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Contributed Mini Review

Myocardial tissue engineering using electrospun nanofiber composites

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Emerging trends for cardiac tissue engineering are focused on increasing the biocompatibility and tissue regeneration ability of artificial heart tissue by incorporating various cell sources and bioactive molecules. Although primary cardiomyocytes can be successfully implanted, clinical applications are restricted due to their low survival rates and poor proliferation. To develop successful cardiovascular tissue regeneration systems, new technologies must be introduced to improve myocardial regeneration. Electrospinning is a simple, versatile technique for fabricating nanofibers. Here, we discuss various biodegradable polymers (natural, synthetic, and combinatorial polymers) that can be used for fiber fabrication. We also describe a series of fiber modification methods that can increase cell survival, proliferation, and migration and provide supporting mechanical properties by mimicking micro-environment structures, such as the extracellular matrix (ECM). In addition, the applications and types of nanofiber-based scaffolds for myocardial regeneration are described. Finally, fusion research methods combined with stem cells and scaffolds to improve biocompatibility are discussed. [BMB Reports 2016; 49(1): 26-36]

INTRODUCTION

For the body to properly perform its functions, the appropriate vessels must supply sufficient nutrients and oxygen to each part of the body. The heart, which acts as a pump to supply blood to the body, requires its own constant supply of necessary nutrients and oxygen, which are delivered through the coronary artery (1). However, if the coronary artery does not supply the required amount of blood, inhibited cardiac function is caused by myocardial ischemia due to metabolite accumulation and hypoxia in the heart muscle. This results in a failure of heart function, known as "heart disease" or "coronary artery disease" (2, 3). After a myocardial infarction (MI), if part of the heart muscle has died, it is replaced by scar tissue over the next few weeks (4). It is difficult for an impaired heart to recover because cardiac muscle tissue does not have the capacity to regenerate, leading to significant pain and disability. Although there are direct and indirect treatment approaches, including surgery, medicine, and transplantation, several hurdles remain, such as a risk of immune reactions and re-stenosis and a severe limitation in the number of available donors. To develop a universal cure for heart disease and reduce mortality, alternative strategies are required.

Since the introduction of regenerative medicine in the 1980s, tissue engineering has yielded safe and readily available strategies for the replacement of damaged tissues or organs (5). As a part of regenerative medicine, stem cell-based cell therapies have shown promising results for novel biomedical treatments of various diseases, including MI, hind limb ischemia, and stroke. However, although stem cells have shown remarkable therapeutic effects, they are characterized by some problematic factors (6, 7). Stem cells that are transplanted for cardiovascular tissue regeneration face harsh conditions that limit their survival in the body, such as oxidative stress-induced cellular damage, enzyme degradation, and a limited microenvironment in which stem cells cannot migrate and or differentiate, such as the extracellular matrix (ECM), for the induction of endothelialization onto target tissue (8). Additionally, bio-artificial organ transplants into the human body also present the problem of revascularization.

To solve these problems, many approaches have been introduced to develop advanced biomaterials to mimic extracellular three-dimensional structures or enhance stem cells survival via hypoxic preconditioning, genetic modification, and drug combination. Among these approaches, biomaterial synthesis and microfabrication have made it possible to pattern cells by using appropriate scaffolds, which are used as the templates of biomedical applications of nanofibers for tissue regeneration. There are two types of fabrication methods to gen-

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erate nanofibers, known as the top-down and the bottom-up approach (9). A nanofiber is single block of scaffolds and one of the most biocompatible formulations to generate the fibrous scaffolds as ECM substitutes. Nanofibers can be produced by a variety of methods such as self-assembly nanofibers, emulsion freeze-drying, gas foaming, computer-aided design technology, phase separation, and electrospinning (9).

In this review, we will focus on material sources, surface modification methods for fabricating multifunctional nanofiber-based scaffolds and the potential of this technique to be applied to myocardial regeneration by forming artificial *in vivo*-like microenvironments. Regarding the development of nanofiber-based scaffolds for use with therapeutic strategies using stem cells, drugs, and growth factors, we attempt to summarize the production process of nanofibers and the potential application of these regenerative factors for heart failure treatments (Fig. 1).

ELECTROSPINNING FOR NANOFIBER CONSTRUCTION

In the last decade, electrospinning has been applied to a variety of fields such as regenerative medicine, the textile industry, and energy storage. One of the major challenges in the regenerative medicine field is to design and fabricate a suitable nanofiber-based scaffold (10, 11). The electrospinning method, first known as electrostatic spinning, is a simple, versatile technique to produce non-woven fibrous mats that remarkably mimic the size (fibers with diameters down to the nanoscale) and scale of the natural ECM (12, 13). To form nanofibers using electrospinning, four major components are required: i) a high voltage power supply (up to 30 kV), ii) a syringe pump (for ejecting the polymer solution with/without therapeutic materials), iii) a needle (a Taylor cone), and iv) a collector (such as metal screen, plate or rotating mandrel) (Fig. 2).

When the electric field between the needle capillary and the collector is produced by the high voltage of the power supply, a Taylor cone will be formed, indicating the formation of low surface tension. Low voltage induces the formation of a pendent droplet (similar to beads) from the Taylor cone. As a result of stretching by electrostatic repulsion and whipping, the liquid jet is continuously reduced in size until it has been solidified or deposited on the collector. Furthermore, by adjusting the experimental parameters, such as the concentration of the

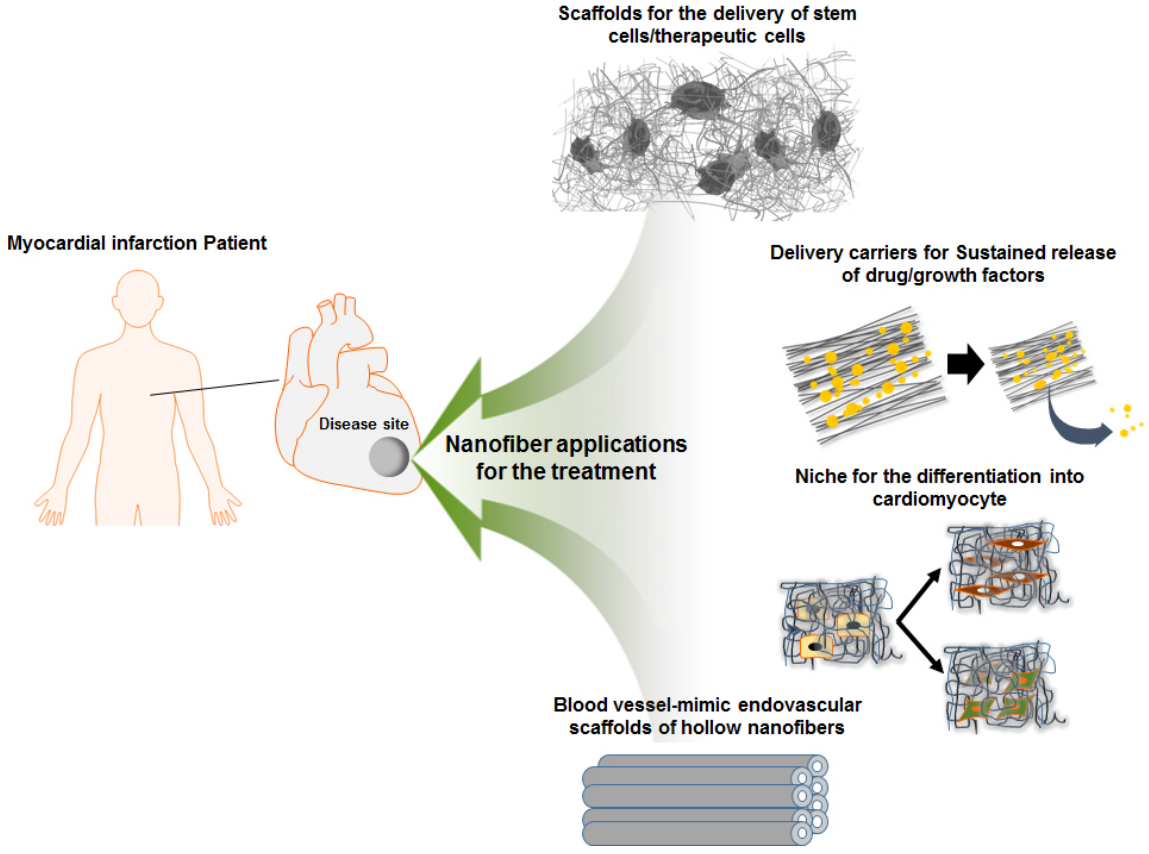

Fig. 1. Schematic representation of applicable strategies of multi-functional fiber-based scaffolds for myocardial regeneration.

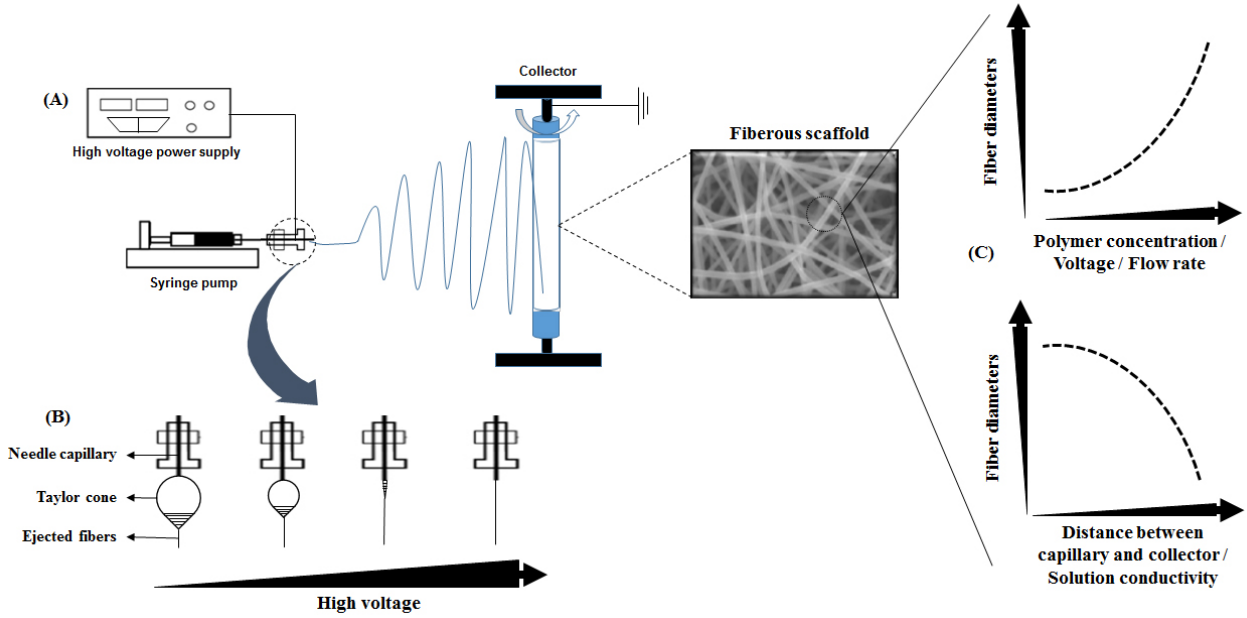

Fig. 2. Scheme of the electrospinning composed of major four parts diameter (A), the change of Taylor cone (B) and fiber diameter according to a broad range of voltage. The diameter of fiber fabricated by electrospinning is variously changed depending on the polymer concentration, voltage, flow rate, distance between capillary and collector, and solution conductivity (C).

polymer solution, voltage, the flow rate, and distance between the needle capillary and the collector, fibers with uniform diameters can be generated. The morphology and characteristics of the fibers can be varied according to the purpose of the application. Additionally, modifying the surface of the fibers and the therapeutic factors within the fibers allows the fibers to be applied to a broad range of therapies, including cardiac regeneration.

THE MATERIAL SOURCE OF NANOFIBERS

Nanofibers fabricated for biomedical applications have been primarily used as scaffolds and are characterized by their nanometer scale and architectural resemblance to the ECM. Several regenerative biomaterials for *in vivo* applications have developed to improve safety and biocompatibility and to achieve the desired function of a tissue or organ. Table 1 summarizes the characteristics of each polymer that is discussed in this section.

Natural polymers

Natural polymer-based nanofibers are ideal for *in vivo* applications due to the non-toxicity of their degradation products and low immune response. The most commonly used natural polymers are collagen, alginate, chitosan, and gelatin.

Collagen is one of the major components of the ECM, existing in many different forms depending on its tissue of origin and often forming nanofibers. Collagen is naturally found in connective tissue where it provides mechanical support that mimics the extracellular matrix in the body. However, its application is limited due to its weak mechanical properties as a supportive scaffold and its rapid *in vivo* degradation (14, 15). To overcome these disadvantages, collagen has been primarily utilized in conjunction with other polymers.

Alginate is a naturally derived biocompatible polysaccharide isolated from brown algae. It can form a hydrogel upon ionic crosslinking with divalent cations such as calcium, as the cations cause the G-units on neighboring polysaccharide chains to interact (16). However, alginate is primarily utilized in conjunction with another polymer as a blended material because it does not adhere to cells and cannot be electrospun alone due to a lack of chain entanglements. Therefore, to increase cell adhesion, the Arg-Gly-Asp (RGD) motif is conjugated to alginate (17).

Chitosan, a natural polysaccharide derived from the deacetylation of chitin, is a non-toxic and cationic with favorable biocompatibility, biodegradability, antibacterial activity, low immunogenicity and wound healing capacity. It is composed of two subunits, D-glucosamine and N-acetyl-D-glucosamine (18, 19). For cardiac tissue engineering, Chen *et al* reported chitosan nanofiber scaffolds used as a 3D cardiac co-culture model system. They first generated fibronectin-coated chitosan fibers via electrospinning to enhance cellular adhesion to the fibers and migration into the interfibrous milieu. The results demon-

Table 1. The material sources for the formation of nanofibers

	Polymers	Characteristics		References
Natural polymers	Collagen	- The major components of ECM - Found connective tissue - Weak mechanical properties - Fast degradation		(14, 15)
	Alginate	- Polysaccharide isolated from brown algae - Hydrogel formation with divalent cations - Low cell adhesion - No formation of nanofiber itself		(16, 17)
	Chitosan	- A natural polysaccharide obtained by deacetylation of chitin - Non-toxic, cationic - Biodegradable and antibacterial activity and low immunogenecity		(18, 19)
	Gelatin	- Biocompatibility - One of components of ECM		(20)
Synthetic polymers	polyglycolide (PGA)	- A biodegradable synthetic polymer - The use of organ solvent - Good mechanical properties	Fast degradable than PLA and PLGA	(21, 22)
	poly(L-lactide) (PLA)		Intermediate degradable between PGA and PLGA	
	poly(lactide-co-glycolide) (PLGA)		Slow degradable than PGA and PLA, large diameter nanofiber (760 nm)	
	poly(ε-caprolactone) (PCL)		- Slow degradation	(23, 24)
Combinatorial polymers	PLGA/gelatin/elastin	- Enhanced cell adhesion		(25)
	PCL/gelatin	- The increase of EC spreading and proliferation		(26)
	PLGA/collagen	- Good stability for tissue formation		(27)

strated that the chitosan nanofibers retained their cylindrical morphology in long-term cell cultures and that neonatal rat cardiomyocytes on the fibers exhibited good cellular attachment and spreading, because of the formation of large tissue-like cellular networks by co-cultures with fibroblasts, indicating that 3D chitosan nanofibers can be used as a potential scaffold to regenerate heart tissue.

Gelatin has been used for many years in biomedical applications in biodegradable grafts, and it is now possible to create artificial analogs of ECM proteins (20). Li *et al* demonstrated the long-lasting proliferation of fetal rat ventricular cells on 3D gelatin mesh matrices, and human ventricular cardiomyocytes survived within the gelatin mesh matrices with no increase in proliferation. In an *in vivo* test, improved cardiac function was identified in rat myocardial scar tissue after implantation (21).

Synthetic polymers

Compared with natural polymers, synthetic polymers are beneficial because they are minimally immunogenic, highly reproducible at a low cost, and have a simple quality control process. Moreover, synthetic polymers can control the biodegradability of biomaterials for long-term therapeutic periods and modify restricted flexibility of biomaterials for tissue regeneration.

Among electrospinning biodegradable polymers, polyglycolide (PGA), poly(L-lactide) (PLA), and poly(lactide-co-glycolide) (PLGA) have been widely used in clinical fields from uses in surgical sutures and implant materials to drug carriers and scaffolds due to their good mechanical properties and biocompatibility. Notably, these polymers have received Food and Drug Administration approval for use in medical devices. PLA and PGA are characterized by different rates of degradation due to differences regarding the hydrophobic methyl groups of their backbone structure. PLA degrades slower than PGA. Park's group showed the degradation rates of nanofibers fabricated by PLA, PGA, and PLGA (L-lactide/glycolide = 50/50). PGA exhibited the fastest rate of degradation followed by PGA > PLGA >> PGA. Additionally, PLGA can be fabricated into nanofibers with larger diameters (760 nm) than those of PGA and PLA (−300 nm) in a broad distribution range of 200-1,800 nm (22).

Poly(ε-caprolactone) (PCL) is a biodegradable synthetic polymer that can be electrospun and has been widely used in a large range of medical devices and implants. PCL also has a long-term degradation period, providing a sustained microstructure for prolonged therapeutic effects in some aspects of tissue engineering (23). PCL-based nanofibers have been applied to sites of heart failure as patches and meshes.

Combinatorial polymers

A recent trends of scaffolds for the application of tissue engineering is to combine natural and synthetic polymer materials as blending polymer. A combinatorial approach to take advantage of the interplay between natural and synthetic polymer systems has been investigated to improve the therapeutic efficacy and scaffold functions *in vivo*. The common purpose of combinatorial polymers is to support increased cell adhe-

sion, survival, migration, effects on cell morphology by PCL/collagen nanofibers as well as to increase cell growth and enhance the penetration into the center of scaffolds of rat bone marrow stromal cells (BMSCs) and cardiac myoblasts by PLGA/gelatin/elastin (PGE) nanofibers (24, 25). In addition, PCL nanofibers have been grafted with gelatin to improve their compatibility with endothelial cells (ECs) for the enhancement of EC spreading and proliferation in a blood vessel tissue-engineering scaffold (26). PLGA knitted mesh-reinforced collagen-chitosan scaffolds for the increase of scaffold function promoted faster cell infiltration and ECM formation, resulting in increased tissue regeneration and angiogenesis in wound healing, although the mechanical functions of the scaffolds have not been reported (27).

NANOFIBER MODIFICATION FOR MULTI-FUNCTIONAL SCAFFOLDS

Nanofibers applied as scaffolds in tissue engineering have been used to support tissue-specific cell functions and tissue-mimicking systems for tissue/organ regeneration. However, although composite nanofibers provide both mechanical properties and biological responses, these qualities are not sufficient to achieve full therapeutic effects. For use in biomedical fields, the surface must be modified to enhance cell adhesion and their drug loading ability. In this section, fiber surface modification will be introduced. Table 2 summarizes the surface modifications that are discussed in this section.

Physical adsorption

The porous structure caused by the fibers in a fabricated scaffold provides a good environment for incorporating drugs and therapeutic cells and inducing sustained release. Bioactive molecules can be incorporated into scaffolds by both chemical and physical methods. The simplest approach for loading biomolecules onto fiber and fiber-based scaffolds is physical surface adsorption by dip-coating (Fig. 3A) (28, 29). In this approach, biomolecules are physically adsorbed onto nanofibers via electrostatic interactions, hydrogen bonding, hydrophobic interactions, and van der Waals interactions (28). Although this method is simple to employ and it is easy to manufacture functional fibers with highly active biomolecules, the incorporated biomolecules are rapidly released, leading to a fast burst release that cannot be maintained for long-term therapeutic effects. Wang *et al* reported that the burst release of human bone morphogenetic protein-2 (hBMP-2) attached to the surface of PLAG/HA scaffolds occurred within 5 days (30). To overcome this problem, chemical surface modifications can be employed.

Blend electrospinning

Another method for generating functional fibers is blending electrospinning (Fig. 3B). This method mixes bioactive molecules in polymer solutions, and then the mixture is fabricated into hybrid fibers by electrospinning. The fabricated functional fibers are used for various applications, such as sustained delivery or release and immobilization of therapeutic materials for the enhancement of cell proliferation and functions. Blend electrospinning involves the encapsulation of biomolecules, resulting in drug-loaded fibers. The biomolecules encapsulated within the fibers exhibit a more sustained release profile without an early burst release compared with the release profile observed for the physical adsorption technique. Encapsulated biomolecules can maintain long-term release for protein delivery because most of the protein or peptides have a short half-life *in vivo*. However, the disadvantage of fibers fabricated by blending electrospinning is the loss of function and activity of incorporated biomolecules due to denaturation or aggregation resulting from conformational changes in the organic solvent environment. These effects decrease the therapeutic effects of fibers fabricated by blending electrospinning. However, this limitation can be overcome by changing the structure of the polymer backbone to control the degradation profile (31). Toh *et al* generated PLGA nanofibers incorporated with

Table 2. The surface modification methods of nanofibers for the attachment of biomolecules

Surface modification methods		Characteristics	References
Physical adsorption		- Very simple - Low efficiency of coatingunstable of modified layer	(28, 29)
Blend electrospinning		- Inhibition of burst release in gibers - The possibility for the loss of function and activity of incorporated biomolecules	(31)
Coaxial electrospinning		- Effective encapsulation into fibers - The possible for the programmed release of biomolecules - Homogeneous bioactive molecules distribution	(35, 36)
Surface-grafted modification	by Plasma	- Effective procedure - The change induction of surface morphology by plasma etching	(41)
	by Radiation	- Simple and no clean process - The difficulty in inner space coating due to penetration depth	(45)
	by Chemical	- Very consierable for biocompatibility	(42-45)

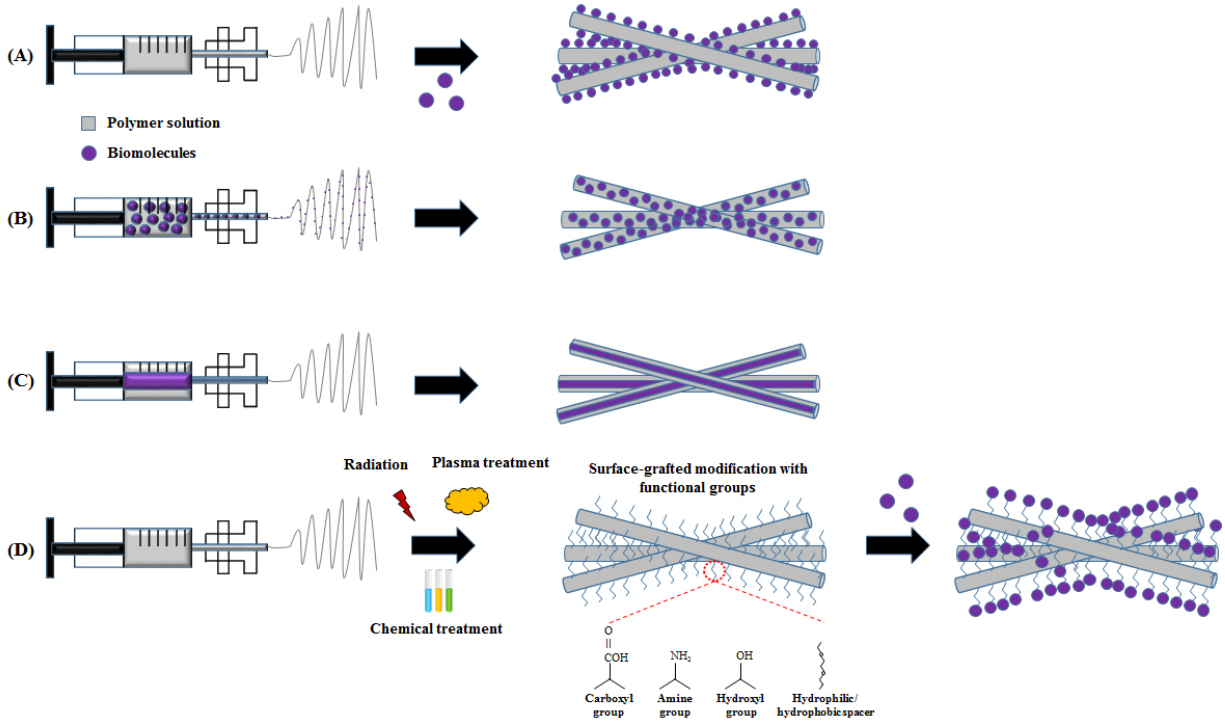

Fig. 3. Fiber surface modification for the generation of multi-functional nanofibers. (A) physical adsorption, (B) blend electrospinning, (C) coaxial electrospinning, and (D) surface-grafted modification by radiation, plasma, and chemical treatment for the formation of functional groups.

bFGF by blending electrospinning to deliver growth factors for tissue engineering applications (32). The PLGA nanofibers containing bFGF exhibited release of the encapsulating protein over 1-2 weeks and were associated with increased collagen production and an upregulation of gene expression of ECM-related proteins.

Coaxial electrospinning

Coaxial electrospinning, also known as co-electrospinning, is a method for fabricating core-shell nanofibers with bioactive molecules (Fig. 3C). Polymer and biomolecule solutions are prepared in different capillaries. They are conducted through one nozzle via electrospinning, generating composite nanofibers with a core-shell structure based on the interfacial tension and viscoelasticity of the two solutions (29, 33, 34). The core-shell structure of nanofibers via coaxial electrospinning is controlled by various conditions, such as applied electric field strength, solution viscosity/concentrations and flow rate. Bioactive molecules are encapsulated in the inner part of the fabricated fibers, and the polymer makes up the outer part. Compared with uniting biomolecules at one site in blend spinning, coaxial electrospinning provides a homogeneous distribution of bioactive molecules throughout the fibers. Moreover, the activity of the biomolecules entrapped in the fibers is similar with that of fibers fabricated by blend spinning, but the burst release rate is low compared with blend spinning, and coaxial electrospinning allows for a prolonged sustained releasing profile (35, 36). Co-electrospinning may be able to control the programmed release of bioactive molecules. Various studies have used coaxial electrospinning to fabricate fibers containing growth factors, plasmid DNA (pDNA), and viruses for gene delivery.

For example, dextran (DEX) and poly (L-lactide-co-epsilon-caprolactone) (PLCL) were produced via co-electrospinning to generate core/shell fibers with platelet-derived growth factor-bb (PDGF-bb), resulting in enhanced cell attachment (37). For gene delivery, mesh scaffolds of poly(ethylenimine)-hyaluronic acid (PEI-HA) in the outer part and plasmid DNA-enhanced green fluorescent protein (EGFP) in the inner part were formed via coaxial electrospinning (38). In another study, PCL fibers generated with a GFP-expressing adenovirus were created via coaxial electrospinning to overcome limitations of the virus, such as host immune response, low infection efficiency and toxicity (39).

Surface modification

Compared with the physical adsorption method, chemical modifications of the fiber surface can prevent the burst release

and covalently immobilize bioactive molecules to control the release of drugs by enzymatic cleavage (40). The surface modification of fibers must expose the reactive functional groups capable of covalently binding to the bioactive molecules (Fig. 3D). These functional groups, such as carboxyl, amine, hydroxyl groups, and hydrophilic/hydrophobic spacers, can be artificially created by plasma, radiation, and chemical treatment (wet chemical methods). The biomolecules attached to the functional groups of the fiber surface can improve biocompatibility for enhanced cell adhesion and a controlled drug release profile.

Plasma treatment

Plasma treatment capable of generating carboxyl or amine groups on nanofibers or polymer substrates have used oxygen, ammonia, argon, or air as the plasma source to modify the characteristics of scaffold surfaces (41). This treatment induces the covalent immobilization of several biomolecules, leading to enhanced cell adhesion and proliferation. With respect to plasma treatment, the appropriate selection of the plasma source is important for the introduction of diverse functional groups on the target surface. For example, PCL nanofibers were treated with air plasma to introduce carboxyl groups on the surface, and reactive carboxyl groups were grafted with gelatin, leading to enhanced endothelial cell (ECs) spreading and proliferation (26).

Wet chemical method

Wet chemical treatment is frequently used with acidic or basic liquid reagents to generate reactive functional groups on the fiber surface through surface graft polymerization, resulting in the creation of carboxylic and hydroxyl groups on the surface by random chemical excision of ester linkages (42-44). This method is more useful for surface modification to deeply located fibers in comparison with the plasma treatment due to the limited penetration depth of plasma (45). However, covalent attachment method to surface of nanofiber is the technical complexity according to the functional group in biomolecules. Additionally, there is a chance to cause partial inactivation of the immobilized molecules by covalent modification at active sites of biomolecules. For example, poly(D,L-lactic acid) (PDLLA) was treated in strong alkaline conditions to introduce hydroxyl groups, and then it was grafted with chitosan to enhance cell affinity, showing the increased adhesion and growth of osteoblasts as well as improved biocompatibility of PDLLA fiber film (46).

NANOFIBER APPLICATIONS AND TYPES FOR MYOCARDIAL REGENERATION

Fibrous scaffolds

Fibrous scaffolds incorporating the basic types of fibers have been utilized to achieve long-term survival when applied to treat an injured heart. These scaffolds overcome the limitations and complications of fibers after *in vivo* transplantation, such as immune response and rejection. Moreover, fiber-based scaffolds can be modified for enhanced cell adhesion and proliferation because they mimic the ECM structure. An important function of scaffolds in cardiac regeneration is to support the environment capable of providing the synchronized beating of cardiomyocytes and contractile properties of the cardiac tissue and the anisotropic structure of myocardial architecture (47).

Based on this purpose, a previously published study reported elastomeric biodegradable poly(glycerol sebacate) (PGS):gelatin nanofibrous scaffolds fabricated by electrospinning (48). They first demonstrated anisotropy capable of mimicking the left ventricular myocardial architecture, and then improved its functionality using neonatal rat cardiac fibroblast cells in PGS:gelatin scaffolds evaluated by the cell attachment, proliferation, differentiation, and contractile function of the cardiomyocytes.

Patch type

At the early stages, a synthetic biodegradable patch is used to repair the living heart with cardiomyocytes surviving on the heart for limited periods (49, 50). Recently, bioengineered heart patches have been improved with polyester-based thermoplastic polymers, such as PGA, PLA, and PCL, to increase their long-term elasticity and mechanical characteristics (51). A synthetic biodegradable patch is widely used with cells or implanted onto the infarct regions. These cardiac patches for cell delivery and left ventricular restraint must be highly biocompatible and must be able to sustain the constant beating of the heart via suitable mechanical properties during cardiac reconstruction (52). Boccaccini *et al* developed biocompatible, degradable and superelastic heart patches from poly(glycerol sebacate) (PGS). The authors assessed the mechanical performance and degradability of the 3D myocardial tissue engineering construction and reported the ability of the elastomeric patch and a reduction in wall stress (53).

Nanofibrous hydrogel

Tissue engineering using 3D functional scaffold systems can generate 3D heart tissue. To date, the hydrogel method involves generating scaffold-based tissue. The characteristics of hydrogels resemble the natural ECM, improving biocompatibility (54). Additionally, hydrogels provide a porous environment due to swelling upon absorption of a large amount of water (55), helping the cells migrate and proliferate. Nanofibrous hydrogels for combining fibers with hydrogels can be generated via various methods, such as layering, mixing of short fibers, and concurrent electrospinning and electrospraying (56), although this system is not widely used and has low mechanical stability compared to scaffold-based techniques. However, the methodology to make nanofibrous hydrogels is easily miniaturized and automated with composite structures (56, 57). A study regarding the combination of fibers and hydrogels used the combination in a supporting system of cardiomy-

ocytes for cardiac tissue engineering (58). In this study, one of six different collagen-like synthetic self-assembling nanofiber hydrogels supported the culture of both neonatal rat cardiomyocytes and human embryonic stem-cell-derived cardiomyocytes, as observed via cell attachment, growth, and function.

Endothelial cell-seeded scaffolds

Smooth muscle cells (SMCs) and HUVECs are an important source for the formation blood vessels (59, 60). Jayakumar *et al* generated a unique scaffold consisting of multilayers for vascular tissue engineering. The inner layer was made up of poly (lactic acid) (PLA) nanofibers, and the outer layer was composed of PCL/PLA. The multi-layers had large pores for SMC penetration. The PLA fiber showed enhanced cellular functionality, and the PCL/PLA outer fiber supported SMC adhesion and proliferation (61). In a separate study, the authors explained SMC penetration and blood compatibility by hemolysis-coagulation and platelet activation (62). Their studies have demonstrated that designed tubular scaffolds can mimic the morphology of native vessels.

One group generated microvascular-scale scaffolds capable of supporting and stimulating endothelial cell (EC) adhesion and growth by the direct-write technique (63). The functionality of these microvascular-scale-diameter (5-20 μm) scaffolds were evaluated for cellular adhesion, proliferation, and scaffold degradation over the course of one week.

Delivery of growth factors

Endothelial cell-seeded and the large or small porous fiber-based scaffolds promote vessel generation by EC penetration into the scaffolds. Another strategy for forming blood vessels is to deliver growth factors at the injured or diseased site using scaffolds. The delivery of biomolecules requires persistent neovascularization or angiogenesis (3). Representative factors that can be used for myocardial regeneration include insulin-like growth factor-1 (IGF-1) and hepatocyte growth factor (HGF) (64, 65). Cohen *et al* administered dual growth factors (IGF/HGF) using an injectable affinity-binding alginate biomaterial to maximize their therapeutic effects (66). The dual IGF-1/HGF affinity-bound alginate prevented cardiomyocyte apoptosis *in vitro*, and intramyocardial injection of the dual IGF-1/HGF affinity-bound alginate increased angiogenesis and mature blood vessel formation in rat myocardial infarction (MI). Zhang's group reported heparinized chitosan/poly ε-caprolactone (CS/PCL) nanofibers immobilized with vascular endothelial growth factor (VEGF) to construct a biomimetic vascular microenvironment, resulting in the rapid induction of endothelialization (67).

STEM CELL-BASED CELL THERAPY WITH FIBERS

Stem cell-based cell therapy is a promising treatment method due to pluripotency, such as the ability of stem cells to differentiate into various types of cells, the ability to self-renew, and their immunomodulatory properties (68, 69). Among stem cells derived from various origins, mesenchymal stem cells (MSC) have been widely used. However, despite these outstanding advantages, several obstacles must be overcome when used in treatments *in vivo*. Transplanted stem cells face critical barriers, such as poor oxygen levels in the implanted site, low interaction with artificial scaffolds, low cell survival rate, and lack of nutrients. These disadvantages lead to limited therapeutic effects. Scaffolds can help overcome the disadvantages of stem cell therapy (70). Several papers have demonstrated a variety of applications using fibers and stem cells according to various applications, fabricated methods, and differentiation routes for applications in heart tissue regeneration, vascular tissue regeneration, bladder replacements, and cartilage repair (2, 71).

First, cardiomyogenic differentiation of human MSC seeded on scaffolds has been assessed in fibers composed of poly(L-lactic acid)-co-PCL, gelatin and VEGF (PLCL/GV) (72). Promising cardiomyogenic differentiation of MSCs on the scaffolds was observed for cardial regeneration. Second, injectable a-cyclodextrin/PEG–b-PCL-(dodecanedioic acid)-polycaprolactone–poly(ethylene glycol) (MPEG–PCL–MPEG) has been used in hydrogels to investigate cell transplant retention and survival, to reduce infarct expansion and to inhibit left ventricle (LV) remodeling by Okello's group (73). Third, a porous biological scaffold composed of multilayers with MSCs was used as a bioengineered cardiac patch in a syngeneic rat model (74). This strategy using cell multilayers was similar to Okano's group using a cell sheet of monolayered mesenchymal stem cells (MSCs) (75, 76). The multi-layered stem cell cardiac patch-treated rats exhibited improved cardiac function (angiogenic cytokines and cardioprotective factors) with no cell death.

CONCLUSION

Tissue engineering will pave the way for the future of regenerative biomedicine and promote the development of biological alternatives to restore function of damaged tissue/organs. Electrospun nanofibers fabricated by electrospinning, a remarkably simple and versatile technique, can provide the architecture required in the field of cardiovascular tissue engineering. Replacing heart tissue or creating new blood vessels is possible by mimicking the 3D ECM structure according to nanofiber fabrication methods and materials. Transplantation and injection sites must be considered, such as in left-ventricular restraint or intracoronary treatment. Moreover, biodegradable polymeric fiber structures, biomolecules, and stem cells in tissue engineering help increase biocompatibility at the disease site. Future improvements will require the design and fabrication of biomaterials capable of supporting local myocardial microenvironments to enhance the recruitment of resident progenitor cells.

The development of biomaterials is important for scaffold

fabrication. Biomaterials and scaffolds maintain a symbiotic relationship. New implanted material development refers to the creation of new methods. However, most research is focused on cell survival, adherence and migration within a scaffold, and the delivery therapeutic agents. Although the advancement of biomedicine and nanotechnology has great potential, the outcomes are unknown. Currently, we are closer to understanding the conditions capable of inducing heart regeneration within the native heart using various sources, such as stem cells, bioactive molecules, and the characteristics and results learned through new applied studies. Accordingly, the clinical use of nanofiber-based scaffolds remains challenging.

Major challenges remain to be explored regarding *in vivo* applications. The technique parameters for fabricating small-diameter nanofibers need to be optimized, and patient's sensitivity to these treatments must be considered for personalized diagnoses and therapies due to individual physiological and physical differences.

Finally, myocardial regeneration will require a combination of new therapies with existing treatment to induce both endogenous and exogenous effects for maximum efficacies. In this sense, nanofiber-based scaffold approaches may serve as an intriguing repair tool for cardiac tissue engineering. With all of these efforts, tissue engineering will impart a positive impact in the near future.

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