FEATURE ARTICLE

Recent Advances in Electrospun Nanofibrous Scaffolds for Cardiac Tissue Engineering

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Cardiovascular diseases remain the leading cause of human mortality worldwide. Some severe symptoms, including myocardial infarction and heart failure, are difficult to heal spontaneously or under systematic treatment due to the limited regenerative capacity of the native myocardium. Cardiac tissue engineering has emerged as a practical strategy to culture functional cardiac tissues and relieve the disorder in myocardium when implanted. In cardiac tissue engineering, the design of a scaffold is closely relevant to the function of the regenerated cardiac tissues. Nanofibrous materials fabricated by electrospinning have been developed as desirable scaffolds for tissue engineering applications because of the biomimicking structure of protein fibers in native extra cellular matrix. The versatilities of electrospinning on the polymer component, the fiber structure, and the functionalization with bioactive molecules have made the fabrication of nanofibrous scaffolds with suitable mechanical strength and biological properties for cardiac tissue engineering feasible. Here, an overview of recent advances in various electrospun scaffolds for engineering cardiac tissues, including the design of advanced electrospun scaffolds and the performance of the scaffolds in functional cardiac tissue regeneration, is provided with the aim to offer guidance in the innovation of novel electrospun scaffolds and methods for improving their potential for cardiac tissue engineering applications.

1. Introduction

Cardiovascular diseases (CVDs) have become the leading cause of morbidity and mortality worldwide in the past two decades.[1] Myocardial infarction (MI) and heart failure caused by frequently occurred MI are the major causes leading to death among CVDs.[2,3] Due to the limited regenerative capacity,[4] ischemic cardiac tissues cannot self-renew and restore to normal functions after MI. The most effective method to restore heart functions is through heart transplantation,[5] which, however, has been limited by the shortage of organ donors and potential side-effects post transplantation, such as infection and cancers.[6,7] The emergence of tissue engineering technology has offered a practical strategy to develop functional cardiac tissues that can be potentially implanted to treat the dysfunctional myocardium. Besides, there is an urgent demand for engineering of biomimicking healthy and diseased cardiac tissues as in vitro tissue models to reveal the mechanism of CVDs and find non-invasive treatment (e.g., drug development) for diseased cardiac tissues.[8]

A variety of approaches have been explored to engineer 3D cardiac tissues in vitro. One of these strategies is to design biomimetic matrix systems for cardiac tissue construction, such as via the hydrogel technique,[9] prefabricated matrices,[10] decellularized heart tissues,[11,12] and cell sheets.[13,14] Great potential has been demonstrated with different systems, such as developments of large-scaled cardiac constructions using 3D hydrogels, functionalized cardiac tissues by highly decellularized heart tissue, and thicker cardiac patches using multi-layer cell sheets. However, these systems are also associated with several limitations, such as insufficient mechanical strength and suturability of the hydrogel system, low production and immunogenicity of decellularized systems,[15] and massive procedures of culturing 3D tissues in cardiac cell sheet approaches.[16] Prefabricated matrices (e.g., nanofibrous and microporous scaffolds) have offered a feasible strategy to address these challenges in cardiac tissue engineering. Particularly, nanofibrous scaffolds fabricated using electrospinning techniquea have been increasingly explored for engineering functional cardiac tissues, given their structures that strongly mimic the structures to the extracellular matrix (ECM) of native myocardium, excellent mechanical properties, easy manipulation of fiber properties, great material handling and suturability for implantation, and scalable production. More importantly, recent advances in fabricating electrospun scaffolds with complex structures (e.g., aligned, spring-like fiber) and compositions (e.g., biomolecules, nanoparticles) have made it versatile to endow them with extra properties for facilitating the organization and functionalities of the cardiac tissues. For instance, aligned conductive electrospun polyaniline (PANi)/poly (lactic-co-glycolic acid) (PLGA) scaffolds have been demonstrated to promote the organization

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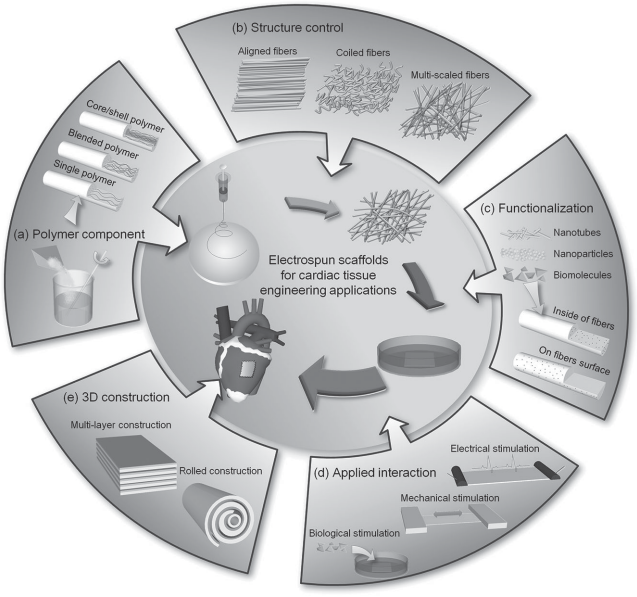

Figure 1. Tissue engineering strategies for regeneration of functional cardiac tissues based on electrospun scaffolds. a) The polymer component of electrospun fibers can be manipulated to form single polymer, blended and core/shell fibers to obtain electrospun scaffolds with suitable mechanical and biological properties for cardiac tissue engineering applications. b) Further control of the fiber structures, including aligned, coiled and multi-scaled nanofibers, can be manipulated to achieve desirable fiber orientation, mechanical properties and pore size to provide structural cues for cultured cells. c) The functionalization of electrospun scaffolds by using nanoscale substances or biomolecules can endow them with additional functions for engineering functional cardiac tissues, such as improved biological properties and electrical conductivity. d) Various stimulations (e.g., biological, mechanical and electrical) during cell culture have shown potential for improving the function and maturation of engineered cardiac tissues. e) As cardiac tissues form on electrospun mats, thicker cardiac patches can be obtained by 3D electrospun construction in multi-layer or rolled manners.

and coupling of cardiomyocytes into cardiac tissues, and their synchronous beating in response to electrical stimulation.[17] Thus, electrospun scaffolds exhibit great potential for cardiac tissue engineering applications in both engineering cardiac tissues in vitro and providing initial structural and conductive support for sustaining their contraction after implantation in vivo (**Figure 1**).

Although there exist several reviews on the approaches for engineering cardiac tissues such as hydrogels,[18] cell sheet,[14,19] and various biomaterial systems,[20,21] the regeneration of functional engineered cardiac tissue by well designed electrospun nanofibrous scaffolds have not been specifically reviewed. In this review, we aim to present an overview of recent studies on electrospun scaffolds with a focus on their applications in cardiac tissue engineering. First, we present various methods to fabricate multi-component (e.g., core/shell and blended), structurally advanced (aligned, multi-scaled and coiled), and functionalized (bioactive and conductive) nanofibrous scaffolds, which all have shown potential to improve the performance of electrospun scaffolds for cardiac tissue engineering applications. Then, we discuss the current applications of these electrospun scaffolds for the construction of functional cardiac tissues, with a focus on the design and manipulation of nanofibrous scaffolds for cardiac tissue regeneration. Finally, the challenges and future perspectives for the development of electrospun materials for engineering functional cardiac tissues are also addressed.

2. Methods of Fabrication of Electrospun Scaffolds

Electrospinning is a feasible technique for fabricating ultrafine polymer fibers (i.e., nanofibers) from polymer solution/melts through a self-assembly process governed by a high electrical field, which is different from conventional techniques involving mechanical force, coagulation chemistry or high temperatures (e.g., wet spinning, melt spinning). An electrospinning system consists of three major components: a high voltage direct current power supply (5 to 50 kV), a spinneret (typically a hypodermic syringe needle) connected to a high-voltage power supply, and a grounded collector. When a high voltage is applied to a liquid droplet at the spinneret, the charged polymer jet is ejected by overcoming the surface tension and stretched into ultrafine fibers due to electrostatic repulsion, which are finally deposited on the grounded collectors.[12] The properties of the electrospun fibers are determined by the pre-electrospinning polymer solution (i.e., molecular weight, concentration, and the solvent) and process parameters (i.e., voltage, flow rate, distance between the spinneret tip and the collector, humidity and temperature). More complex or multi-functional nanofibrous materials can be prepared by using special spinnerets, collectors, or different methods of fiber collection, thus making electrospun materials suitable for a range of application in various industries, especially for biomedical applications. In this part, the strategies on the fabrication of electrospun scaffolds that could be potentially utilized for cardiac tissue engineering will be detailed.

2.1. Design of the Polymer Component

To date, more than 200 polymers have been successfully electrospun to nanoscale or microscale fibers. However, only those with excellent biocompatibility have been widely utilized for tissue engineering applications, including both synthetic (e.g., polycaprolactone (PCL), poly-L-lactic acid (PLLA) and polyurethane (PU)) and natural (e.g., gelatin, collagen and silk fibroin) polymers. Besides biocompatibility, the electrospun scaffolds also need to offer additional properties required for cardiac tissue engineering, including a degradation rate that is comparable to the regeneration rate of the native ECM for providing sustained support for cardiac tissue regeneration, mechanical properties that match those of human myocardium (Young's modulus and tensile strength are up to ≈0.5 MPa and ≈15 kPa, respectively), and improved scaffold conductivity for inducing the electromechanical function of the engineered cardiac tissues. **Table 1** lists all the polymers that have been fabricated into electrospun scaffolds and explored for cardiac tissue engineering. Given the rigorous requirements for the scaffolds used in cardiac tissue engineering, it is challenging to find a single electrospinnable polymer that can fulfill all the needs, and great interest has been paid for the fabrication of electrospun scaffolds using multiple polymers, including blended or core/shell electrospun scaffolds.

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Table 1. The polymers used for fabricating the electrospun scaffolds for cardiac tissue engineering applications.

Polymer	Solvent	Fiber diameter [nm]	Advantages	Advanced properties	Ref
Single polymer					
PCL	THF/DMF	≈2500	Biocompatible, controllable on fiber diameter and mechanical properties	Conductive by CNTs	[54]
	DCM/DMF	300–5000		Spring-like fibers, and functionalized by gold nanoparticles	[45,47]
	chloroform/ methanol	2500–4100		Functionalized by plasma	[72]
PLLA	Dichloromethane	900–1700	Biocompatible, suitable mechanical properties of its electrospun fibers for cardiac tissue engineering	Aligned, or functionalized by G-CSF	[49,70]
PU	Dichloromethane	1000–7000	Biocompatible, elastomeric tensile mechanical properties, flexible on its chemistry component	Aligned fibers	[42]
PLGA	Chloroform/acetone	2000–4000	Biocompatible and biodegradable, FDA-approved and suitable mechanical properties for cardiac tissue engineering	Multi-scale fibers, or conductive by PANi	[17,46]
	HFP	400–1300		Functionalized by YIGSR and RGD	[61]
PHB	CHCl3	500–3000	Biocompatible, angiogenic capacity and prevention of negative remodeling in infarcted tissue		[73]
Albumin	TFE/water		Excellent biocompatibility and biodegradability, elastic mechanical properties		[74]
Blended polymers					
PCL–PGS	Ethanol/anhydrous chloroform	2000–5000	Controllable degradation rate, mechanical and thermal properties		[32]
PCL–gel	HFP	200–300	Controllable mechanical properties and hydrophilicity, promote cell attachment	Aligned fibers, or conductive by PPy	[25,44,57,75]
PGS–gel	Acetic acid /water	500–1000	Controllable mechanical and structural properties, promote tissues formation, cardiac-specific protein expression and contraction of cardiomyocytes	Aligned fibers	[22]
PLGA–col	HFP	240–360	Suitable mechanical properties and hydrophilicity, promote cell differentiation of ESCs to cardiomyocytes		[27]
PLGA–gel	HFIP	110–220	Suitable mechanical properties, promote cardiac-specific protein expression and tissues formation		[28]
POC–PLCL	HFP.	400–580	Biocompatible, 40:60 POC/PLCL sample show highly suitable tensile strength and elastic modulus comparable to human heart		[29]
PVA–BSA	Water	250–400	Suitable mechanical properties, promote cell attachment, proliferation and cardiac-specific protein expression	Functionalized by gold nanoparticles	[26]
Fib–gel	HFP	120–320	Reinforced mechanical properties compared to Fib fibers, excellent biological properties	Functionalized by TGFβ_2	[24,76]
Core/shell polymers					
PGS/gel	HFP, TFE	350–450	Highly elastic compared to Gel fibers, hydrophilic, and promote cell proliferation and cardiac specific protein expression	Buckled structures	[23,77]
PGS/col	HFP, HFP	900–1400	Mechanical properties comparable to that of human heart, hydrophilic, cell proliferation and differentiation of MSCs were improved compared to Col fibers		[59]
PGS/PLLA	THF, chloroform/ DMF	1000–2400	Reinforced tensile strength, rupture elongation, and stiffness comparable to myocardium		[69,78]
PLCL/gel	Chloroform, water	300–400	Suitable mechanical properties for cardiac tissue engineering, and controlled release of functional agent	Functionalized by VEGF	[79]

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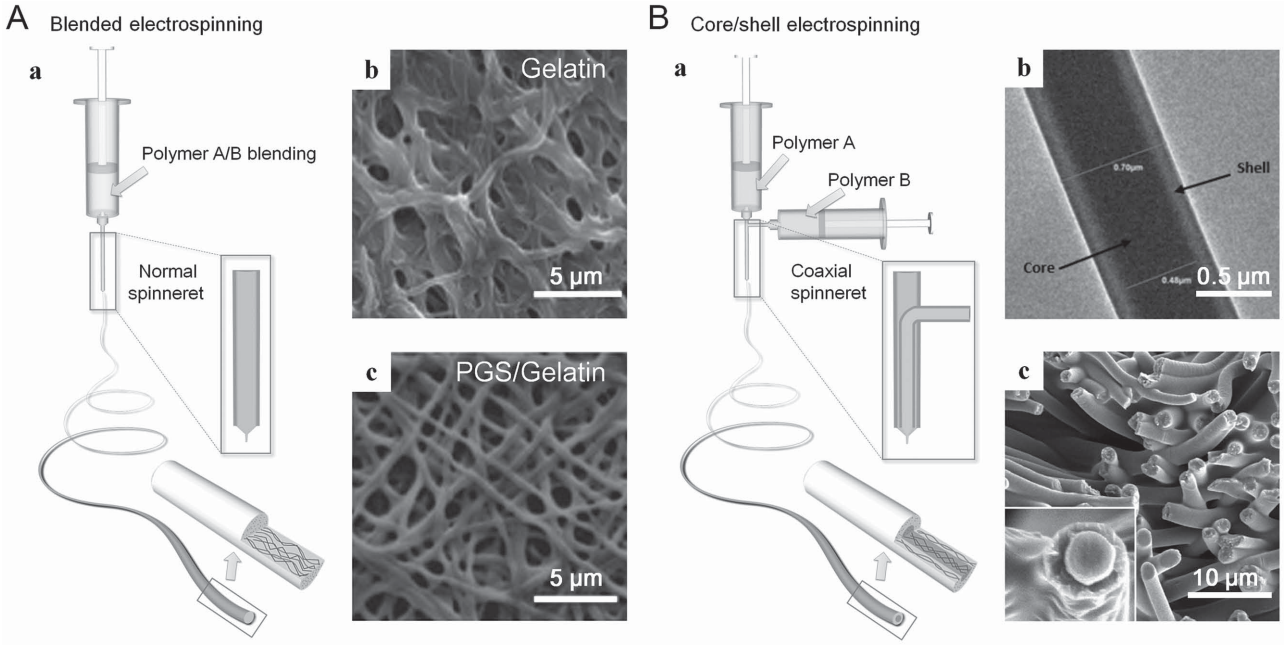

Figure 2. Blended and core/shell electrospinning. A) Blended electrospinning. a) Schematic of blended electrospinning, where polymer blending is electrospun by normal spinneret to form blended electrospun fibers. b,c) Scanning electron microscope (SEM) images of electrospun gelatin fibers and PGS/gelatin fibers showing their different morphology (scale bar: 5 μm). Panels (A-b,c) reproduced with permission.[22] Copyright 2013, Elsevier. B) Core/shell electrospinning. a) Schematic of core/shell electrospinning, in which shell polymer solution (polymer A) and core polymer solution (polymer B) flow through a coaxial spinneret and are electrospun into core/shell fibers. b) TEM image showing the core/shell structure inside of a fiber (scale bar: 0.5 μm). Reproduced with permission.[68] Copyright 2015, Elsevier. c) SEM image showing the cross section of core/shell scaffolds (scale bar: 10 μm). Reproduced with permission.[69] Copyright 2013, Elsevier.

2.1.1. Blended Electrospun Scaffolds for Cardiac Tissue Engineering

A blended electrospun scaffold can be fabricated by using a polymer blend (**Figure 2**A), aiming to fine-tune the mechanical, chemical and biological properties of the scaffolds to promote cardiac tissue regeneration. The most typical blended electrospun scaffolds are made of natural and synthetic polymer blends, leveraging the intrinsic good biocompatibility of natural polymers and the excellent mechanical properties of synthetic polymers. For instance, gelatin has been frequently used to fabricate electrospun scaffolds for engineering cardiac tissues, which, however, have limited clinical applications due to their insufficient mechanical strength (≈0.1 MPa) and fast degradation rate (water soluble).[22] Although further cross-linking treatments can slightly improve the mechanical strength of the scaffolds, this has raised another concern of using non-biocompatible cross-linker (i.e., glutaraldehyde).[23,24] To overcome these challenges, various blended PCL/gelatin and similar scaffolds have been electrospun,[25–28] such as poly(glycerol sebacate) (PGS)/gelatin (Figure 2A), poly(lactic-co-glycolic) acid (PLGA)/collagen, PLGA/gelatin, polyvinyl alcohol (PVA)/albumin from bovine serum (BSA).

Besides adjusting different polymer combinations, the ratio of polymer blends can also be changed to fine-tune the mechanical properties and the degradation rate to match natural ECM. For example, poly(1,8-octanediol-cocitrate) (POC)/poly(L-lactic acid)-co-poly-(3-caprolactone) (PLCL) blended electrospun scaffolds with mechanical properties (tensile strength of 1.04 MPa, Young's modulus of 0.51 MPa) comparable to native cardiac tissue have been fabricated by adjusting their weight ratio to 40:60, while the degradation time of PGS/PCL blended electrospun scaffolds can be controlled from fast (less than 100 days) to slow (2–4 years dependent on the molecular weight) in a similar way.[29]

Blended nanofibrous scaffolds using more than two polymers have also been electrospun to achieve better control the properties of scaffolds, or to achieve additional functions for cardiac tissue engineering applications.[30,31] For instance, the ability of polyethylene glycol (PEG)/PCL/carboxylated PCL (CPCL) electrospun scaffolds to promote the differentiation of murine ESCs to cardiomyocytes has been improved by controlling their weight ratio to 4%, 86%, and 10% respectively, which may be due to the optimized mechanical and chemical properties. In another case, hemoglobin/gelatin/fibrinogen blended scaffolds improved O_2 transport capability, exhibiting great potentials in promoting the cardiomyogenic differentiation of MSCs. These improvements are most attributed to the closely mimicking of the scaffolds to the native ECM in human myocardium, including mechanical strength and biological functions.

2.1.2. Core/Shell Electrospun Scaffolds for Cardiac Tissue Engineering

Core/shell electrospinning is another strategy to obtain multi-component electrospun scaffolds, where the core/shell electrospun scaffolds can be fabricated by using a coaxial spinneret to eject two or more polymer solutions simultaneously, thus

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forming a layered structure along the radial direction of the fibers (Figure 2B). The outer solution, which forms the shell of the fibers, should be electrospinnable to promote the formation of core/shell structure, while the inner solution for the-fiber core does not have to be. By using coaxial electrospinning, fibers with advanced structures, including core/shell fibers, hollow fibers, and core fibers in which the shell is removed post-fabrication, can be obtained by carefully selecting the types of polymers to form the core and shell of the fibers. In addition, the diameters of the core and shell of the coaxial fibers can be controlled by manipulating the relative flow rates of polymer solutions.

For biomedical applications, the core polymer mainly determines the mechanical properties and stability of the core/shell fibers, while the shell polymer determines their biological properties. Therefore, the polymers for the core or shell usually have certain characteristics, which can be indicated from the polymers utilized by coaxial electrospinning for engineering cardiac tissues. For example, PGS has been intensively used as the core polymer due to its excellent mechanical properties, while natural polymers (e.g., collagen, gelatin and chitosan) have been widely employed to form the fiber shell given their excellent biological properties.

Core/shell electrospun scaffolds offer several advantages compared to the blended electrospun scaffolds, leveraging the merits of both core and shell polymers that may be attenuated by each other in the blended electrospun scaffolds. For instance, the PGS/gelatin core/shell scaffolds offer similar mechanical strength to PGS and similar biological properties to gelatin. Besides, the degradation rate of the PGS/gelatin fibers is mainly determined by the cross-linked gelatin (shell), whereas the blended fibers consisting of PGS and other polymers may suffer from mass loss and reduction in mechanical strength as induced by the relatively faster degradation rate of PGS.[32] Therefore, the core/shell scaffolds would have better stability than the blended scaffolds made from the same polymers, which is critical for the long-term cyclic stretch of engineered cardiac tissues upon implantation in vivo.[33] However, most natural polymers require an extra crosslinking step to become insoluble in aqueous solutions when serving as the shell materials, which may impact their biocompatibility due to the introduction of inbiocompatible cross-linkers. Thus, natural polymers such as silk fibroin with excellent biological properties, controllable degradation rates, and no need to use cross-linkers can be ideal candidates for cardiac tissue engineering applications.[34]

2.2. Electrospun Scaffolds with Controlled Structure

The control over the structural properties of electrospun scaffolds (e.g., fiber morphology, fiber diameter, and fiber orientation) has demonstrated as an important factor in improving their performance for tissue engineering applications. By manipulating the process and parameters of the electrospinning, scaffolds with special patterns such as aligned, coiled, or multi-scaled fibers can be obtained, which could provide structural cues to the cultured cells for promoting their maturation to functional cardiac tissues.

2.2.1. Electrospun Scaffolds with Aligned Fibers

Electrospinning has been demonstrated as a versatile technique with the capability of fabricating fibres aligned in parallel by controlling the method of fiber collection. The aligned fibrous materials are of great interests for tissue engineering applications, as these structures possess unique electrical, optical, and mechanical properties, similar to most native tissues (**Figure 3**A). For instance, the aligned fibers mimicking the parallel orientation of native tissues have demonstrated favorable cell adhesion, migration and proliferation for cardiac, neural, and skeletal muscular tissues.[35] A number of approaches have been explored for the alignment of polymeric fibers, including the utilization of rotating collectors with or without ancillary setups (e.g., insulated cylinder collector and adjacent counter electrode,[36] wire drum collector,[37] and two spinnerets with opposite voltages and directions,[38] and non-rotating collectors (e.g., metal frame and two separated conductive substrates.[39,40]

For native myocardium, the aligned feature is critical for the transmission of electrical signals and realization of systematic contractions, which is important for the physiological functions. By using aligned electrospun scaffolds, several improvements on cultured cardiac tissues have been observed, including the orientation and organization of cultured cells,[41] and the electrophysiological function of cardiac tissues.[22] For instance, more highly organized cardiac tissues (anisotropic and improved sarcomere organization) have been formed on aligned polyurethane (PU) scaffolds from murine embryonic stem cells (ESCs) compared to random fiber orientation,[42] and better synchronized beating of cardiac tissues has been observed on aligned PGS/gelatin blended electrospun scaffolds compared to random scaffolds.[22] Similar phenomena were also observed in many other investigations.[30,43,44] All these studies indicate that the aligned electrospun scaffolds could be ideal matrices for guiding the cardiomyocytes into organized tissues that closely mimic the native myocardium.

2.2.2. Electrospun Scaffolds with Multi-Scaled Structures

The diameter of nanofibers in scaffolds is also important because it can influence many properties of scaffolds, such as biocompatibility, mechanical strength, pore size, the ratio of area–volume, etc. As the diameter of proteins in native ECM is at the nanoscale, the scaffolds used for cardiac tissue engineering applications mostly consists of nanofibers with diameters of several hundred nanometers. But microfibers have also been applied in cardiac tissue engineering with promising results. For example, microscale PCL electrospun fibers have shown ability for prompting the formation of functionalized cardiac tissues,[45] maybe due to the significantly reinforced mechanical strength and increased pore size of microfibers compared with nanofibers. Therefore, the fabrication of electrospun scaffolds with larger fiber diameter, by selecting suitable polymer and solvent, could be an alternative for cardiac tissue engineering applications.

It is also possible to take advantage of microfibers by blending microfibers with nanofibers to produce multi-scaled

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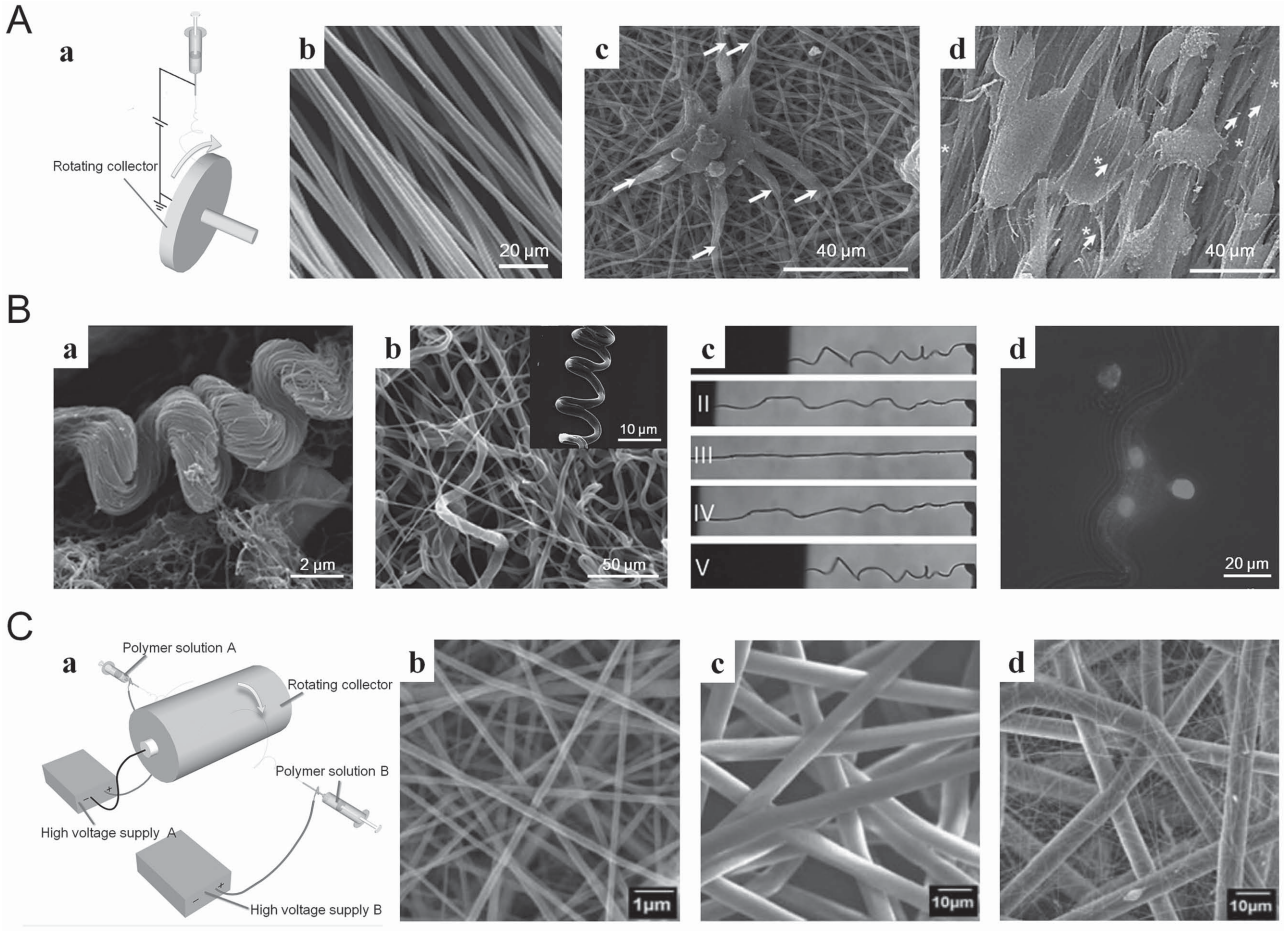

Figure 3. Structural control on electrospun fibers. A) Aligned electrospun scaffolds and their guidance for cell orientation. a) Schematic of aligned electrospun scaffold fabrication by using high-speed rotating collector. b) SEM image of aligned fibers collected by rotating collector (scale bar: 20 μm). Panel (A-b) reproduced with permission.[64] Copyright 2011, Elsevier. c,d) SEM images of cultured cells on random electrospun scaffolds and aligned electrospun scaffolds respectively (scale bar: 40 μm). Reproduced with permission.[70] Copyright 2005, Elsevier. B) Coiled electrospun scaffolds. a) SEM image of coiled perimysial fibers in decellularized hearts (scale bar: 2 μm). b) SEM image of coiled electrospun PCL fibers (scale bar: 50 and 10 μm). c) Photographs of coiled fibers stretched using an AFM tip showing its spring-like properties. d) Immunostaining of cardiomyocytes attached to a coiled fiber; sample was stained for α-actinin (pink) and nuclei (blue) (scale bar: 20 μm). Panel (B) reproduced with permission.[45] Copyright 2013, Elsevier. C) Multi-scaled electrospun scaffolds. a) Schematic of a multimodal electrospinning system, where the electrospun fibers A/B blending can be obtained on a slow-speed rotating collector. b) SEM image of nanoscale electrospun scaffolds (fabricated by 12 wt% PCL in $CHCl_3$/CH_3OH) (scale bar: 1 μm). c) SEM image of microscale electrospun scaffolds (fabricated by 30 wt% PCL in $CHCl_3$) (scale bar: 10 μm). d) SEM image of multi-scaled electrospun scaffolds fabricated by multimodal electrospinning using 12 wt% PCL solution and 30 wt% PCL solution (scale bar: 10 μm). Panels (C-b–d) reproduced with permission.[71] Copyright 2010, Taylor & Francis.

scaffolds (Figure 3C), which has shown potential for tissue engineering applications.[20] By using a multimodal electrospinning system, scaffolds consisted of fibrin nanofibers (50–500 nm) and PLGA microfibers (2–4 μm) were fabricated,[46] which significantly improved the differentiation of umbilical cord blood mesenchyme stem cells (UCBMSCs) to cardiomyocytes and cell attachment and infiltration. Another PU scaffold with multi-scaled structure has also shown beneficial effect in the differentiation of mouse embryonic stem cell-derived cardiomyocytes (mESCDCs) to mature phenotype.[42] The significantly increased pore size in multi-scaled electrospun scaffolds could be helpful for cell infiltration, thus be beneficial for the formation of cardiac tissues.

2.2.3. Electrospun Scaffolds with other Fiber Patterns

The fibers with special patterns, such as coiled (Figure 3B) and buckled fibers,[23,45,47] can also be obtained by electrospinning. Such fiber structures have exhibited the capacity to tune the mechanical properties of the electrospun scaffolds to match that of the native myocardium. For instance, Fleischer et al. fabricated PCL electrospun scaffolds with coiled fibrous morphology by controlling the flow rate (0.5 mL h^{-1} for coiled fibers vs. 7 mL h^{-1} for straight fibers), and found that the cardiac tissues grown on the coiled fibrous scaffolds exhibited stronger contraction forces, higher beating rates and lower excitation thresholds than those on the straight fibrous

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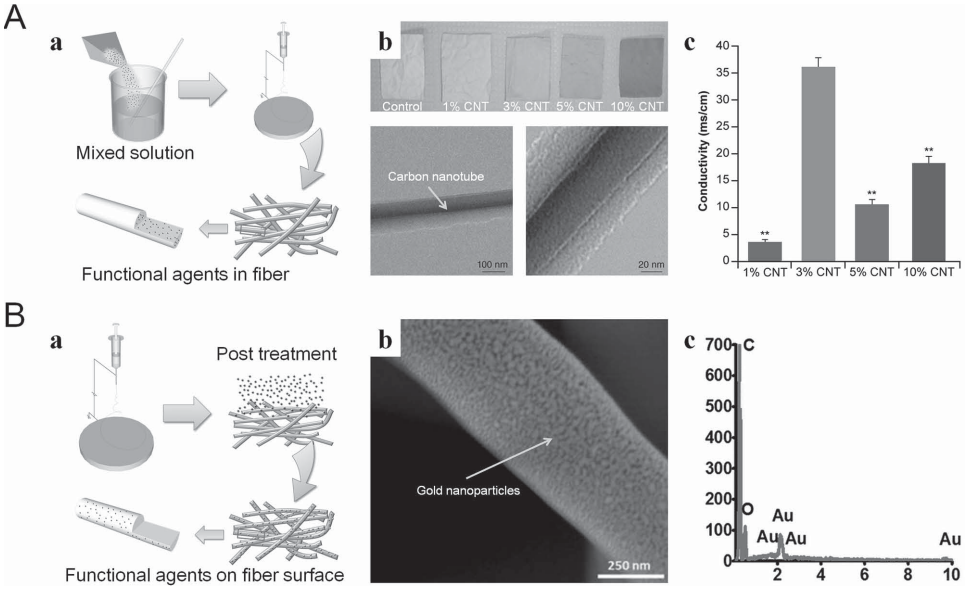

Figure 4. Functionalization of electrospun scaffolds during and post fabrication. A) The functionalization of electrospun scaffolds by introducing functional agents into the polymer solution during fabrication: a) Schematic of the procedure for fabricating functionalized scaffolds where functional agents are embed inside of fiber; b) Carbon nanotube-functionalized PCL electrospun scaffolds by electrospinning of blended CNTs/PCL solution; photograph shows the appearance of PCL scaffolds functionalized by CNTs at different concentrations (top);transmission electron microscope (TEM) images show carbon nanotubes encapsulated in a fiber with different magnification (bottom) (scale bar: 100 and 20 nm). Panel (A-b) reproduced with permission.[54] Copyright 2013, Elsevier. c) Effect of carbon nanotubes on the conductivity of PCL/CNTs electrospun scaffolds. Reproduced with permission.[54] Copyright 2013, Elsevier. B) The functionalization of electrospun scaffolds by post-treatment of scaffolds. a) Schematic of the procedure for functionalizing electrospun scaffolds by depositing the functional agents on fiber surface. b) TEM image shows the gold nanoparticles deposited on the surface of a PCL fiber (scale bar: 250 nm). Panel (B-b) reproduced with permission.[47] Copyright 2014, Royal Society of Chemistry. c) EDX spectrum further shows the existence of Au in post-treated electrospun scaffolds. Reproduced with permission.[47] Copyright 2014, Royal Society of Chemistry.

scaffolds.[45] This could be attributed to their morphology and mechanical properties mimicking the coiled perimysial fibers in the natural heart matrix.[48] In another study, a charged (15 kV) metal ring (diameter 30 mm) was set around the tip of the spinneret concentrically to obtain orthogonal and looped oriented PGS/gelatin core/shell scaffolds. These scaffolds exhibited mechanical properties comparable to those of the native myocardium, with Young's modulus of 3.59 MPa and 2.07 MPa, respectively. Moreover, these scaffolds also displayed the capability of inducing cardiogenic differentiation of adipose-derived stem cells (ADSCs) with higher expression of cardiac-specific markers at both gene and protein levels. Therefore, such patterning of electrospun scaffolds could also provide them with unique and useful properties for engineering functional cardiac tissues.

2.3. Functionalization of Electrospun Scaffolds

Besides employing multiple polymers, addition of functional agents (i.e., nanoparticles and biomolecules) into the electrospun materials has shown to be another practicable strategy to modify the scaffold properties and endow them with specific functions for different applications. There are generally two ways to add the additives to the electrospun materials, i.e., by directly adding the additives into polymer solutions before electrospinning or by embedding the electrospun scaffolds in an additive solution/dispersion to absorb additives (**Figure 4**). Both methods have shown great success in realizing the functionalization of the electrospun scaffolds. For cardiac tissue engineering applications, improvements in scaffold conductivity is as important as their biological and mechanical properties to promote the functionalization and maturation of the regenerated cardiac tissues, which can be achieved by adding conductive agents, such as carbon nanotubes and graphene to the scaffolds.

2.3.1. Electrospun Scaffolds with Improved Biological Properties

Functionalizing the electrospun materials with bioactive molecules offers an option to endow specific biological functions to the materials in addition to their intrinsic biocompatibility, such as the abilities to promote cell homing, survival, or differentiation to specific cell types. The incorporated biomolecules usually take effect in a controlled fashion with high efficiency along the degradation of the electrospun materials. For instance, Yu et al. conjugated YIGSR and RGD (adhesive peptides derived from laminin) to poly-L-lysine (PLL) and then blended with PLGA solution to fabricate functionalized scaffolds, which enhanced the adhesion of cardiomyocytes and the formation of functional cardiac tissues. PLLA electrospun scaffolds functionalized with granulocytes colony-stimulating factor (G-CSF) have also demonstrated

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the ability to promote the differentiation of C2C12 murine skeletal myoblasts to cardiomyocyte phenotype.[49] In addition, post-treatment of electrospun scaffolds with bioactive substance is also practicable, e.g., fibronectin post-treated chitosan electrospun scaffolds improved the elongated shape and adhesion of cardiomyocytes.[50] Therefore, incorporation of biomolecules into electrospun materials either during the fabrication process or through the post-treatment can effectively provide biological cues for the maturation of the regenerative cardiac tissues.

2.3.2. Electrospun Scaffolds with Improved Conductivity

The powerful contraction force of ventricle comes from the synchronous contraction of cardiomyocytes, which is driven by the electrical signal and dominated by the excellent electromechanical functions of the ECM in native myocardium. Due to the low conduction velocity of the traditional scaffolds utilized *in* cardiac tissue engineering, the engineered tissues often lack the capability of keeping pace with the contraction rhythm of the native myocardium, therefore arrhythmia is frequently observed after the implantation of engineered cardiac tissues.[51] Electrospun scaffolds with excellent conductivity could be helpful to reduce the chance of post-implantation arrhythmia because of the improved sensing of the native electrical signals. Besides, the rhythmic contraction of cardiomyocytes can also promote cell alignment and electrical coupling,[52] thus be beneficial to promote the differentiation of cultured cardiac tissues in vitro. Therefore, the improvements in the conductivity of electrospun scaffolds would be crucial for the performance of the regenerated cardiac tissues.

Unfortunately, there are no such polymers that simultaneously possess excellent conductivity, mechanical and biological properties suitable for cardiac tissue engineering applications. Therefore, functionalizing the electrospun scaffolds with conductive and biocompatible additives could offer an alternative approach to overcome these challenges. For instance, carbon nanotubes (CNTs) were widely used to fabricate conductive composite biomaterials because of their excellent conductivity and acceptable biocompatibility (Figure 4A).[53–55] Based on this, CNTs have been used to obtain conductive electrospun scaffolds (e.g., PCL-CNTs electrospun composite scaffolds with conductivity up to 35 mS/cm,[54] which significantly improve their potential for engineering functional cardiac tissues. Other conductive nano-structured substances were also incorporated into the electrospun materials, such as AuNPs (Figure 4B), which showed potential in promoting the differentiation of MSCs to cardiomyogenic cells mainly due to the improved conductivity and mechanical strength of the AuNP-loaded scaffolds.[26,47] Besides inorganic conductive agents, some conductive and biocompatible polymers such as polyaniline (PANi) and polypyrrole (PPy) have been utilized to obtain conductive blended electrospun scaffolds.[17,56,57] Such scaffolds can be easily electrospun, and their conductivity manipulated by controlling the component ratio of conductive polymer; however, the biological and mechanical properties of electrospun scaffolds may be compromised due to the change on their chemical composition.[58]

3. Applications of Electrospun Scaffolds for Cardiac Tissue Regeneration

3.1. Cell Survival and Differentiation of Cardiomyocytes

The scaffolds for cardiac tissue engineering should support cell survival and promote cell differentiation to cardiac phenotype, which can be achieved by electrospun scaffolds due their biomimicking nanofibrous structure and excellent biocompatibility.[59,60] Moreover, the functionalization of electrospun scaffolds by bioactive factors can further enhance their biological properties. For instance, the adhesion and growth of cardiomyocytes can be significantly enhanced by incorporation of YIGSR (**Figure 5**B) and RGD,[61] and fibronectin.[50]

Some electrospun scaffolds have also shown ability to promote the expression of cardiac specific marker proteins (i.e., α-sarcomeric actinin, troponin), which represents the cardiomyogenic differentiation of cultured cells. For instance, ESCs seeded on blended electrospun scaffolds of 4%PEG–86%PCL–10%CPCL exhibited highest α-MHC expression due to suitable mechanical properties compared to other polymer compounding ratios.[31] In another case, multi-scaled fibrin-PLGA scaffolds fabricated by simultaneous electrospinning of fibrin and PLGA can enhance the expression of α-sarcomeric actinin, troponin, tropomyosin, and desmin.[46]

Electrospun scaffolds functionalized by some bioactive molecules are also good for cardiac phenotype differentiation of cultured cells. For instance, PLLA scaffolds functionalized with granulocytes colony-stimulating factor were fabricated by electrospinning of G-CSF/PLLA solution blending, which enhanced the co-expression of cTnI and Cx43 of C2C12 murine skeletal myoblasts, indicating pre-differentiated phenotypes of cardiomyocytes.[49] Conductive electrospun scaffolds, achieved by adding carbon nanotubes, also showed potential for promoting cardiac differentiation of human mesenchymal stem cells (hMSCs) even without electrical stimulation.

3.2. Cardiac Tissue Formation

As the cells cultured on electrospun scaffolds are capable of survival and differentiation into cardiomyocytes, further attention should be paid to induce the formation of organized cardiac tissues, which is essential for electrical signal transmission and synchronous and powerful myocardium contractions (Figure 5). The presence of gap junction between cells is an evidence for tissues formation. For instance, expression of gap junction protein Cx43 can be promoted by functionalizing electrospun scaffolds with G-CSF,[49] or conductive PANi (Figure 5C).[17] Co-culture of cardiomyocytes and fibroblasts on electrospun scaffolds is another strategy to induce cardiac tissues formation, as confirmed by the polarized cardiomyocyte morphology and synchronized contractions over long-term culture.[50]

The morphology and orientation of cardiac tissues formed on electrospun scaffolds can also be controlled to mimic native myocardium, which is highly ordered and organized by rod-shaped cardiomyocytes. Anisotropic cues generated by structural design or mechanical and electrical stimulations

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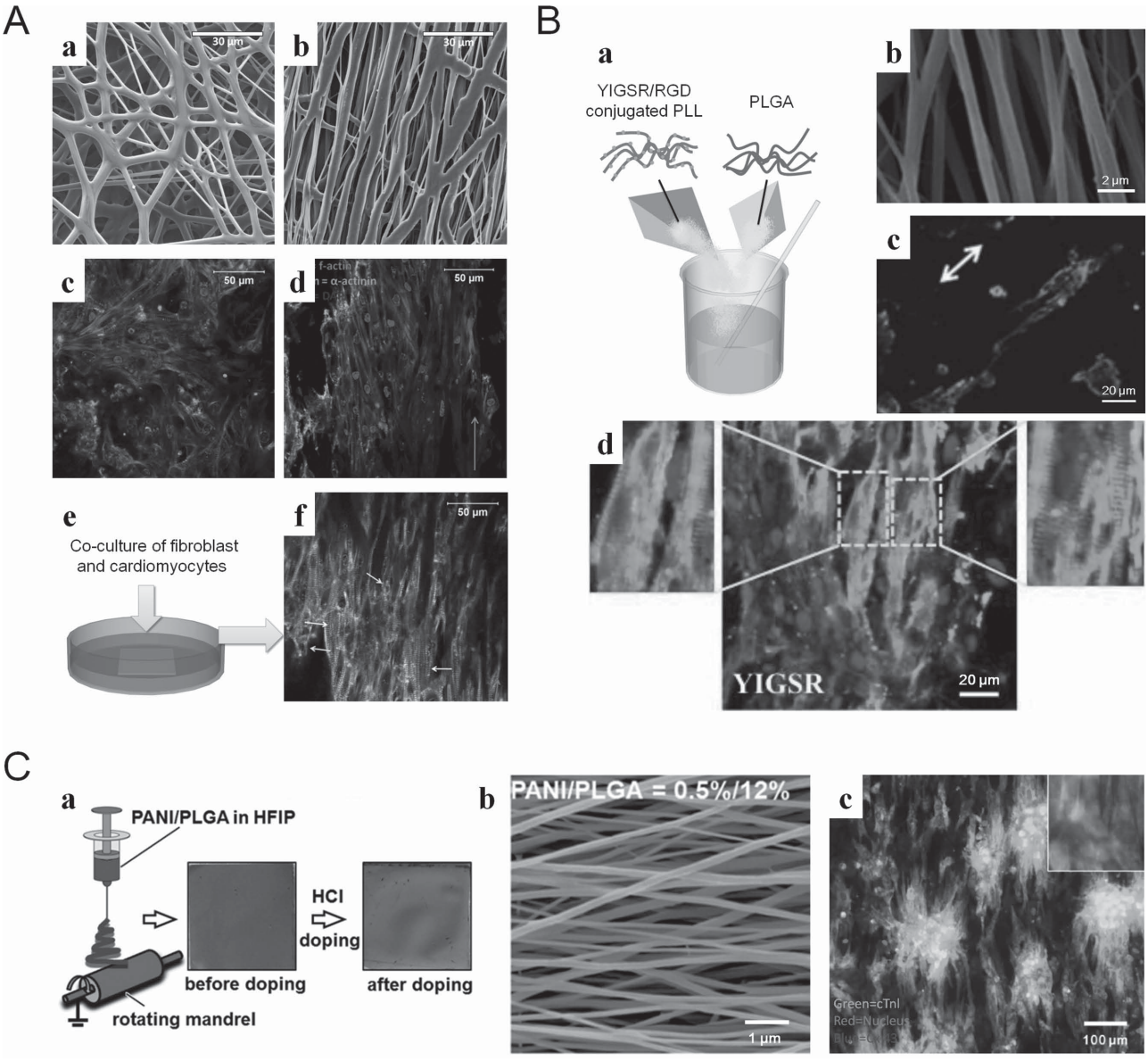

Figure 5. Formation of cardiac tissues on electrospun scaffolds. A) Tissue formation on aligned electrospun polyurethane scaffolds. a) and b) SEM images of aligned and random electrospun polyurethane scaffolds respectively (scale bar: 30 μm). c,d) Immunofluorescent images show cardiac tissues formed on aligned scaffolds represent higher degree of cardiac-specific protein expression, rod-shape of cells and sarcomere formation (scale bar: 50 μm); e) Co-culture of fibroblast and cardiomyocytes, and f) immunofluorescent image shows co-culture of fibroblasts with cardiomyocytes further promotes the changing of cells morphology to rod-shape and the formation of sarcomere (scale bar: 50 μm); Samples were stained for f-actin (red), nuclei (blue) and α-actinin (green). Reproduced with permission.[42] B) Tissues formation on YIGSR/RGD functionalized electrospun PLGA scaffolds. a) Schematic of the solution preparation for fabricating YIGSR/RGD functionalized PLGA scaffolds. b) SEM image of aligned YIGSR/RGD functionalized PLGA scaffolds (scale bar: 2 μm). c) Immunofluorescent image shows cardiomyocytes cultured on control PLGA scaffolds (scale bar: 20 μm). d) Immunofluorescent image shows much higher cardiac-specific protein expression and sarcomere formation of cardiomyocytes cultured on YIGSR functionalized PLGA scaffolds (scale bar: 20 μm); Samples were stained for α-actinin (green) and nucleus (blue). Reproduced with permission.[61] Copyright 2014, Mary Ann Liebert, Inc. C) Tissues formation on conductive electrospun PANi/PLGA scaffolds. a) Schematic of PANi/PLGA electrospun scaffolds fabrication. b) SEM image of aligned electrospun PANi/PLGA scaffolds (scale bar: 1 μm). c) Immunofluorescent image shows organized cardiomyocytes clusters formation and Cx 43 expression intercellularly in clusters (scale bar: 100 μm). Samples were stained for cTnI (green), nucleus (red) and Cx 43 (blue). Reproduced with permission.[17] Copyright 2013, Elsevier.

through electrospun scaffolds hold great potentials in inducing reorganization and maturation of cardiac tissues. The most frequently used method is patterned electrospun scaffolds, which provide cultured cells with a structural cue.[17,22,42,62] For instance, aligned PU scaffolds have demonstrated to promote the anisotropic organization of mESCDCs, confirmed with sarcomere formation and cell morphology change to an elongated and rod shape (Figure 5A).[42] In addition, co-culture of fibroblasts along cardiomyocytes on electrospun scaffolds can further improve the ordered sarcomere formation,[42,50] indicating

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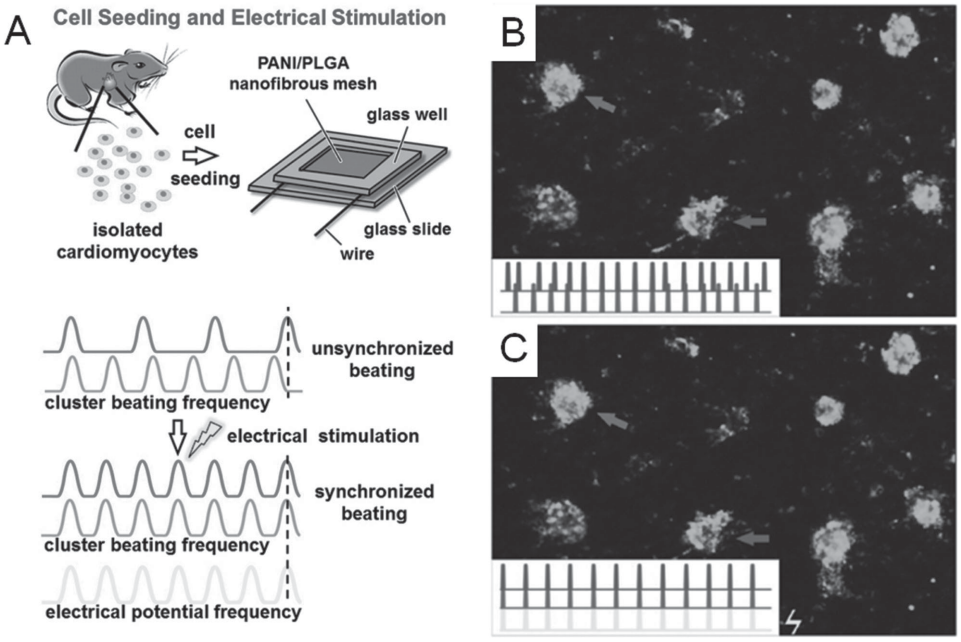

Figure 6. The electrophysiological function of cardiac tissues cultured on conductive electrospun scaffolds. A) Schematic of cardiomyocytes seeding on conductive PANi/PLGA scaffolds and the application of electrical stimulation (top), and the change on cluster beating frequency of two cell clusters upon rhythmic electrical stimulation. B) The beating frequency of two separated cell clusters without the electrical stimulation (red and blue arrows indicates the two recorded clusters, and their beating frequency is showed by red and blue lines respectively). C) The beating frequency of two separated cell clusters when apply the rhythmic electrical stimulation shows the beating of the two clusters become synchronous with the electrical stimulation (the frequency of electrical stimulation is showed by yellow lines). Reproduced with permission.[17] Copyright 2013, Elsevier

high-level organization of cardiomyocytes within the engineered cardiac tissues.

3.3. Enhancement of the Functionality of Cardiac Tissues

The advanced goal of cardiac tissue engineering is to regenerate the synchronously rhythmic beating function of myocardium. By selecting suitable cell source, including primary cardiomyocytes or ESC-derived cardiomyocytes,[17,42,50] spontaneous contraction can be observed in cardiac tissues cultured on electrospun scaffolds. As cardiac tissues are formed on electrospun scaffolds, the organization of cardiomyocytes is highly related with their contraction. For instance, the cardiomyocytes cultured on aligned PGS/Gelatin scaffolds after 7 d were found to have significantly fast beating rate and distinct beating pattern compared with those cultured on random scaffolds.[22] The mechanical properties and chemical composition of electrospun scaffolds also has influence on the contraction of cardiomyocytes, as evidenced by the observation that the synchronized beating rate of cardiomyocytes on PGS/gelatin scaffolds was higher than that on gelatin scaffolds.[22]

The synchronous contraction of cardiomyocytes is a sign for the electrical coupling between cardiac tissues, which is important for the rapid transmission of native electrical signal to avoid the occurrence of arrhythmias when the engineered cardiac tissues are implanted in vivo. Control of the conductivity of electrospun scaffolds would be helpful for both the synchronous beating of cardiomyocytes in vitro and the transmission of electrical signal in vivo. Intriguing achievement has been made by adding a conductive polymer (PANi) into a PLGA solution to fabricate composite electrospun scaffolds (**Figure 6**).[17] Primary cardiomyocytes seeded on the aligned PANi/PLGA scaffolds formed clusters at day 3, with cardiomyocytes among each cluster beating spontaneously and synchronously. Although the beating rates between individual clusters were asynchronous initially, the beating rate of clusters cultured on highly conductive scaffolds became synchronous upon electrical stimulation. This finding indicates that the cardiac tissues formed on conductive electrospun scaffolds could potentially beat synchronously with the native myocardium by the stimulation of internal electrical signal after implantation.

3.4. Construction of 3D Cardiac Tissue

Engineering of cardiac patches with thicknesses of several millimeters is required for clinical applications. However, the oxygen and nutrients can only diffuse through patches thinner than approximately 400–500 μm. Therefore, there is a great demand for the improvement of culture medium perfusion through thicker 3D cardiac patches. The vascularization of cardiac tissues is a desirable strategy to provide 3D structure with biomimicking channels for liquid perfusion, and have already been achieved on 3D biomaterial construction. For instance, the biotechnology of co-culturing endothelial cells and even fibroblasts with cardiomyocytes to create a vascular network within cardiac tissues has been demonstrated on porous sponges,[63] which can be easily transformed for the vascularization of cardiac patches on electrospun scaffolds.

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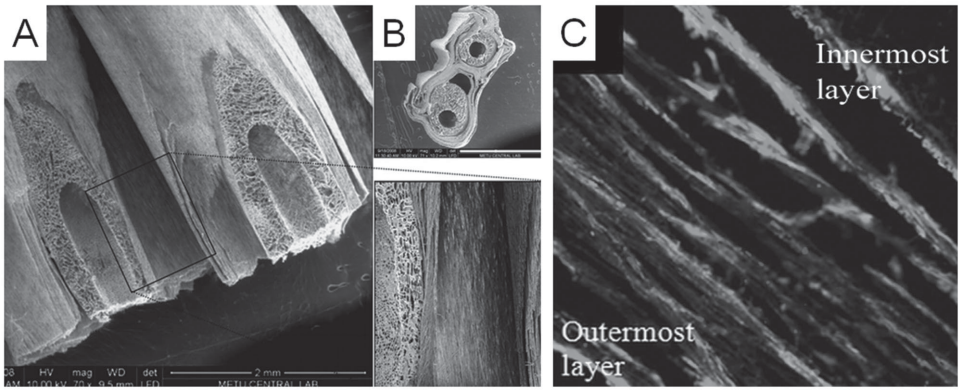

Figure 7. The 3D electrospun scaffolds incorporated with artificial vessel. MSCs is seeded on aligned electrospun PHBV/ P(L-D,L)LA/PGS scaffolds first, and then wrapped on two hollow microporous tubing (9–10 layers, thickness 2 mm) to form this 3D construction. A) SEM image of the 3D electrospun construction from top view (scale bar: 2 mm). B) SEM image shows the cross section (scale bar: 2 mm). C) Immunofluorescent image shows uniform cell distribution from the outermost layer to innermost layer; Sample was stained for F-actin (green) and nucleus (blue). Reproduced with permission.[64] Copyright 2011, Elsevier.

There are also some engineering technologies that could be utilized to improve the perfusion in 3D electrospun scaffolds. The incorporation of artificial channels with 3D electrospun scaffolds has been explored for the enhancement of nutrients and oxygen perfusion. For instance, a hollow and microporous tubing was employed to create vascular channels, and wrapped by aligned electrospun poly(3-hydroxybutyrate -co-3-hydroxyvalerate)/poly(L-D,L-lactic acid)/PGS electrospun scaffolds (9–10 layers and thickness of 2 mm) (**Figure 7**). Improved perfusion of culture medium through the multi-layer electrospun construction is achieved as evidenced by the enhanced uniform cell distribution from the outermost layer to innermost layer.[64] Besides, the electrospun scaffolds with much larger pore size can, intrinsically, better support liquid perfusion, which could be achieved in coiled and multi-scaled electrospun scaffolds.[45,46] In addition, the large pore in electrospun scaffolds can enhance the cell infiltration into the deep of fibers structure, which is beneficial for the construction of 3D cardiac tissues in thick electrospun scaffolds.

4. Conclusions and Future Perspectives

In recent years, extensive and intensive advancements have been achieved in the field of cardiac tissue engineering because of the endeavors from interdisciplinary studies. Although there are still many challenges for the regeneration of functional cardiac tissues, the problems we need to focus on are much clearer than a decade ago. The significance of mimicking the ECM in native myocardium have already been clarified in the design of scaffolds for engineering functional cardiac tissues, thus various materials have been fabricated and tested for their performance in cardiac tissue engineering. The electrospun nanofibrous materials have been developed as practicable scaffolds for cardiac tissue engineering, and been widely studies in recent years. The abilities of electrospun scaffolds with or without functionalities to promote cell attachment, proliferation, differentiation into a cardiac phenotype, formation of organized cardiac tissues and cell response to electrical signal have already been demonstrated, indicating their great potentials for engineering functional cardiac tissues. By integrating the advanced electrospun scaffold design with fast growing biotechnologies, it is possible to partially or fully overcome the existing challenges in cardiac tissue engineering, and make the translation of electrospun-based engineered cardiac tissues for clinical applications more realistic.

Although the electrospun scaffolds have already shown their abilities for engineering functional cardiac tissues in vitro, some efforts are still needed to enhance the potential of electrospun scaffolds for the ultimate goal, engineering functional human cardiac tissues. First, the functionalization of engineered cardiac tissues needs to be improved. Cyclical mechanical stimulation would be a practicable choice since its effect on the organization and contract function of formed cardiac tissues has been demonstrated by other systems.[33,65] Furthermore, the combination of well-designed electrospun scaffolds (polymer component, electrospinning parameters, fiber pattern, and functionalization) and applied mechanical and electrical stimulations during cell culturing could probably improve the performance of electrospun scaffolds for engineering function cardiac tissues. Secondly, the engineering of large-scaled 3D cardiac patches by using electrospun scaffolds is a problem, but is possible to be solved by the cooperation of microchannel system with 3D or multilayer electrospun scaffolds, or by some biological techniques that are capable to promote the vascularization of cardiac tissues in 3D cardiac patch.[66] The 3D electrospun scaffolds with much larger pore size would also be helpful for the perfusion of nutrients and cell infiltration into the depth of fiber construction, thus are worthy to be tried in cardiac tissue engineering. Thirdly, much more attention need to be paid in cardiac tissue engineering clinical research in animal by using electrospun scaffolds to test their biocompatibility, and the performance of cardiac tissues formed on electrospun scaffolds for relieving the symptoms of heart diseases in animal models. Lastly, in order to apply electrospun scaffolds for human cardiac tissue engineering, it is necessary to test the performance of an electrospun scaffold for the formation of human cardiac tissues. The state-of-the-art technique for

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harvesting cardiomyocytes derived from hPSC have provide a desirable cell source;[67] this could be utilized to accelerate the progress of electrospun scaffolds in clinical applications for regenerating human cardiac tissues.

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