology *(9,10)*. Lee et al. *(11)* suggested that antibodies are suitable capture agents for use in molecular filtration. In our ongoing project, antibodies will be used as the molecular recognition agent and will be attached to the surface of the nanofibers. Nanoparticles of carbon rods, fibers, and tubes with single or double walls, open or closed ends, and straight or spiral forms have been synthesized in the past 10 yr. Carbon nanotubes have been shown to have unique properties: stiffness and strength higher than any other material, as well as extraordinary electronic properties. Carbon nanotubes are reported to be thermally stable in vacuum up to 2800°C, to have a capacity to carry an electric current 1000 times better than copper wires, and to have twice the thermal conductivity of diamond (which is also a form of carbon). Carbon nanotubes used as reinforcing particles in nanocomposites also have many other potential applications. They could be the basis for a new era of smaller and more powerful electronic devices than any previously envisioned. Nanocomputers based on carbon nanotubes have already been demonstrated *(12)*.

Polymer Nanofibers for Biomedical Applications

Medical application of electrospinning is the subject of a patent claimed by Smith et al. *(13)*. They produced a skin mask by directly electrospinning fibers onto the skin surface in order to protect or heal eventual wounds. Electrospun fiber mats were also explored as drug delivery vehicles, with promising results. Mats were made from either PLA, poly(ethylene-*co*-vinyl alcohol (PEVA), or a 50:50 blend using tetracycline hydrochloride as a model drug *(14)*. PLA has been widely used in various biomedical applications owing to its biodegradability, biocompatibility, good mechanical properties, and ability to be dissolved in common solvents for processing *(15,16)*. Silicone rubber, nylons, polyesters, polyurethanes, acrylics, and other polymers have been used in biomedical applications *(17)*. Some of the uses of biomaterials include dental implants, bone replacements and cements, heart valves, cosmetic surgeries, and vascular grafts *(18)*.

Electrospinning can even be used to create biocompatible thin films with useful coating design and surface structure that can be deposited on implantable devices in order to facilitate the integration of these devices with the body. Silk-like polymer with fibronectin functionality (ECM proteins) has been electrospun for making biocompatible films used on prosthetic devices aimed for implantation in the central nervous system *(19)*. Of particular interest are electrospun membranes composed of elastomeric fibers, for the development of several protective clothing applications. Much work is being done with the aim of developing garments for soldiers that reduce risks of chemical exposure. The idea is to lace several types of polymers and fibers to make protective ultrathin layers that would enhance chemical reactivity and environmental resistance.

Polymer Nanofibers for Tissue Engineering

Engineering Skin

Tissue engineering provides interdisciplinary research worldwide because of the potential for obtaining living tissue replacements, thereby reducing the reliance on donor tissue and organs. Natural and synthetic fibers have been used widely for tissue repair, and these fibrous scaffolds are mechanically stable and capable of functioning biologically in the implant site *(20)*. Mechanical stability is dependent primarily on the selection of biomaterial, the architectural design of the scaffold, and the cell material interactions *(21)*.

Dressings for human wounds have been aimed at protection, removal of exudates, inhibition of exogenous microorganism invasion, and improved appearance. Polyurethane is frequently used in wound dressings because of its good barrier properties and oxygen permeability. Wound dressing with electrospun nanofibrous membrane can meet the requirements such as higher gas permeation and protection of wound from infection and

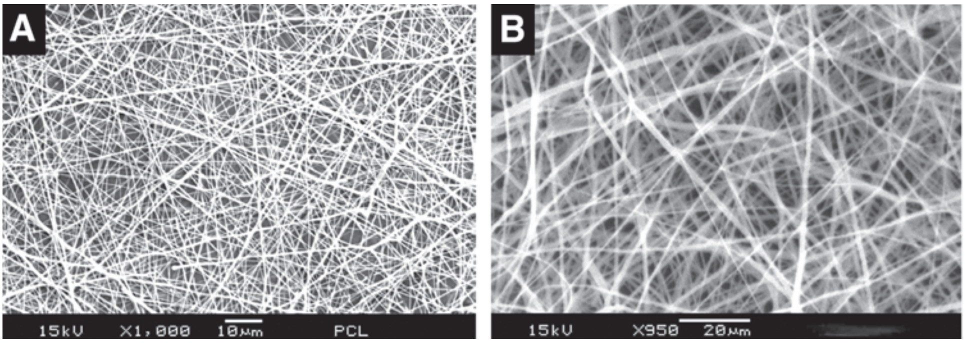

Fig. 1. **(A)** PCL nanofibers (480 nm); **(B)** collagen nanofibers (797 nm).

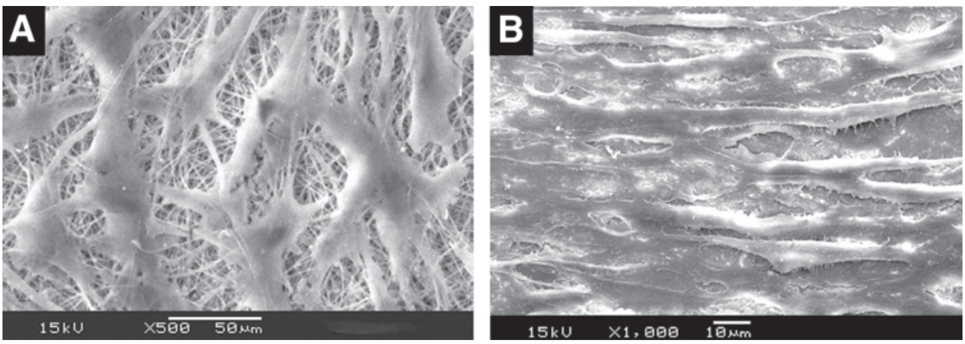

Fig. 2. **(A)** Human dermal fibroblast on PCL nanofibers; **(B)** human dermal fibroblast on collagen nanofibers.

dehydration. The goal of wound dressing is the production of an ideal structure that gives higher porosity and a good barrier. To reach this goal, wound-dressing materials must be selected carefully, and the structure must be controlled to confirm that it has good barrier properties and oxygen permeability. The PCL nanofibers support the fibroblast cell culture compared to PCL films (Fig. 1A). The preparation and characterization of collagen: PCL biocomposite and nanofiber membranes support human dermal fibroblast and keratinocytes in tissue-engineered skin in regenerative medicine *(22)*. Cells perform amoeboid movement to migrate through the pores and push the surrounding fibers aside to expand the hole as the small fibers offer little resistance to cell movement. This proves that the pores with smaller diameters in this structure may not hinder cell migration and that the scaffolds may show the dynamic nature with the means of external force (amoeboid movement of the living cell). This dynamic architecture may provide the cells with an opportunity to adjust optimally the pore diameter and grow into the scaffold (Fig. 2A,B). PCL is characterized by a resorption time in excess of 1 yr but is known to be susceptible to enzymatic degradation *(23,24)*. Electrospun nanofibrous membrane showed good and immediate adherence to wet wound surface. The membrane attained uniform adherence to the wound surface without any accumulation of fluid, and it provided a good means for wound healing.

Engineering Blood Vessel

The electrospinning technique is anticipated to be generally useful for producing novel biomaterials and biodegradable matrices for supporting cell attachment and proliferation. This process has the ability to produce nonwoven, nanofibrous structure and is well suited for tissue engineering *(25)*. Most of the natural ECM is composed of randomly oriented collagen nanofibers in nanometer-scale diameters (Fig. 1B), and the building components of the scaffolds are made of biodegradable PCL fibers. Attempts have been made, for many years, to develop viable synthetic or tissue-engineered prostheses for small blood vessels, but all had high failure rates for one reason or another. The nanomatrix designed and fabricated three-dimensional (3D) scaffolds out of collagen, and the cell-secreting natural material can be used to seed the smooth muscle cells (SMCs) to mimic natural small-diameter blood vessels. Studies suggested that muscle cells once implanted in the scaffold developed the function, shape, morphology, and cellular architecture of the normal vessel *(26)*. Poly(glycolide-*co*-ε-caprolactone) scaffolds possess elastic mechanical properties and allow the promotion of SMC adhesion and subsequent tissue formation *(27)*. Typical materials currently used as vascular grafts include poly(ethylene terephthalate) and poly(tetrafluoroethylene) *(28)*. Mo et al. *(29)* and Xu et al. *(30)* suggest that synthetic polymer scaffold poly(L-lactide-*co*-ε-caprolactone) is composed of aligned and random nanofibers that mimic the native ECM, and this scaffold showed the favorable behavior of SMC and endothelial cell interaction between the scaffold. The electrospinning method used to fabricate the scaffold is simple for mass production and has huge potential for application in blood vessel engineering. Various methods have been developed to support the seeding and growth of endothelial cells on polyurethane or other biomaterial surfaces such as surface modifications by plasma treatment and photochemically grafted GRGD peptide on a modified polyurethane surface or chitosan surface. These surface modifications may be categorized as chemical or biologic surface modifications to enhance endothelialization on biomaterial surfaces. On the other hand, modifications of physical properties of surfaces such as enhancing hydrophilic properties, changing the porosity of materials, and increasing roughness of the surface may also enhance endothelialization of biomaterials *(31)*. The above study supports to construct the blood vessel scaffold to create the functional tissue-engineered blood vessel substitute for the replacement of diseased organs.

Engineering Cartilage

Cartilage defects resulting from aging, joint injury, and developmental disorders cause joint pain and loss of mobility. The tissue-engineering approach provides a cell-based therapy to repair articular cartilage defects and restore joint functions *(32)*. Many different techniques have been developed to produce nanostructured biodegradable materials such as microspheres, foams, and films. It has been demonstrated that the molecular structure and morphology of PLA, PGA, PCL, and their copolymers can play a major role in the degradation and mechanical properties of the final products *(33)*. Modifications of physical properties of surfaces such as enhancing hydrophilic properties, changing the porosity of materials, and increasing roughness of the surface may also enhance endothelialization of biomaterials. Polymer scaffolds are primarily used for the delivery and retention of chondrogenic cells in cartilage tissue engineering *(34)*. Many naturally derived and synthetic polymers are currently used as scaffolds for regeneration of articular cartilage, and many others are under development.

In cartilage tissue engineering, chondrocytes and mesenchymal stem cells (MSCs) are commonly used for cartilage regeneration. PCL nanofibrous scaffolds structurally similar to ECM may represent promising structures for tissue-engineering applications. 3D nanofibrous scaffolds are characterized by high porosity with a wide distribution of pore diameter, high surface area-to-volume ratio, and morphologic similarities to natural collagen fibrils. These nanofibrous scaffolds (Fig. 1A,B) are readily fabricated in any shape and size as needed clinically and also provide sound mechanical stability to provide a carrier for MSC transplantation in tissue-engineering cartilage repair. Collagen, PLA, poly(lactic-*co*-glycolic acid) (PLGA), and PGA were shown to provide a scaffold for in vitro cartilage regeneration, as demonstrated by cell densities equivalent to those found in natural tissues and by continued cellular production of type II collagen *(35,36)*.

Engineering Bone

Bone grafts have been used to fill defects in bone caused by clinical states or trauma, such as fractures with bone loss, bone infection, or bone tumors. Autologous bone is the current "gold standard" graft material without the risk of transfer of disease for the treatment of skeletal defects and fracture repair. By contrast, the use of allogenic bone may transmit diseases and cause an immune response, which can lead to a high failure rate. One of the main goals in tissue engineering has been to develop biodegradable materials as bone graft substitutes for filling large bone defects. These materials should maintain adequate mechanical strength over critical phases of the tissue-healing process, should be oesteoconductive, and should degrade at a controlled rate to provide space for the formation of new bone. Engineering living tissue for reconstructive surgery requires an appropriate cell source, optimal culture conditions, and a biodegradable scaffold as the basic elements. Although specialized cells remain an important source, stem cells have emerged as a promising new alternative. Recent advances in stem cell biology have shown that MSCs can differentiate into cells of mesenchymal tissues such as bone, cartilage, muscle, tendon, ligament, and fat and are expected to play an important role in the repair of skeletal defects *(37–39)*. Osteoblasts derived from MSCs of neonatal rats were cultured on poly(DL-lactide-*co*-glycolide) foams, and mineralization as well as 3D bone formation was observed *(40)*. Cell adhesion is the most important aspect of cell interaction with a biomaterial because it is the prerequisite for further cellular activity such as spreading, proliferation, and differentiation. Initial oesteoblast/material interactions may be conveniently characterized by four stages: (1) protein adsorption to the surface, (2) contact of rounded cells, (3) attachment of cells to the substrate, and (4) spreading of cells. Initial cell attachment will be influenced by the original surface characteristics of the materials *(41)*. In a later phase of cell development, however, other factors such as polymer degradation products or bulk properties might play a more crucial role.

Biodegradable polymers often have been combined with bioceramics to produce materials for bone repair. Biodegradable polymers such as PGA, PLA, and PLGA have found application as resorbable sutures *(42)*, bone fixation screws, plates, and drug carriers; and they have been widely investigated as bone graft substitutes, with positive results *(43,44)*. It is important to reiterate that poly(ε-caprolactone) has been investigated mainly for long-term implants for drug release and support of mineralized tissue formation and may be a suitable candidate for the treatment of bone defects *(45)*. An improvement in the mechanical properties of PCL has been achieved by copolymerization with PLA, enabling its use for orthopedic applications, such as the repair of bone defects. There has been widespread use of bioceramics such as hydroxyapatite and tricalciumphosphate for bone regeneration applications, and their biocompatibility is thought to be owing to their structural similarity to the mineral phase of bone. Biodegradable ceramics are used in dental and orthopedic surgery as fillers for bone defects and as coatings on metallic implants to improve implant integration in host bone. Biodegradable polymers have been used as binders for hydroxyapatite and tricalciumphosphate to overcome the problems of brittleness and the difficulty of shaping hard ceramic materials to fit bone defects *(46)*. At the same time, bioceramic reinforcement of polymers has been shown to improve mechanical properties and osteoconductivity *(47)*. Biodegradable polymer/bioceramic composites are promising materials for bone graft replacement, and this has been intensively investigated in the last decade *(48)*.

Carbon nanotubes and nanofibers have several properties that suggest these materials may be of value in the development of novel devices for bone reconstruction *(49)*. Carbon fibers with nanometer dimensions simulate dimensions of collagen fibrils (0.1–8 μm in diameter) in bone. Price et al. *(49)* suggest for the first time that smaller-diameter carbon nanofibers without the pyrolytic layer (i.e., PR-24 PS fibers) could be suitable for use in orthopedic/dental implant material designs owing to the increase in osteoblast and decrease in osteoblast competitive cell line (fibroblasts, chondrocytes, and SMCs) adhesion. More osteoblast adhesion and less competitive cell adhesion could lead to faster integration of the bone to the implant surface in vivo. In addition, other in vitro studies have shown increased osteoblast alkaline phosphatase activity and calcium deposition on nanometer carbon fibers compared with conventional fibers *(50)*. This is further evidence that nanometer carbon fibers enhance osteoblast activity for possible increased integration of bone on the orthopedic material surface.

Nerve

Nerve tissue repair is a precious treatment concept in human health care because it directly impacts the quality of life. Polymer scaffolds can be used as scaffold to promote cell adhesion, to maintain differentiated cell function without hindering proliferation, to provide template to organize and direct the growth of cells, and to help in the function of ECM *(51)*. Nanofibrous porous scaffold is prepared by phase separation using poly(L-lactic acid) with a similar structure of natural ECM in nerve tissue engineering *(52)*. Spilker et al. *(53)* demonstrated that cells from nerve explants were able to construct and restructure the walls of porous collagen-glycosaminoglycan (GAG) matrices in vitro, suggesting that contractile cells may be capable of restructuring the extracellular component of the nerve wound environment in vivo. The nondegradable conduits have been made of materials including stainless steel, poly(acrylonitrile-*co*-vinylchloride), polycarbonate, and silicone. However, conduits fabricated with these durable materials remain *in situ* as a foreign body and may elicit an inflammatory response or cause compression injury, thus limiting nerve regeneration. Biodegradable polymer scaffolds provide an alternative and have been used for the repair of nerve defects. These temporary conduits have been composed of materials such as polyurethane, polyorthoester, glycolide trimethylene carbonate, poly(lactic-*co*-glycolic acid), poly(glycolic acid), poly(DL-lactic acid), and poly(lactic acid-*co*-ε-caprolactone) *(54)*. Fabre et al. *(55)* recently demonstrated that seeding a resorbable implant (trimethyencarbonate-co-ε-caprolactone) with Schwann cells significantly increases growth, ensheathment, and peripheral nerve myelination.

Conclusion

Nanotechnology has been considered a wide and interdisciplinary area of research and development for the past few years, because creating new materials and devices from nanoscale building blocks could access new and improved properties and functionalities. The electrospinning technique provides an inexpensive and easy way to produce nanofibers from many types of polymers on a low basis weight, with a small fiber diameter, variable pore size, high surface area, and choice of fiber chemistry in nanotechnology. Polymer nanofibers are important tools in the development of new products such as barrier fabrics, microaerodynamic accelerators based on permeable nanofiber mats, nanofiber-based filters, biosensors, and protective clothing. Nanofibers are used in biomedical applications including wound dressings, nanocomposites for dental restoration, drug delivery systems based on nanotubes, and structural elements in artificial organs. Control of cell interaction with bioengineering scaffolds may achieve a variety of engineered tissues. The importance of bioengineering research lies in its ability to improve health care and restore function in those with physical impairments.

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