4 Using Nanofiber Scaffolds for the Differentiation of Induced Pluripotent Stem Cells into Cardiomyocytes: The Latest Approaches in Tissue Engineering

Rumysa Saleem Khan, Taha Umair Wani, Anjum Hamid Rather, Mushtaq A. Beigh, and Faheem A. Sheikh

Abstract

Organ and tissue replacement therapies are complicated by immune rejection that restricts the long-term effectiveness of implanted devices. With advancements in nanotechnology and tissue engineering, its applications in the biomedical field have gradually increased. To increase the immunologic acceptance of these devices and to mitigate diseased conditions, stem cells have arisen as a suitable choice. The heart is known to recover its function after myocardial infarction with stem cell transplantation. The cardiomyocytes (CMs) to be used can be generated and applied in regenerative medicine by creating tissue-engineered cardiac patches with the evolution of human-induced pluripotent stem cell (hiPSC) technology. Several novel 3D scaffolds have been introduced as stem cell carriers with favorable surface morphologies. Electrospinning-mediated fabrication of tissue engineering scaffolds is considered a method of choice as it can make fibers that best mimic the extracellular matrix of the heart. Stem cells combined with nanofiber carriers to regenerate cardiac tissues show a vast potential to treat cardiac diseases. This chapter gives insights into the production of hiPSC-derived CMs on nanofibrous scaffolds and how these biomaterials can improve stem cell function in the cardiac tissues with potential applications in cardiac regenerative medicine.

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4.1 Introduction

Cardiovascular disease (CVD) is a heart and blood vessel disease group and is a significant reason for deaths in the USA. Millions of people have coronary heart disease, and more than 0.7 million new cases are registered of myocardial infarction (MI) every year in the USA (Benjamin et al. 2018). Some of the well-known CVDs are given in Fig. 4.1. When blood flow to the heart is blocked because of damage to the heart muscle, it results in MI and causes necrosis of cardiac tissue (Fig. 4.2). Scar formation and defective responses after MI cause a decrease in function of the left ventricle and, eventually, complete heart failure (Lloyd-Jones et al. 2009; Prabhu and Frangogiannis 2016) (Fig. 4.3). If the heart cannot supply sufficient blood to prepare the needs of the body, it causes problems in heart functions (Kemp and Conte 2012). For the failure of the heart, the fundamental reason is high blood pressure (Nabel and Braunwald 2012). In the past, trials to repair cardiac function using therapeutic delivery did not benefit the pumping function of the heart (Dimmeler et al. 2008). After MI, a quarter of heart cells vanish, and a considerable cell supply is needed for its regeneration (Kajstura et al. 1998; Yoon et al. 2006). To

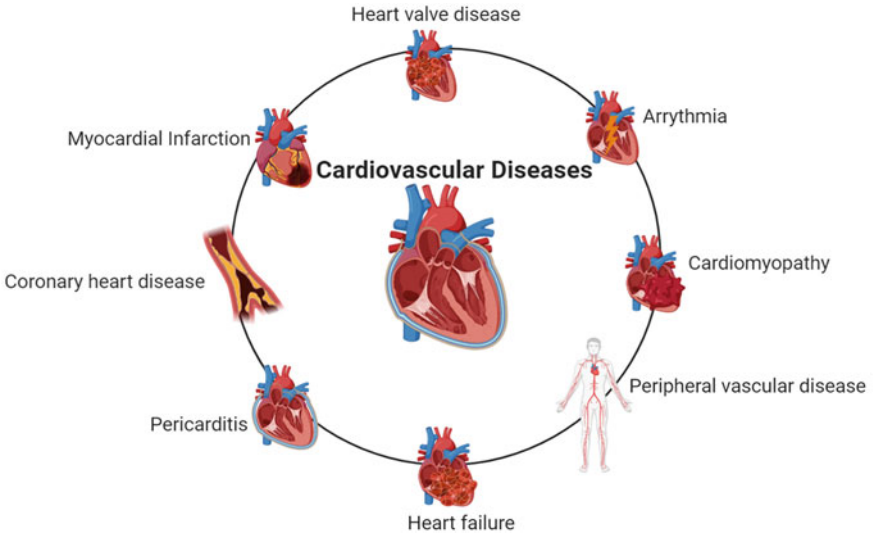

Fig. 4.1 CVDs—heart and blood vessel diseases. These constitute heart valve disease, arrhythmia, cardiomyopathy, peripheral vascular disease, heart failure, pericarditis, coronary heart disease, and MI

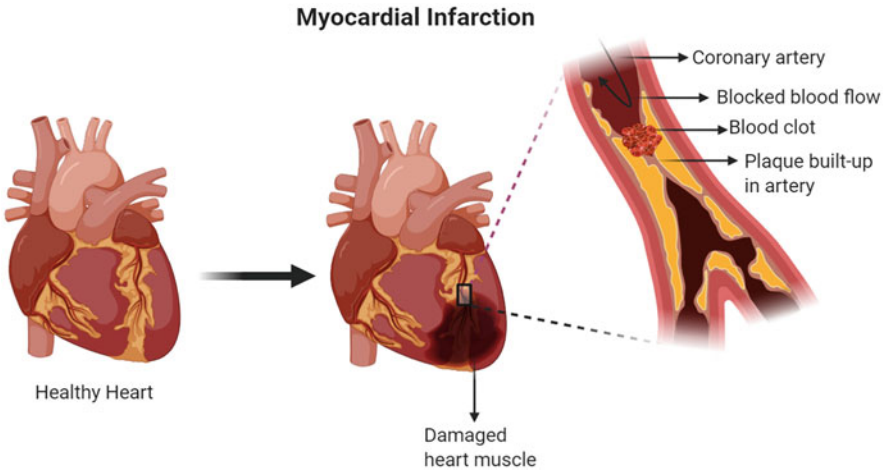

Fig. 4.2 MI occurs on the accumulation of atherosclerotic plaque in the inner lining of a coronary artery, which then bursts, leading to thrombus formation; blood flow to the heart is blocked, resulting in necrosis of cardiac tissue. (Image created using BioRender)

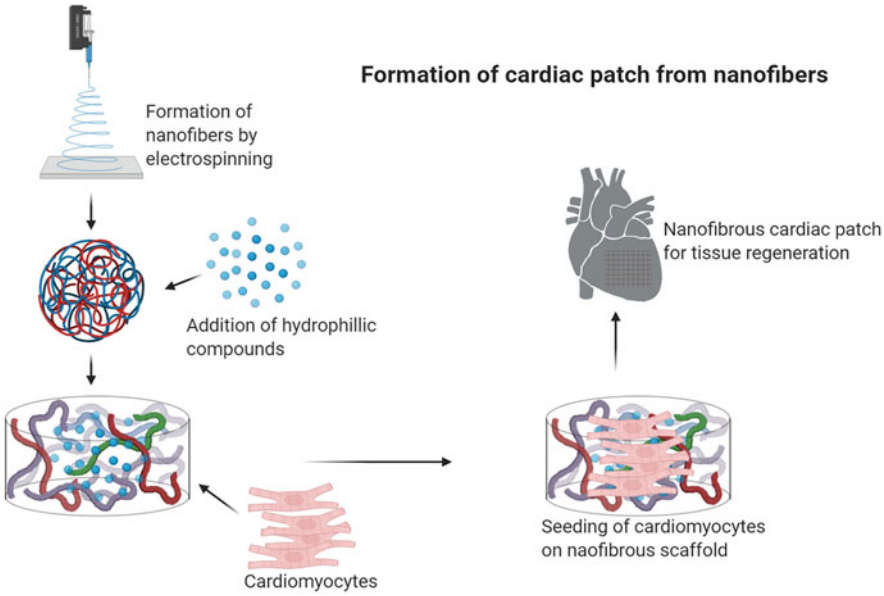

Fig. 4.3 MI refers to the death of the heart muscle tissue by lack of oxygen in myocardial tissue, leading to subsequent consequences. (Image created using BioRender)

repair cardiac tissue and treat heart failure, cardiac regeneration is seen as the best option (Laflamme and Murry 2011). To treat MI, fabricated cardiac tissue-like constructs have been established by culturing cardiomyocytes (CMs) on nanofibers

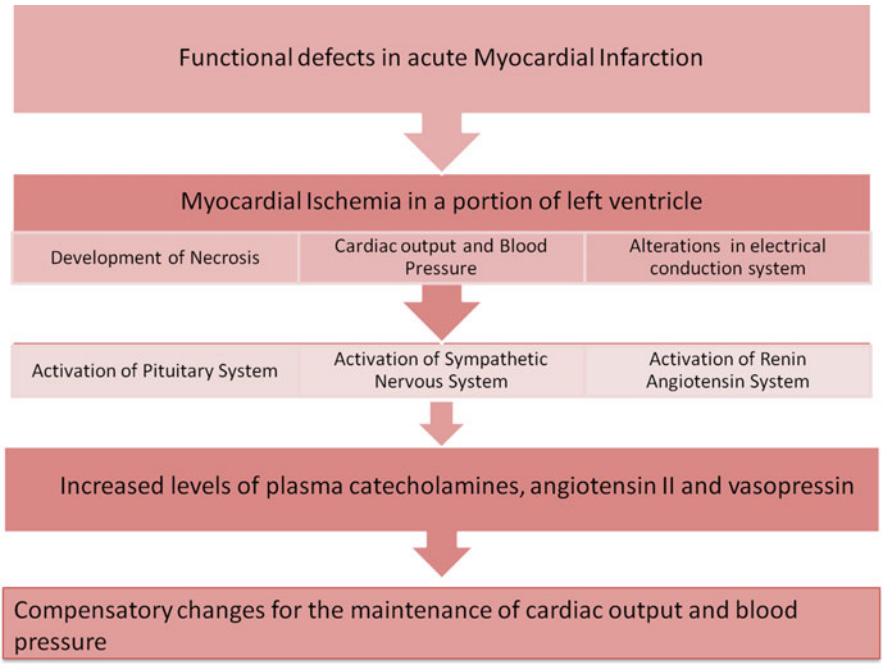

Fig. 4.4 Fabrication of cardiac patch using electrospun nanofibers: Cardiac patches are made by suspending the CMs in a scaffold of a biomaterial fabricated to resemble the ECM. Patches produced with aligned scaffolds are more easily vascularized by the circulatory system of the host

(Fig. 4.4) (Radisic and Christman 2013; Martins et al. 2014; Li et al. 2017a; Gao et al. 2018). The therapeutic potential of embryonic stem cells (ESCs) is enormous, but their use is limited on account of immunological rejection by the host (Boheler et al. 2002). Yamanaka and colleagues discovered a novel approach to induce stemness in fibroblasts by incorporating genetic factors and named them induced pluripotent stem cells (iPSCs) (Takahashi and Yamanaka 2006), which are like ESCs in appearance and differentiation potential. New myocardium and enhanced cardiac function in rats, mice, pigs, and primates have been formed by transplantation of ESCs- or iPSCs-derived cardiac cells and patches (Plowright et al. 2014; Breckwoldt et al. 2016; Foo et al. 2018).

There are mainly parallel-aligned CMs in the cardiac muscle, interspersed with parallel-aligned microvessels (Kaneko et al. 2011). Investigations have been done on the consequences of anisotropic myocardial fabricated tissues on primary (Engelmayr et al. 2008; Bian et al. 2014; Kai et al. 2014; Lin et al. 2014) or stem cell-derived CMs (Parrag et al. 2012). ECM is the essential component of engineered tissues, and it provides signals to cells. Parallel-aligned (anisotropic) scaffolds reflect the native cellular organization and give directions for the cell rearrangements. This leads to the elongated CM morphology (Kai et al. 2011; Orlova et al. 2011; Kharaziha et al. 2014; Khan et al. 2015; Ruan et al. 2016; Li et al. 2017a; Lemoine

et al. 2017). Spatially oriented electrospinning is an approach to fabricate anisotropic scaffolds (Zong et al. 2005). When CMs are cultured on these spatially fabricated scaffolds, these arrange the cytoskeleton according to the alignment of fiber (Zong et al. 2005; Parrag et al. 2012; Wanjare et al. 2017; Allen et al. 2019). As the energy consumption of myocardium is high, an uninterrupted blood supply from capillaries is needed for its sustenance (Parker and Ingber 2007). The CM and vascular endothelial cell interaction is necessary in order to establish contact with host vasculature (Sekine et al. 2008; Zamani et al. 2018; Huang et al. 2018). Endothelial cells in engineered heart tissue lead to angiogenesis after myocardial injury because of the presence of endothelial cells within them (Sekine et al. 2008; Gao et al. 2018). Regarding this, stem cell transplantation has drawn enormous attention with the discovery of iPSCs (Takahashi and Yamanaka 2006). Due to the improvement in the direct differentiation of hPSCs, now there is a certainty of producing stem cell-derived CMs and endothelial cells from ESCs or iPSCs with better effectiveness (Burridge et al. 2014). The induced CMs and endothelial cells resemble their appearance and functions to native cells and are tested in animal models for heart tissue regeneration (Rufaihah et al. 2011; Nakayama et al. 2018; Ishida et al. 2019). Many stem cells have the potential to differentiate into cardiac cells, like mesenchymal stem cells (Müller-Ehmsen et al. 2002; Orlic et al. 2003), ESCs (Caplan and Dennis 2006), iPSCs (Lahti et al. 2012), and CPCs (Miyahara et al. 2006), so a lot of attention has been drawn toward PSCs (Braam et al. 2010; Minami et al. 2012; Liang et al. 2013; Navarrete et al. 2013; Mathur et al. 2016). CM constructs that resemble tissues are necessary, not the non-organized clusters of cells (Braam et al. 2010; Matsa et al. 2011; Liang et al. 2013; Navarrete et al. 2013).

The ESCs are taken from the inner mass of cells of the blastocyst (Fig. 4.5). The inner cell mass grows to form ectoderm, endoderm, and mesoderm of the embryo proper, in vivo (Kingham and Oreffo 2013). Because ESCs are mainly produced from preimplantation embryos (Olson 2006; Kattman et al. 2011; Noseda et al. 2011) and iPSCs are generated from somatic cells (Lian et al. 2012; Zhang et al. 2012), these have gained more attention. Recently, there has been a strong surge in the production of hiPSC using fibroblasts under defined factors (Jaffe 2008). Also, there is an increasingly sophisticated capacity of iPSCs to easily differentiate into cell types related to diseases such as CMs (Burridge et al. 2012; Mordwinkin et al. 2013; Matsa et al. 2014).

4.2 Gene Expression and Signaling Pathways in CM Differentiation

Many studies have been performed on model organisms that demonstrate that the signaling pathways like Wnt, BMP, and Activin/Nodal/TGF-β play essential roles in establishing the cardiovascular system (Olson 2006; Evans et al. 2010; Noseda et al. 2011). From a mixed population of iPSCs, purification of CMs is attained by non-genetic methods, for example, cell-surface markers (Kattman et al. 2011; Lian et al. 2012; Zhang et al. 2012; Abilez et al. 2014; Sanchez-Freire et al. 2014),

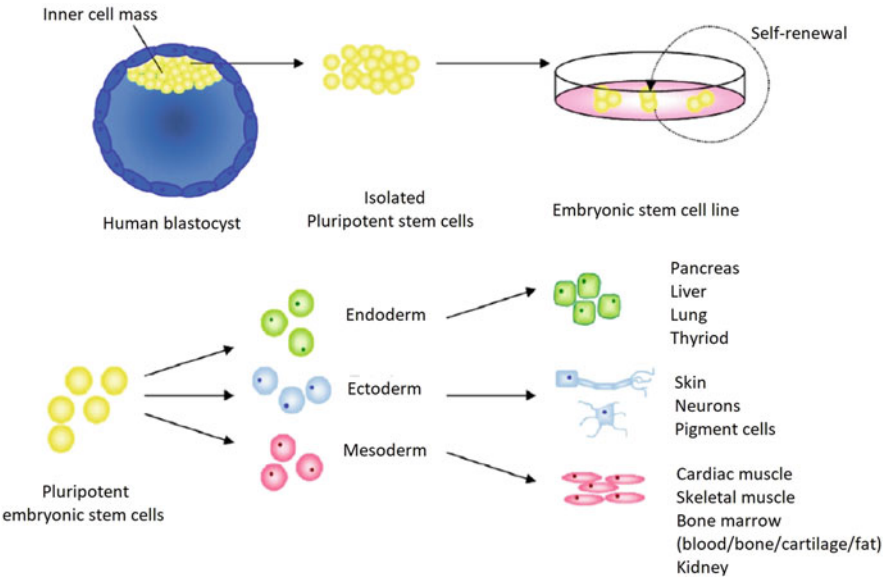

Fig. 4.5 Differentiation of human ESC lines: ESCs are derived from the cells of the blastocyst. With their maintenance in culture, they experience self-renewal and proliferation and retain their stem cell state. (They are adapted with permission from (Kingham and Oreffo 2013))

mitochondria-specific cells (Uosaki et al. 2011), fluorescent probes (Ban et al. 2013), and glucose deprivation (Tohyama et al. 2013). iPSC differentiation toward CMs at the molecular level is coordinated by the patterned expression of various genes at certain steps, which include genes for the establishment of mesoderm, mesoderm for cardiogenesis, cardiac-specific progenitors, and genes for muscle-related proteins of CMs, respectively (Kattman et al. 2011; Liang et al. 2013; Abilez et al. 2014). The ion channel genes of cardiac tissues are in the left ventricle, such as sodium, potassium, and L-type calcium channels (Liang et al. 2013). iPSC-CMs express genes for Ca^{2+} cycling machinery, such as inositol triphosphate receptor, sarcoplasmic reticulum Ca^{2+} ATPase, ryanodine receptor, calsequestrin 2, junctophilin 2, calreticulin, phospholamban, sodium exchanger, and triadin (Itzhaki et al. 2011; Jung et al. 2012a; Rao et al. 2013). Mitochondrial complexes I–V and genes for cholesterol metabolism and genes against apoptotic and oxidative stress processes are expressed in iPSC-CMs (Rana et al. 2012). ROCK signaling pathway, which downregulates cell migration and cell-cell adhesion, is often targeted in cardiac engineering (Riento and Ridley 2003). On screening a diverse compound library using hPSC-CPC, it was found that inhibitor of Wnt pathway signaling (XAV939); bone morphogenetic proteins; a dorsomorphin inhibitor of AMP-activated kinase, RepSox, which acts as an inhibitor of TGF-β type 1 receptor; ALK5; or other inhibitors of ALK5 (Drowley et al. 2016) enhance differentiation of hPSC-CPC.

4.3 Electrospinning Is a Preferable Method for Nanofiber Fabrication

Among many methods employed for tissue engineering, electrospinning has attracted the most attention as it produces nonwoven meshes in the form of scaffolds that structurally resemble the ECM of the heart (Ali et al. 1993; Czyz and Wobus 2001). For the fabrication of fibers, electrospinning is a well-known nanotechnology technique that utilizes electrically charged polymeric solution and is widely used to produce biomaterials for tissue engineering (Ali et al. 1993; Dorfman et al. 1998; Xie et al. 2009) (Fig. 4.6). Electrospun nanofibers produce nanoscale structures that are highly porous and interconnective and have high surface area to volume ratio, imparting the properties of attachment with the cells, better proliferation, and, lastly, their differentiation (Han et al. 2016). It is also a successful technique to fabricate anisotropic scaffolds (Barnes et al. 2007) rather than other conventional methods like soft lithography, microfluidics, photolithography, and two-photon initiated polymerization (Kim et al. 2010; Ma et al. 2014; Xiao et al. 2014).

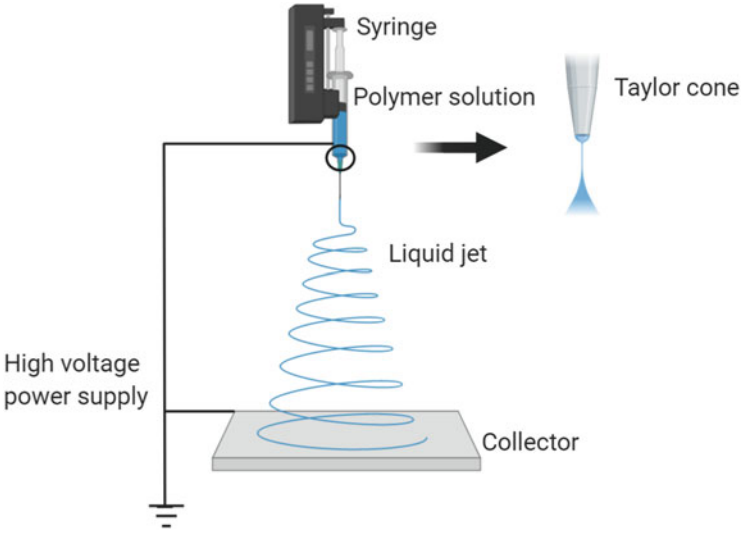

Fig. 4.6 Electrospinning is a versatile micro-nanofiber production method that uses electric force to form threads of the polymer solutions of a hundred nanometers. On applying a high voltage, the liquid is charged due to the electrostatic repulsion and surface tension; the droplet gets stretched, and then at a point, a stream of liquid flows from the surface. This point is where the Taylor cone formation occurs. The liquid jet, which is now produced, gets elongated by a whipping process due to electrostatic repulsion. Finally, it is deposited on the collector, and uniform fibers of nanometer-scale diameters are perfectly formed. A brief review of the studies done on the differentiation of stem cells into CMs using different nanomaterials is given in Table 4.1.

Table 4.1 Differentiation of CMs from stem cells on different nanomaterials

S. no.	Polymer	Application	Reference
1	Gelatin	Crosslinked gelatin nanofibers supported by poly(ethylene glycol) diacrylate honeycomb frame prevent heterogeneous cardiac clusters and larger cardiac clusters	Tang et al. (2016b)
2	Gelatin	Gelatin nanofibers are suitable for the long-term expansion of human pluripotent stem cells under the feeder and serum-free culture conditions	Yu et al. (2019)
3	Polycaprolactone	The 3D-aligned polycaprolactone nanofiber scaffolds show improved CM differentiation of hiPSC-CPCs	Ding et al. (2020)
4	Polycaprolactone	There is a predominant topography role over endothelial culture in cell survival, angiogenesis, and vessel formation as the aligned scaffolds provide the directions to the formation of anisotropic vessels	Willerth et al. (2019)
5	Polycaprolactone	3D polycaprolactone nanofibrous scaffolds directly promote CM differentiation, which might be mediated by the activation of canonical Wnt/β-catenin signaling during early differentiation	Chen et al. (2015)
6	Polycaprolactone	Electrospun anisotropic fibrous scaffolds induce efficient alignment of hPSC-CMs	Han et al. (2016)
7	Immobilized fibronectin on polycaprolactone	The implantation of umbilical cord blood-derived mesenchymal cells with fibronectin on polycaprolactone causes a reduction in MI size and fibrosis and causes a rise in thickness hence an effective carrier for stem cell transplantation to treat MI	Kang et al. (2014)
8	Chitosan-polycaprolactone	hESCs on aligned chitosan-polycaprolactone substrates enhance their myogenic differentiation and show MyoD expression better than those on collagen or polycaprolactone alone, hence showing the importance of ECM topology in directing the myogenic differentiation of hESCs	Leung et al. (2013)
9	Polydimethylglutarimide	There is an increment in the expression of cardiac maturation markers in CMs cultured on polydimethylglutarimide-aligned fibers compared to the control	Li et al. (2016)
10	Poly(lactic-co-glycolic acid)	Poly(lactic-co-glycolic acid) scaffold raises the hydrophilicity and biodegradability of scaffold and finally	Torabi et al. (2020)

(continued)

Table 4.1 (continued)

S. no.	Polymer	Application	Reference
		leads to better CM differentiation potential of hiPSCs	
11	Poly(lactic-co-glycolic acid)	Culturing ESCs on poly(lactic-co-glycolic acid) with collagen nanofibers shows better differentiation to CMs than poly(lactic-co-glycolic acid) nanofibers	Prabhakaran et al. (2014)
12	Poly(lactic-co-glycolic acid)	Efficient quality cardiac tissue-like constructs are formed by culturing hiPSCS-CMs on grounded and aligned poly(lactic-co-glycolic acid)	Li et al. (2017b)
13	Polyaniline/ polyethersulfone	Biocompatible polyaniline/ polyethersulfone scaffolds conduct electricity and, just like the bundles in heart pacemakers, help in delivering electrical pulses to the cells. Using these aligned electroactive nanofibrous scaffolds, a large increase in differentiation of hiPSCs to CMs is observed	Mohammadi Amirabad et al. (2017)
14	Hydrogel based on polyethylene glycol	Hydrogel injections enhance heart functioning post-MI and do not disrupt normal heart functions	Chow et al. (2017)
15	Hydrogel based on RAD16-I	The hydrogel in the patch made of RAD16-I hydrogel with hiPSCs acts on the cells in a pro-cardiogenic manner. It improves cell distribution in order to assist in the uniformity of colonization of the membrane pores and assist in differentiating progenitor cells to CMs	Puig-Sanvicens et al. (2015)
16	Chitosan hydrogel	Temperature-responsive chitosan hydrogel is used in delivering stem cells to the damaged myocardium and increasing graft size	Lu et al. (2009)
17	Polydimethylsiloxane	The substrate of intermediate elasticity causes the cell-substrate and cell-cell interaction, which enhances embryoid body-like formation, and its elasticity is the same as that of the native tissue	Wang et al. (2019)
18	Atelocollagen	Electrospun atelocollagen scaffold seeded with hiPSC-CMs is feasible for the stabilization of the dilated cardiomyopathy with potential for its clinical use in the future	Joanne et al. (2016)
19	Fibrin-collagen microthreads	hiPSC-CMs are capable of attaching to fibrin microthreads; however, a collagen IV protein coating improves their attachment to fibrin microthreads	Hansen et al. (2018)
20	Collagen	The vascular grafts on aligned nanofibers can amend the arrangement of primary	Nakayama et al. (2015)

(continued)

Table 4.1 (continued)

S. no.	Polymer	Application	Reference
		and iPSC-derived vascular lineages and restrain the inflammatory reaction of primary endothelial cells and iPSC-derived endothelial cells	
21	RAD/PRG and RAD/KLT peptide nanofiber	The co-transplantation of mesenchymal stem cells with RAD/PRG promoted their localization and survival in the infarcted myocardium. The therapeutic effect was improved with either RAD/PRG or RAD/KLT	Li et al. (2017c)
22	PA-RGDS Peptide nanomatrix	PA-RGDS enhances the survival of endothelial stem cell-derived CMs and enhances heart functioning after MI	Ban et al. (2014)
23	Polyurethane	Pre-differentiation of mesenchymal stem cells into CMs before injection results in better cardiac regeneration than only injecting undifferentiated mesenchymal stem cells into the heart	Guan et al. (2011)
24	Polyethylene terephthalate	hiPSC-CMs grown on polyethylene terephthalate textiles with gelatin coating demonstrate superior structural properties like rod-shaped structure and enhanced sarcomere orientation	Pekkanen-Mattila et al. (2019)

4.4 Polymers and Bioactive Agents Used in Tissue Engineering

Nanofibrous scaffolds with a diameter range of nanometer to a few microns are utilized for heart muscle generation more than other scaffold types, like sponge scaffolds (Prabhakaran et al. 2011). Whether natural or synthetic, numerous polymers have been successfully used in the production of mammalian culture suitable nanofibers. However, due to the close resemblance of natural polymers to structural proteins of ECM, they are widely used, e.g., collagen, laminin, and gelatin (Jung et al. 2012b; Boccaccini et al. 2015). Synthetic polymers, such as polycaprolactone, poly-lactic-co-glycolic acid, and polyurethane, are mostly used in the production of biocompatible scaffolds (Khil et al. 2005; Vasita and Katti 2006; Song et al. 2008). Other synthetic polymers include chitosan, polyaniline, polyethersulfone, polydimethylglutarimide, peptide amphiphile, and some other peptide nanofibers. Polymers are combined to create co-polymers to be used in the making of scaffolds of desired properties. Other than polymers, e.g., synthetic hydrogels, sometimes based upon polyethylene glycol, are utilized as a scaffold for drug delivery and cell culture (Lin and Anseth 2009). They are biocompatible and have excellent safety records and have established well in the medical field (Van Tomme et al. 2008; Hoffman 2012; Frey et al. 2014). Bioactive agents, like hormones, growth factors, and other small molecules, are introduced within the

scaffold matrix to make an ECM-like environment for the proliferation of cells to enhance cell survival, proliferation, integration, and differentiation (Laflamme et al. 2007). Erythropoietin is an example of a bioactive agent successful in clinical studies to reduce cell death and remodel post-MI (Brines and Cerami 2008). A crucial ECM molecule for stem cell differentiation and adhesion is fibronectin (Tate et al. 2002; Wijelath et al. 2004; Van Dijk et al. 2008), which is expressed in the normal heart also. Platelets are the growth factors that are involved in blood clotting, immune response, angiogenesis, and recovery of the damaged tissues in the body. This has attracted the attention of physicians in damaged engineering tissues because the probability of a reaction is near to the ground due to the use of personal blood (Boswell et al. 2012; Lang et al. 2018).

4.5 Differentiation of CMs from Stem Cells Using Nanofibers

4.5.1 Gelatin Nanofibers

PSCs demand more acclimated 3D cellular microenvironments than conventional 2D surfaces to keep their pluripotency or differentiation homogeneity. Matrigel (gelatinous protein compound of mice tumor cells) (Hughes et al. 2010) recombinant proteins like laminin (Rodin et al. 2014) or vitronectin (Rowland et al. 2010) were introduced for iPSC growth and differentiation. Additional substrates have also been used, like oxygen plasma etched plates (Mahlstedt et al. 2010), porous materials (hydroxyapatite scaffolds) (Kim et al. 2007a), and electrospun nanofibers (Kumar et al. 2015; Li et al. 2017c), which have textured surface morphology. Without improvement to conventional approaches, these will not overcome the risks of genetic instability and tumorigenicity (Okita and Yamanaka 2011; Liyang et al. 2013). These methods do not show the production of rigid and thick cardiac sheets because these have a limitation of cardiac diffusion (Shimizu et al. 2006). For the differentiation of hiPSCs to motor neurons, a patch culture method has been proposed, which has shown an enhanced upregulation of gene expression of the neurons and smooth maturation of motor neurons (Tang et al. 2016a). Tang et al. extended the patch process to culture and differentiated hiPSCs toward functional CMs. A crosslinked monolayer of gelatin nanofibers backed by a poly(ethylene glycol) diacrylate honeycomb frame consisted of the patch. Poly(ethylene glycol) diacrylate, a derivative of polyethylene glycol, could be utilized for multiple drug delivery and tissue engineering-based purposes. UV-based molding method was used for the preparation of the poly(ethylene glycol) diacrylate frame and gelatin nanofibers, electrospinning was used (Fig. 4.7). On the poly(ethylene glycol) diacrylate frame gelatin, nanofibers were electrospun. Due to the wide-sized pores of the patch and natural polymer used, the crosslinked gelatin nanofibers minimized the exogenic substance contact of hiPSCs. Vitronectin or additional extracellular matrix proteins can be used to coat the culture patch if required for more functional cell-nanofiber pairing. As the culture patch was within off-ground conditions, the crosslinked monolayer nanofibers allowed significantly enhancing the exposure field

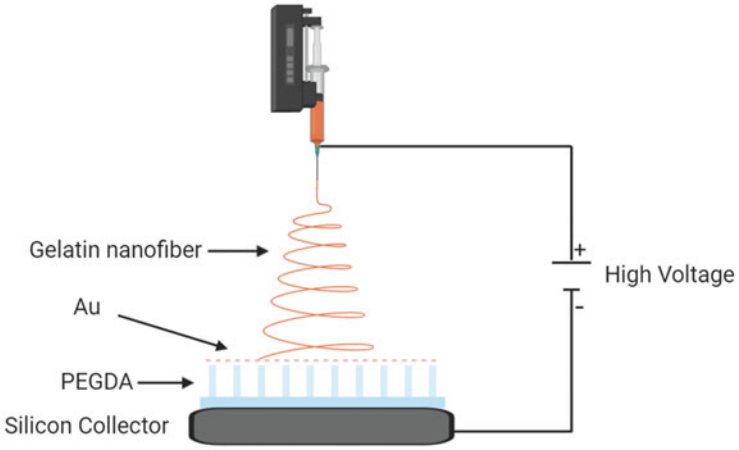

Fig. 4.7 Nanofibers of gelatin formed by electrospinning on honeycomb frame made of polymer poly(ethylene glycol) diacrylate

of hiPSCs upon the culture medium. They had also shown in their previous work that preferentially, cells remained confined in the nano-patterned region due to intensified distribution of nutrients and cell uptake (Hu et al. 2010; Tang et al. 2016b). Due to the increased exposure area with the help of monolayer of crosslinked nanofibers supported, cell metabolites could more efficiently be diffused, and in this manner, the culture patch is favorable over other 3D substrate types (Hu and Li 2007). Due to good mechanical stability and weak in-plane resistance to cardiac contraction, the honeycomb structure was chosen (Nishikawa et al. 2003; Arai et al. 2008; Engelmayr et al. 2008), and to aid hiPSC clustering, poly(ethylene glycol) diacrylate was used. Poly(ethylene glycol) diacrylate has been popularly adopted to outline the cell culture surfaces for their weak cell adhesivity and inadequate protein absorption and smooth chemical alteration (Moon et al. 2009). Colonies of ~250μm diameter (~1300 cells) refer to the most suitable size of hiPSC colonies for cardiac differentiation, and a honeycomb structure patch size of 500μm was chosen to generate the hemisphere colonies of this size (Dahlmann et al. 2013). In this study, ROCK inhibitor Y-27632 was utilized to adjust the impact of vitronectin, promoting the integrin-mediated cell adhesivity to the substrate. The cell-substrate interaction decreased due to low vitronectin surface coating and a short interval Y-27632 application given to the cells. It also supports cell to cell adhesion and then augments the cell grouping and development of hemispheric hiPSC colonies. As Y-27632 lasted for 4h or longer, flat hiPSC colonies were generated. However, when the application was restricted to 1–2h, hemisphere colonies with a diameter of around 225μm were obtained. These colonies were selected for cardiac differentiation due to the morphological similarity of hemisphere colonies with embryoid bodies. These are more resembling to in vivo development process of embryos and can assist in CM induction (Mummery et al. 2002, 2003). Poly (ethylene glycol) diacrylate honeycomb compartment prevents the formation of

heterogeneous cardiac clusters because of necessary fusion until the later stage of cardiac differentiation.

Cells belonging to the same population exhibit a considerable heterogeneity degree (Janes et al. 2010; Wilson et al. 2015). The determination of the fate of stem cells is also influenced by their inherent heterogeneity (Warren et al. 2006; Musina et al. 2006; Franco et al. 2010). Variations among donor cells result in operative variability and diverse differentiation potentials among other hPSC lines (Adewumi et al. 2007; Kim et al. 2007b; Cahan and Daley 2013). Also, those obtained from different types of tissues, for example, various germ layer tissues, show lineage bias as they go through directed differentiation (Kim et al. 2011), suggesting that hPSCs may hold on to distinct memory about their origin. Furthermore, reprogramming is a complicated and multi-step procedure that adds additional modifications (Liang and Zhang 2013). Building single cell-derived clones could diminish this heterogeneity (Narsinh et al. 2011; Lecault et al. 2011; Smallwood et al. 2014). Leqian et al., in their study (Yu et al. 2019), developed a single hPSC separation and culture platform of gelatin nanofibers. These nanofibers are appropriate for the long-term development of hPSCs supporting the feeder and serum-free culture environments. They established a single cell-derived sub-clone that proved to possess a discrete morphology related to other sub-clones. When this clone was used for differentiation toward CMs, it demonstrated much greater differentiation capability, maturation, and more substantial beating than those obtained from the other sub-clones. These observations present a suitable approach for single-cell separation and culture and illustrate those disparities in differentiation biases among sub-clones belonging to a cell line (Fig. 4.8).

4.5.2 Polycaprolactone Nanofibers

Polycaprolactone is a high-molecular-weight synthetic polymer, which is recognized by the US Food and Drug Administration for therapeutics (Kuppan and Sethuraman 2013). Moreover, the 3D-polycaprolactone nanofibrous scaffolds fabricated by electrospinning are employed for tissue engineering based on stem cells because of their good mechanical and biodegradable characteristics (Hashemi et al. 2009; Lim et al. 2009). These scaffolds provide a possibility to design scaffolds of micro- to nanoscale topography with a significant porosity similar to that of native ECM (Sill and von Recum 2008). Ding et al. (2020) examined the influence of 3D-aligned polycaprolactone nanofiber scaffolds upon cardiac differentiation of hPSC-CPCs. Cells treated with Wnt signaling inhibitors on 3D-aligned nanofiber scaffolds displayed enhanced CM differentiation of hPSC-CPCs. A notable rise in cTnT-positive cells on the 14th day in the 2D culture of differentiation related to cells administered with DMSO vehicle control resulted from treating hPSC-CPCs with Wnt inhibitors (53AH and XAV939). The results show that the Wnt signaling pathway performs an essential role in cardiac differentiation (Patsch et al. 2015; Wang et al. 2011; Willems et al. 2011; Ao et al. 2012; Lian et al. 2013). When the cells were treated with 53AH (i.e., a distinct structural inhibitor of Wnt signaling),

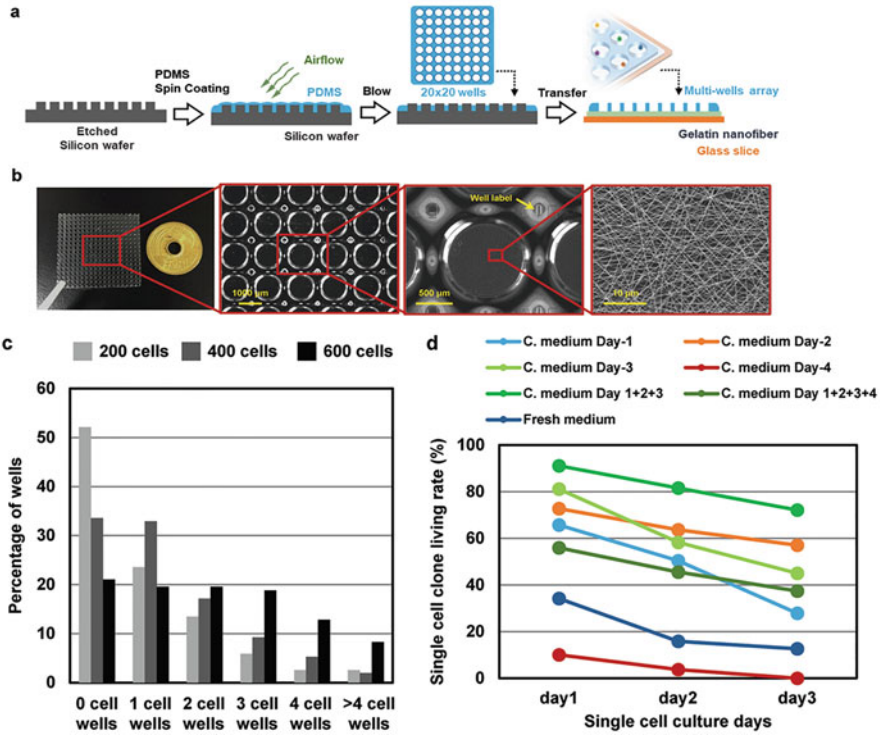

Fig. 4.8 Single-cell isolation system. (**a**) The process of making culture device for cell isolation. (**b**) Photograph of the culture device and single-cell isolation. The device is made of a polydimethylsiloxane multiwell array (400 wells) for single-cell isolation, and for the single-cell culture, it consists of gelatin nanofiber substrates. (**c**) The single-cell distribution rate of the multiwall array. (**d**) The highest viability was shown by the single cells with a mixture of days 1, 2, and 3 conditioned medium (C. medium) in the beginning 3 days of culture ($n \geq 20$). (Adapted from (Yu et al. 2019))

the expression of CM marker genes TNNT2 and MYH7 that are predominantly expressed in fetal ventricles was higher, and when the cells were treated with XAV939, the expression of MYH7 was also elevated (Fig. 4.9). These 3D-aligned nanofiber scaffolds mimic the structure of the native ECM.

Wanjare et al. (2019) fabricated microfibrous polycaprolactone by electrospinning to simulate the established physiological cellular organization of the entire myocardium to control the assemblage of induced-ESCs (iESCs) and induced-CMs (iCMs). By reprogramming peripheral blood mononuclear cells by transduction of cardiac differentiation-related genes (Sox2, Oct3/4, KLF4, and c-myc) mediated by Sendai virus, the hPSC (P356) cell line was developed. hESC (H9) was used along with hPSCs. After the cell seeding, visualization of the in vitro establishment of iCMs and iESCs within scaffolds was achieved according to phenotypic markers of troponin-T (TNNT) for CMs and CD31 for embryonic cells

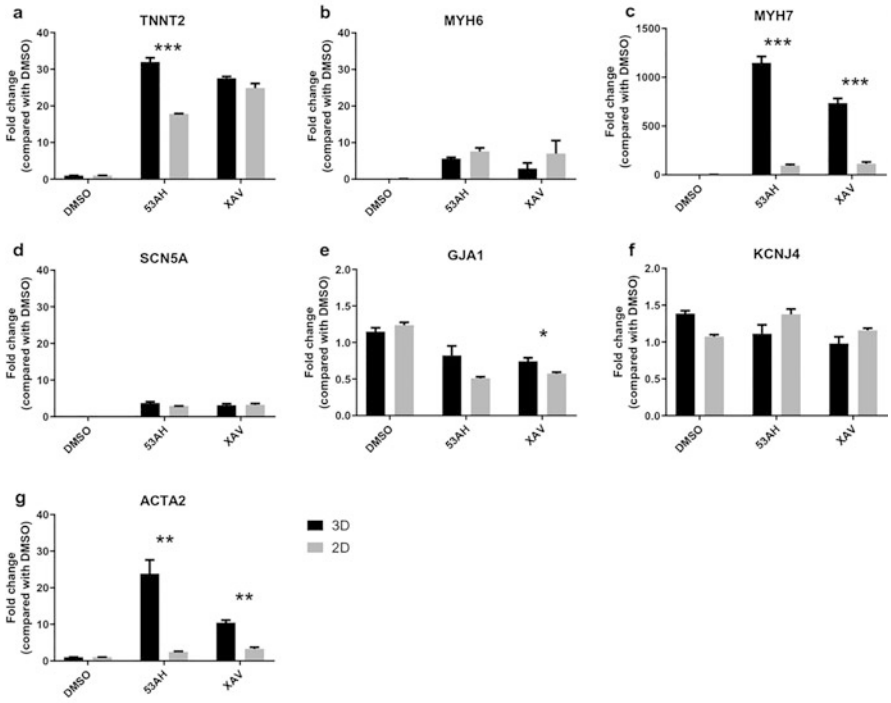

Fig. 4.9 Real-time RT-PCR analysis of CPC differentiation and proliferation in 3D vs. 2D culture. (**a**–**e**) Expression of different cardiac expression markers at day 7 of differentiation in 2D and 3D cultures. (Obtained with permission from (Ding et al. 2020))

(Fig. 4.10). NOD SCID mice (nonobese diabetic-severe combined immunodeficiency mutant mice) were utilized toward subcutaneous implantation studies. These scaffolds successfully induced and precisely directed the differentiation in the experimental cells.

With the use of an electrospun nanofiber as the substrate instead of tissue culture polystyrene plates (TCPs), CMs display more stable and long-lasting, spontaneous syncytium (Şenel Ayaz et al. 2014). Jingjia et al. (Han et al. 2016) designed the aligned and isotropic polycaprolactone fibers. The fibrous scaffolds were gold or palladium coated and were analyzed for characterization. After the culture of hiPSC-CMs was done on scaffolds coated with Matrigel, the alignment of cells on these substrates was confirmed using a polystyrene substrate as the control. Yan et al. (Chen et al. 2015) studied the impact of 3D polycaprolactone scaffolds on the CM differentiation of murine-iPSCs while performing in vitro examinations. A unique CM-inducing effect exists in the exchanges between the nanofibers of 3D polycaprolactone and the intracellular Wnt/β-catenin signaling of iPSCs. It was found that the gelatin-coated 3D scaffolds were suitable for iPSC cultivation and differentiation. Also, the conventional TCPs are less effective than 3D scaffolds for inducing the CM differentiation of iPSCs using the monolayer culture method.

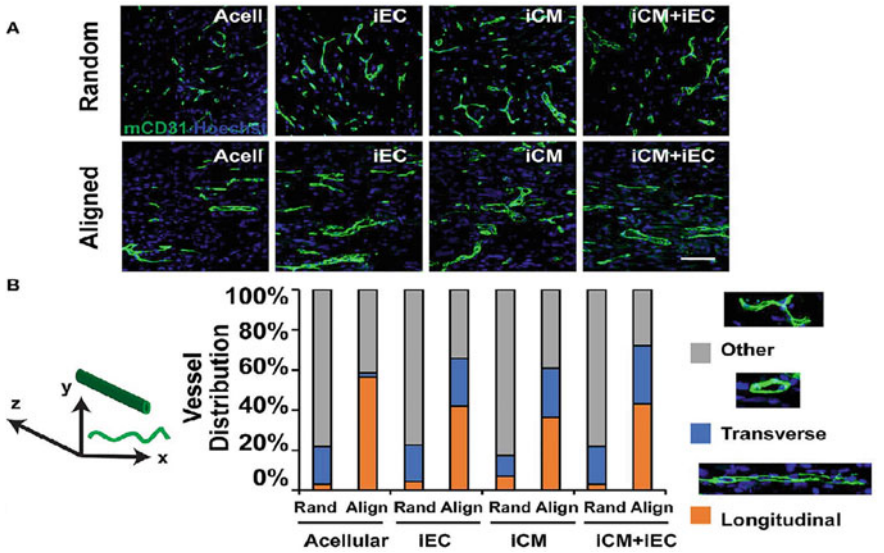

Fig. 4.10 The vascularization of cardiac tissue after successful implantation in mice model (**a**). Confocal microscopy images of CD31 staining (green) of engineered tissues obtained from scaffolds containing iCMs, iECs, or iCM+iECs after implantation. Acell means acellular scaffold (**b**). The orientation of vessels in engineered myocardial tissue relative to the axis of the aligned fibers. (Adapted with permission from (Wanjare et al. 2019))

To increase myogenic differentiation of hESCs, Leung et al. (2013) used chitosan-polycaprolactone fibers resembling the native muscle ECM microenvironment along with the Wnt3a protein. Polycaprolactone nanofibers were fabricated by electrospinning, and hESCs were seeded on this scaffold in media containing Wnt3a. An elongated morphology of hESCs was observed along fiber direction as compared to control substrates. Cells cultured on chitosan-polycaprolactone with Wnt3a expressed a high percentage of myogenic proteins over total hESCs after 2 days of cell culture.

4.5.3 Polydimethylglutarimide Nanofibers

Polydimethylglutarimide is a biocompatible polymer that can be easily electrospun into nanofibers (Orlova et al. 2011). Li et al. (2016) investigated hiPSC-CMs on fibers made of polydimethylglutarimide and pursued their cardiac tissue-like construction. Electrospinning was performed using a rotating drum to prepare aligned polydimethylglutarimide while increasing the rotation speed of the collector. The fibers of concentrations 19% and 16% demonstrated the best alignment than those from lower concentrations. The density of sheets was manipulated by changing the spinning time, and the 90-s electrospun fibers were chosen for CM culture. The as-spun polydimethylglutarimide threads on transferring to the surface of

microelectrode array let the extracellular recording of mimicking activities. Recording electrical activity in CMs enables the evaluation of their electrophysiological characteristics (Meiry et al. 2001).

4.5.4 Poly(lactic-co-glycolic) Acid Nanofibers

This polymer has ample versatility to some drugs, including hydrophobic, hydrophilic, micromolecular, and macromolecular. It also limits drug degeneration and the chance of easy surface modification to give more considerable interaction with biological surfaces (Pick 2009). Poly(lactic-co-glycolic) acid does not cause any inflammatory response and becomes absorbed well, not concentrating in the tissues or organs (Ali et al. 1993). Torabi et al. (2020), in their study, developed platelet-rich plasma-incorporated poly(lactic-co-glycolic) acid nanofibrous scaffold. The results demonstrated that the fabricated poly(lactic-co-glycolic) acid scaffold, in comparison to standard TCPs, exhibits enhanced biocompatibility. The platelet-rich plasma in poly(lactic-co-glycolic) acid is believed to increase biodegradability and hydrophilicity of scaffold and results in a suitable increase in the CM differentiation potential of hiPSCs. Its enhanced biocompatibility caused a rise in proliferation rate and PSCs survival due to the present growth factors. It may be noted here that there has been a positive impact of platelet-rich plasma on the growth and proliferation rate of other cells (Choi et al. 2005; Drengk et al. 2009). Platelet-rich plasma-incorporated scaffolds showed the maximum expression of cardiac genes, such as MLC2A, ANF, and MLC2V. Prabhakaran et al. (2014) analyzed the ability to differentiate ESCs into CMs on the poly(lactic-co-glycolic) acid and poly(lactic-co-glycolic) acid/collagen electrospun fibrous scaffolds as the cardiac patch. They made uniform bead-free fiber of poly(lactic-co-glycolic) acid and poly(lactic-co-glycolic) acid with collagen using the electrospinning technique. The ESC differentiation was induced by embryoid body formation, which proliferated and differentiated into CMs. Higher proliferation on poly(lactic-co-glycolic) acid with collagen scaffolds was observed by scanning electron microscope; the reason behind this could be the small diameter or high surface area of the fiber.

Most hiPSC-CMs morphologically and functionally look like naive CMs rather than adult ones, limiting their administration. Li et al. (2017a) produced high-quality constructs similar to cardiac tissue by culturing hiPSC-CMs on nanofibers of low girth made of biodegradable poly(lactic-co-glycolic) acid polymer. They described that multi-layered and elongated CMs could be arranged at high density with ordered fibers in a one-step seeding process, developing in upregulated cardiac biomarkers and enhanced cardiac functions. Constructs similar to cardiac tissue were used for assessing drugs, and they were more vigorous than the 2D control. They also highlighted the usability of cardiac constructs for in vitro designing of engraftments and in vivo treatment of MI.

4.5.5 Polyaniline and Polyethersulfone Nanofibers

Polyaniline is receiving much attention due to its excellent electrical conductivity. It has gained wide tissue engineering usage due to its electroactive qualities, high endurance, biocompatibility, easy synthesis, and being economical (Qazi et al. 2014). It scavenges reactive oxygen species and can mitigate oxidative stress in a myocardial injury (Gizdavic-Nikolaidis et al. 2004). It can be doped from the nonconductive form of meraldine base into the conductive state of emeraldine salt by protonic acids (e.g., camphor-10-sulfonic (β)) (Khuspe et al. 2014) and has been employed to assist in the transmission of electrical pulses (Balint et al. 2014). Furthermore, among different doped states of the poly(lactic-co-glycolic) acid, this polymer with camphor-10-sulfonic (β) is considerably aligned in the direction of the fiber. Moreover, the better interchain charge transfer occurs in poly(lactic-co-glycolic) acid with camphor-10-sulfonic (β) due to its dedicated polymeric chain packaging (Pouget et al. 1995). These semiconducting polymers concurrently support the adhesion and reproduction of CMs and induce cardiac differentiation (Bidez et al. 2006). Polyethersulfone nanofibers are other biocompatible materials that induce mesodermal differentiation (Ardeshirylajimi et al. 2013). Polyethersulfone is mechanically stable for differentiation but its electrical conductivity is low. Hence, blending polyethersulfone with polyaniline is practiced to create a scaffold with added mechanical stability for cardiac differentiation and electrical conductivity.

Mohammadi et al. (2017) practiced electrical stimulations onto aligned and random scaffolds to see how it affects the differentiation of PSCs to CMs. The effect of multidirectional electrical stimulation generated by random scaffolds on CM differentiation was negative, but the aligned unidirectional electrical stimulation of the aligned scaffold was positive. Recapitulation of the requirements and events leading to cardiac differentiation and maturation is a significant challenge (Courtney et al. 2006). The electrical impulses were applied to hiPSCs taken from patients with cardiovascular disease (referred to as CVD-iPS cells) seeded on aligned polyaniline/polyethersulfone scaffolds. This setup worked as an electrically effective cell culture system, including features similar to in vivo conditions of the heart that have been shown to generate cardiogenesis (Serena et al. 2009; Chi et al. 2010; Hernández et al. 2016). Through embryogenesis, the primary pacemaker cells originate from fetal CMs in the center of the sinoatrial node to create the first electrical impulses. Studies have been performed about exogenous electrical stimulation and cardiac differentiation of diverse kinds of stem cells (Serena et al. 2009; Hernández et al. 2016) by changing intracellular ion concentrations (Trollinger et al. 2002), yielding reactive oxygen species (Serena et al. 2009), or locating growth factor receptors and lipids in the cell membranes (Zhao et al. 2002). In this work, a bioreactor that applied exogenous electrical impulses was designed to apply electrical stimulation to cells via stainless steel electrodes. Analysis of the properties of this bioreactor showed that it is suitable for cardiac differentiation.

4.5.6 Polydimethylsiloxane Nanofibers

An important factor of the culture substrate that plays a vital role in determining the cell fate is the elasticity (Banerjee et al. 2009; Kshitiz et al. 2012; Sun et al. 2012; Li et al. 2017b). The self-renewal and differentiation of hPSCs are affected by the flexibility of cultures and morphology (Liu et al. 2014, 2017; Macrí-Pellizzeri et al. 2015). Engler et al. showed that the differentiation of mesenchymal stem cells toward a particular lineage is significantly based on substrate elasticity (Engler et al. 2006). The matrix stiffness and cell density assist hiPSCs in intercellular network formation, preserving phenotype and contractile function (Lee et al. 2017). A stencil method was revealed to investigate the outcome of substrate elasticity on the clustering and cardiac differentiation of hiPSCs. Dense elastomer pillars of altering stiffness were designed by adjusting the height of the pillars. To form uniform cell clusters, an elastomer stencil with a honeycomb pattern was created before cell seeding. It was demonstrated that both cell clustering and cardiac differentiation are dependent on the elasticity of substrate. They showed that the pillar of moderate elasticity (9 kPa) was better for both stiffer and softer ones.

4.5.7 Collagen Nanofibers

Joanne et al., in one of their works, have shown the feasibility of deriving collagen scaffolds mixed with biological solvents and crosslinking agents (Kitsara et al. 2015). In their other study (Joanne et al. 2016), they generated collagen scaffolds and cultured hiPSC-CMs and injected these scaffolds epicardially in a dilated cardiomyopathy (DCM) mouse model. Atelocollagen extracted from animal dermal tissue was electrospun by applying a proper voltage between the collector and the syringe needle. The suturing of collagen scaffolds on the ventricles of mice was done. The results showed the feasibility of the hiPSC-CM-seeded scaffold for the treatment of DCM. Hansen et al. (2018) developed and characterized hiPSC-CM-seeded fibrin suture that could be used for the delivery platform in repairing cardiac issues. hiPSC-CMs were seeded onto micro fibrin threads, and their contractile properties with time were characterized. These researchers fabricated a fibrin microthread suture for direct cell delivery to the myocardium (Guyette et al. 2013). In their study, various ECM and surface coatings were applied for improving cell attachment. Collagen IV and fibronectin were selected because of their occurrence in the cardiac basement membrane (Moyes et al. 2013; Rodriguez et al. 2014). Other hiPSC-CMs seeded on microthreads showed contraction within 7 days after seeding. The contraction of cells was in the direction of fiber for over 21 days. Also, this ordering was proved by immunohistochemical stains as over 21 days, the cells ordered further close to the thread, having the final alignment within 8° to the thread. Their findings suggest that hiPSC-CMs are able to attach to fibrin microthreads while the collagen IV protein-coating improves the potential of hiPSC-CMs attachment to fibrin microthreads. By the 14th day, the fibers contracted at a frequency that resembled the human heart and generated strains like those developed by

myocardium. Like the previous studies (Guyette et al. 2013; Hansen et al. 2016; Tao et al. 2017), this microthread scaffold could impact cardiac delivery approaches.

A blood vessel is composed of fibrillar ECMs and the other cell composition at the micro-/nanoscale (Stehbens and Martin 1993). Nakayama et al. (2015) designed a bi-layered vascular graft extracted from hiPSCs that reiterates the cell construction, alignment, and anti-inflammatory operation of blood vessels. In this graft, longitudinal-aligned nanofibrillar collagen-containing endothelial stem cells consisted of the luminal layer. Collagen with iPSC-derived smooth muscle cells consisted of the outer layer. Cells aligned on aligned fibers showed the association of F-actin within 8° from the direction of scaffolds. Endothelial stem cells seeded on ordered scaffolds had significantly lowered immune response due to attachment to monocytes. There is a significant influence of anisotropic scaffolds in directing cell construction and function.

4.5.8 Peptide Nanofiber

RADA-16-I (Ac-(RADA)4-CONH2) is a peptide nanofiber that causes the attachment, development, and differentiation of stem cells and mature somatic cells (Davis et al. 2005). RADA16-I can be modified with the addition of functional peptides to its C-terminal end. One useful peptide, e.g., PRG (Ac-(RADA) 4GPRGDSGYRGDS-CONH2), contains RGD (a natural cell adhesion motif to regulate the localization and proliferation of cells by ligation with integrin). Another operative peptide, i.e., KLT (Ac-(RADA)4G4KLTWQELYQLKYKGI-CONH2), augments angiogenesis by copying vascular endothelial growth factor (Liu et al. 2012). Functionalized self-assembling peptide nanofibers are of good biological histocompatibility. They have the capability to sustain the reproduction and differentiation of cells for repairing nervous tissues in vertebrate studies and are having applications for cardiovascular diseases as well. Peptide amphiphile is self-assembling peptides that join a hydrophobic and a hydrophilic peptide sequence exhibiting an excellent approach to gain this purpose (Hartgerink et al. 2001). Self-adhesive ligand Arg-Gly-Asp-Ser (RGDS), when incorporated with peptide amphiphile (PA-RGDS) and matrix metalloprotease-2 (MMP-2) degradable sequence, Gly-Thr-Ala-Gly-Leu-Ile-Gly-Gln (GTAGLIGQ), forms an ECM mimicking injectable nano-matrix. When interjected into PA, RGDS and fibronectin-derived ligand enhance cell adhesion and endurance and are verified by several cell types, including mesenchymal, umbilical, aortic, and pancreatic beta cells (Benton et al. 2009; Yu et al. 2009, 2010; Sapir et al. 2011; Gandaglia et al. 2012; Mihardja et al. 2013). MMP-2 degradable sequences on incorporation into peptide amphiphile cause a gradual degeneration of the fiber and are replaced by ECM of cells because the damaged tissue exhibits enhanced MMP production under ischemic conditions (Bendeck et al. 1994; Spinale et al. 1998; Cheung et al. 2000). This increases the migration of CMs into the myocardium (Jun et al. 2005). PA-RGDS nearly simulates the physical and biochemical complexity of ECM. Peptide amphiphile is amphiphilic and provides assembly into 3D networks of nanofibers similar to ECM

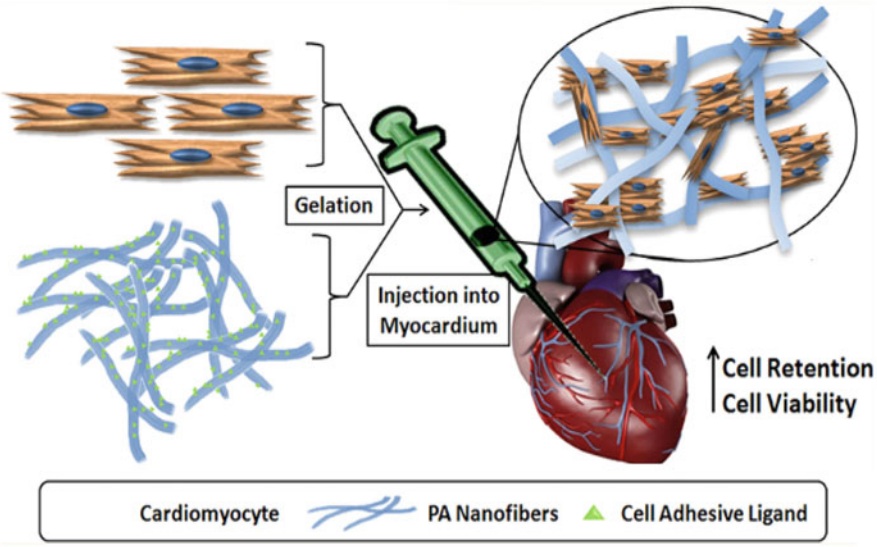

Fig. 4.11 PA-RGDS intensifies the endurance of mouse ESC-CMs and boosts heart function post-MI. (Adapted with permission from (Ban et al. 2014))

proteins under biological conditions. To know the underlying performance and machinery of transplanted stem cells in vivo, molecular imaging by dual-modal or multi-modal is more accepted to comprehend and review stem cell therapy with comprehensive information (Nguyen et al. 2014). To trace the transplanted stem cells and estimate their clinical effects, steadfast dual-modal imaging that utilizes bioluminescence and magnetic resonance imaging is set up (Cao et al. 2015). Li et al. (2017c) co-transplanted the bone marrow-derived mesenchymal stem cells using RAD/PRG or RAD/KLT, which promoted their localization and endurance in the infarcted myocardium, and their therapeutic effect was increased by co-transplantation with either RAD/PRG or RAD/KLT.

Ban et al. (2014) derived the CMS from mouse ESCs and encapsulated them in peptide amphiphile-RGDS to check their usage in MI therapy (Fig. 4.11). The incorporation of RGDS and GTAGLIGQ did the generation of peptide amphiphile-RGDS into peptide amphiphile. The CMs were taken from the rat model. They elucidated that most CMs survived in PA-RGDS for a week. The CMs were injected into the myocardium of mice model with PA-RGDS, and a threefold increase was seen in the incorporation in the models with CM+PA-RGDS compared to those with only CMs. A well-established cardiac function was seen in the group of mice with CM+PA-RGDS from 3 weeks and was sustained for 12 weeks.

4.5.9 Polyurethane Nanofibers

Tissue constructs with similar properties of structure and mechanics as that of myocardium were generated by Guan et al. (2011). In this regard, the electrospun polyurethane nanofibers were produced. The tissue construct mesenchymal differentiation was recorded by analyzing the expression of cardiac markers and the development of ion channels. The differentiation of cardiac cells was seen to be initiated by recording the mRNA expression. Tissue constructs were stretched statically to achieve cell alignment. The strain was increased from 25% to 75%, and it increased the degree of 3D alignment of cells. The RT-PCR determined that with a strain of 75%, the expression of GATA4, Nkx2.5, and MEF2C, which are the markers of differentiation of CMs, increased. Their work suggests that the pre-differentiation of mesenchymal stem cells into CMs before injection results in a higher cardiac regeneration rather than only injecting undifferentiated mesenchymal stem cells into the heart.

4.6 Conclusion and What Is Next

Cardiac tissue engineering is a field that repairs, reconstructs, and replaces cardiovascular structures, especially the heart, with engineered tissues. With the technology of deriving CMs from iPSCs, CVD phenotypes can be modeled, screening of the drugs can be done, and new ways of producing regenerative medicines can be achieved. It also offers ways to isolate iPSC-CMs from patients with genetic mutations or understand their pathophysiology. Direct reprogramming of the somatic cells into CMs without a transitionally pluripotent state is possible now. Genes are introduced at particular loci, and gene mutations are introduced to reverse the mutations that lead to diseases in iPSC-based CVD models in vitro. It is a novel and fast budding technology having thrilling applications. In the future, with more refinements, it will create means for the progress of personalized medicine for CVDs. Biomaterials can enhance stem cell function, and more knowledge of biomaterial engineering will help in molecularly designing biomaterials that resemble naturally occurring ECM of cardiac tissues. This way, biomaterials can guide the differentiation and function of progenitor cells. Biomaterials can be designed molecularly to control many of the factors that drive the differentiation of progenitor cells and function. Injectable nano-matrices could be designed to maintain biophysical and biochemical microenvironments of transplanted cells for better engineered cardiac tissues.

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