Chapter

20

MATERIALS FOR CARDIAC TISSUE ENGINEERING

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Contents

20.1. INTRODUCTION

Stem cell research is poised to revolutionise many aspects of biomedical research [1,2]. In consequence, in this field of study, research output and the number of active researchers have rapidly grown in a broad range of areas, including stem cell-based clinical applications, and basic research on fundamental cell biology and development. Thus, stem cell research has expanded in emerging topics such as embryonic stem (ES) and induced pluripotent stem (iPS) cells, bioethics and social science [3].

This is, for instance, the case of cardiovascular diseases, which are at present the leading cause of death worldwide, according to the World Health Organisation [4]. Once damaged, cardiac muscle has little intrinsic repair capability due to the poor regeneration potential exhibited by remaining cardiac muscle cells (cardiomyocytes). For decades, heart damage in patients has been typically managed using medical (aspirin, β-blockers, or angiotensin--converting enzyme inhibitors) or mechanical means, such as percutaneous coronary intervention and coronary artery bypass graft surgery [5]. Heart function, however, recovers completely only after cardiac transplantation, which is restricted by heart donor availability and deleterious immunological responses. One alternative is promoting repair by the delivery of functional cells to the injured myocardium. Thus, over the last 10 years, there has been tremendous effort in developing therapies based on stem cells and, more recently, tissue engineering [6-8].

In this context, nanostructure materials (within the overall size range of 1–1,000 nm) offer appealing strategies to allow greater means of regenerating cardiac cells and to strengthen weakened scarred heart tissue. For example, researchers recognise carbon nanotubes as reactive to electrical stimulation, and they then use these nanoparticles to create therapeutic cells with the characteristics of genuine cardiac progenitors [9,10]. Moreover, novel engineered cardiac patches are developed by seeding cells with regenerative potential onto porous scaffolds that give the appropriate shape and organisation to the tissue. Nevertheless, while the heart is an electrically conductive organ, the majority of materials used for scaffold assembly are non-conductive; thus, the resultant engineered tissue does not contract as normal heart tissue does. To solve this problem, some researchers have generated gold-infused cardiac patches, whose cells all beat synchronously [11-13]. Taken together, preliminary data from these studies could have implications for millions of people around the world, and show the undoubted potential held by this emerging therapy.

Herein, we examine and discuss the growing demonstrations of damaged heart repair involving approaches to cell- and tissue engineering-based therapy and nanotechnology. In the following text, we thus envision that we are moving

from traditional medicine (that addresses the symptoms of heart diseases) to being rightfully able to heal these debilitating diseases.

20.2. SCAFFOLD-FREE CARDIAC TISSUE ENGINEERING

20.2.1. Cell sheets

The scaffold-free cell sheets consist of a number of monolayers of therapeutic cells cultured on temperature-responsive polymer surfaces that allow non-enzymatic detachment [14]. Remarkably, monolayers or sheets of distinct cell types (*i.e.* mesenchymal stem cell (MSC)), through the establishment of tight and functional cellular connections, are able to propagate electrochemical signals, improving cardiac function when implanted to cover the infarcted myocardium in rodents [14-16]. Moreover, specific layers comprising endothelial cells can be placed between therapeutic cell sheets to enhance vascularisation of the implant in various animal models [17]. A similar strategy of scaffold-free tissue engineering is based on aggregations of embryonic stem cell (ESC)-derived cardiomyocytes, which will generate synchronously contracting cardiac tissue [18]. Despite the beneficial effects of scaffold-free constructs, the translation of this approach to the clinics is challenging, due to the difficulties of adaptation to human heart proportions.

20.2.2. Injectable nanomaterials

One of the most widely used injectable nanomaterials for cardiac regeneration are those referred to as hydrogels. In particular, hydrogels, which can be natural or synthetic, are three-dimensional (3D) cross-linked polymer networks that reproduce the extracellular matrix (ECM) and the natural microenvironment [19]. Hydrogels are used as a vehicle to inject therapeutic cells, proteins and genes into injured myocardium, conferring efficient cell retention into the place of injection. Commonly, hydrogels are injected transendocardially, epicardially or intracoronary for minimally invasive delivery. For this purpose, they are liquid and jellify upon injection *via* physical or chemical factors such as temperature and pH. Injectable hydrogels have been developed using numerous natural and synthetic biomaterials.

- Natural hydrogels: The most common natural hydrogels are collagen, gelatin, laminin, matrigel, hyaluronic acid (HA), ECM, alginate and chitosan. Importantly, the structure of these compounds is highly similar to the molecules found in biological organisms, thus reducing possible harmful immunoreactions after in vivo implantation.
- Synthetic hydrogels include poly(ethylene glycol) (PEG), polylactide (PLA), poly(lactic-*co*-glycolic acid) (PLGA), poly(caprolactone) (PCL), poly(acrylamide), polyurethane, poly(*N*-isopropylacrylamide), and self-assembling peptides hydrogels, just as examples. In terms of

advantages, these polymers can be easily modulated to have physical and chemical properties suitable for cardiac tissue engineering. However, they can induce cytotoxicity. Alternatively, a mixture of natural and synthetic hydrogels can be used, combining the advantages of both types of polymers [20-22].

Pre-clinically, application of hydrogels increases cell survival in cellular cardiomyoplasty [23-32], and contributes to restoring damaged myocardium in both small and large animal models of myocardial infarction (MI) [33-38]. Interestingly, injectable hydrogels also preserve cardiac function when administrated without cells, demonstrating that the cells may not be responsible [39-54]. For instance, promising preliminary results obtained in rodents and pigs using alginate [45,46] led to the first clinical trials in MI patients (NCT00557531 and NCT01226563) [55].

Injectable hydrogels can also be used to deliver proteins, genes and nanoparticles as therapeutics themselves or to improve cell survival and retention further, prolonging the release into the target tissue and ultimately improving cardiac function after MI. To date, a number of growth factors has been delivered using this strategy, including hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF), insulin-like growth factor-1 (IGF-1), stem cell derived factor-1 (SDF-1) and transforming growth factor beta (TGF-β1) [56-63]. Similarly, plasmids containing VEGF and pleiotrophin have been administered as injectable hydrogels, in order to improve transfection efficiency [40].

More recently, the use of other nanomaterials in combination with therapeutic cells has been explored. As examples:

- Superparamagnetic oxide, iron oxide and ferumoxytol nanoparticles: have been developed to deliver therapeutic cells into infarcted animals. Magnetic targeting was used to direct cells with regenerative potential to damaged areas, and to enhance cell retention and engraftment [64-66]. The incorporation of iron oxide nanoparticles into microcapsules comprising MSCs, agarose, ECM proteins, collagen, and fibrin highly increases cell survival and retention [67]. Also, the use of antibodies to target exogenous therapeutic cells and endogenous injured cells has been combined with iron oxide to enhance cell targeting by magnetic attraction [68].
- Magnetic nanobeads*:* have been employed to deliver adenoviral vectors encoding hVEGF to enhance transduction efficiency [69].
- Heparin-presenting self-assembling peptides nanofibers: used to deliver paracrine factors derived from hypoxic conditioned stem cell media into the ischemia-reperfusion model of MI. As a result, a significant preservation of hemodynamic function is obtained [70].

20.2.3. Exosomes

Exosomes are specialised lipid membranous nano-sized vesicles released by many cell types, containing a variety of RNA species (including mRNAs, and miRNAs), soluble (cytosolic) and transmembrane proteins presented in the appropriate and functional orientation. Their intrinsic properties make exosomes an ideal therapeutic candidate in regenerative medicine, such as for MI patients [71]. Exosomes have unique tissue / cell-specific proteins that reveal their cellular source. Their functions are still unknown, but they are believed to be also important for intercellular communication. Exosomes of a particular type of cells can have a therapeutic use on a specific tissue due to their paracrine effect. As an example, delivery of MSC-derived exosomes to the myocardial ischemia / reperfusion mouse model enhanced myocardial viability and prevented adverse remodelling [72]. Moreover, extracellular vesicles derived from human bone marrow MSC promoted angiogenesis in a rat MI model [73].

20.3. SCAFFOLDS FOR CARDIAC TISSUE ENGINEERING

20.3.1. Artificial cardiac tissue

The microenvironment in which cells live is crucial for the maintenance of their basic properties and functions. In particular, the microarray signalling and response of the cells rely on their interactions with the components of the ECM where they reside [74]. This is also the case for regenerative cells. In consequence, cardiac tissue engineering hopes to profit from the formation of new 3D functional artificial heart tissue that mimics the physicochemical and physiological properties of cardiac ECM. In this context, different natural and synthetic biocompatible and biodegradable materials (Figure 1) have appeared in the experimental set.

Figure 1

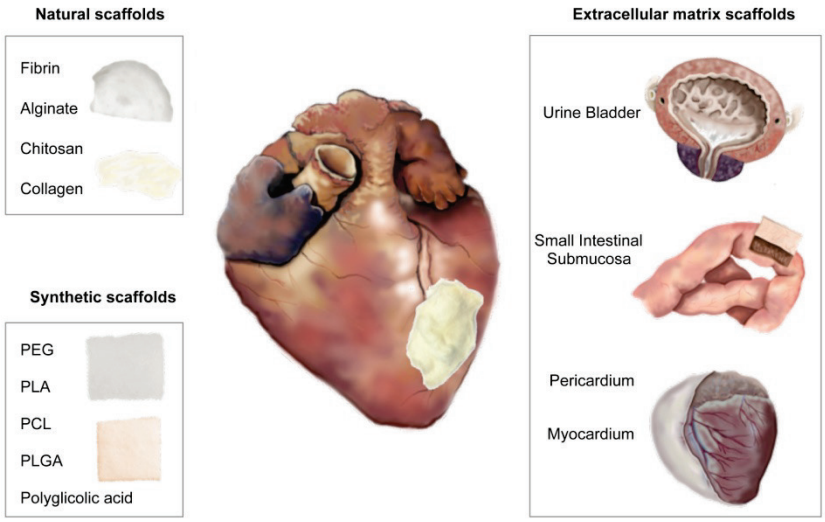

Figure 1. Schematic illustration of different scaffolds used in cardiac tissue engineering. On the left, the most commonly used matrices from natural (top) and synthetic (bottom) materials are listed. Several organs from which ECM scaffolds can be obtained are shown on the right. The figure was designed and hand-drawn by C.G-M.

- Natural materials such as fibrin [75], chitosan [76], alginate [10] or collagen mixtures [77], are highly flexible, allowing different sizes and shapes according to the needs of the individual recipient and the implanted cells. For instance, fibrin patches embedded with cardiac adipose tissue-derived progenitor cells demonstrated significant improvement in cardiac function in MI murine model after 30 days of follow up (Figure 2 A-D) [75].

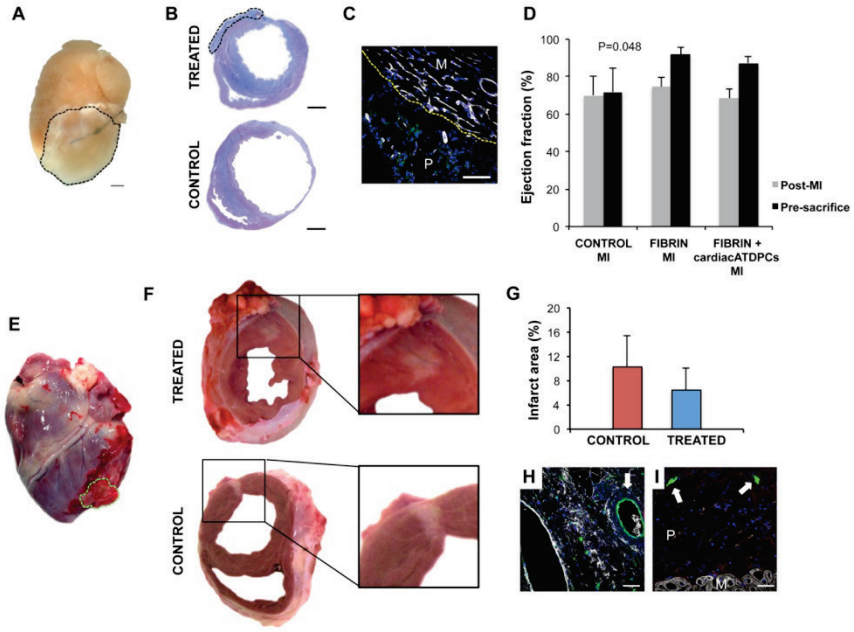

Figure 2. *In vivo* study results. (A) Picture of an excised mouse heart showing fibrin patch (dotted line) covering infarcted area. (B) Masson's thricrome staining cross section of cardiac ATDPCs fibrin (dotted line) treated and control animals. (C) Image exhibiting green fluorescent protein (GFP)-positive cardiac ATDPCs (green) and the presence of Isolectin B4 (white) positive vessels in the patch. (D) Histogram representing percentage of ejection fraction values of control-MI, fibrin-MI and fibrin with cardiac ATDPCs-MI animals at post-MI and pre-sacrifice time points [75]. (E) Image of an excised porcine heart, showing the pericardial descellularised scaffold (green dotted line) adhesion to myocardium after 30 days of follow up. (F) Images of two heart sections from treated and control animals exhibiting the infarct area. Zoomed-in photographs show transmurality degree differences between groups. (G) Histogram representing the mean ± scanning electron microscope (SEM) of infarcted area in control and treated animals after 1 month of follow up. (H) Green positive immunostaining against isolectin B4 (green) and elastin (white) indicates the presence of newly formed vessels (arrow) [110]. (I) Immunofluorescence against βIII tubulin (green), cTnI (white), and elastin (red) labelled nerve fibres (arrows), M, myocardium and P, patch, respectively [111]. *Nuclei* are counterstained with DAPI (blue). Scale bars = 1 mm (A, B), 75 μm (C), 50 μm (H, I).

- Synthetic materials: PLA [78], poly(glicolic acid) [79], PEG [80], PLGA [81], or PCL are the most commonly used for cardiac regeneration purposes [82]. They have been extensively analysed with bioreactors [83] and in animal studies and clinical trials [7,84]. But the aforementioned examples are only a short list of many existing synthetic biomaterials continuously emerging [85]. However, to date,

the U.S. Food and Drug Administration (FDA) has only approved the clinical use of PEG, PLA and PLGA [86].

Collectively, these new generated scaffolds should be porous to ensure the oxygen and nutrients' diffusion, and also should warrant the cell-cell interaction avoiding anoikis process. However, the similarity of the artificial cardiac matrix to cardiac tissue is not perfect, and the implanted cells only colonise the surface or not more than a few microns of thickness [87]. Latterly, in order to unravel this critical point, two different alternatives have appeared, as follows:

- Carbon nanotubes (CNT): have emerged as a novel alternative to improve biochemical and mechanical properties of the aforementioned materials. CNTs are composed of sheets of graphite rolled into cylindrical tubes and could be single- or multi-walled. In addition to their optimal conductive, mechanical and thermal features, CNTs are porous structures (1 nm in single-walled and 4–30 nm in multi-walled) mimicking natural collagen fibers of the ECM, favouring cell adhesion, proliferation and differentiation [88]. Regarding cardiac tissue repair, several studies have included the use of CNTs into their original scaffolds. For instance, Pok and colleagues have developed a chitosan scaffold with biocompatible CNTs acting as electrical nanobridges between cardiomyocytes. An improved electrical coupling, synchronous beating, and cardiomyocyte function were described [89]. Most recently, a CNTs scaffold has been generated to show its cytocompatibility, cell viability, attachment, proliferation and, also, cell infiltration [90].
- Electrospinning technology: has emerged to create new nanostructured scaffolds [91]. This method is based on immobilising different cell types with a wide range of molecules simultaneously within a fibre during the generation of the scaffold [92]. Interestingly, electrospinning technology can process both a small and large number of progenitor cells creating different 3D architectures. In this field of search, several studies have demonstrated the notable advantages of 3D synthetic electrospun scaffolds. As an example, Fleischer and colleagues have developed an electrospun scaffold made of PCL, dichloromethane and dimethylformamide with gold nanoparticles able to favour cell-cell coupling at the electrical level [11]. However, despite the electrospinning of synthetic polymers warranting a porous network of fibers allowing the diffusion of oxygen and nutrients, their mechanical properties do not match those of ECM, lacking the interaction between cells. In contrast, natural materials offer functional bioactive properties supporting tissue assembly, but cannot be electrospun because of their lack of viscoelasticity. Consequently, both materials are used together in search for the ideal electrospun scaffold for cardiac repair [93].

20.3.2. Extracellular matrix derived from natural tissues

ECM is a cell-secreted heterogeneous mixture composed of water, saccharides and numerous functional and structural proteins combined and spatially organised by tissue type [94,95]. Because of their importance in many processes, including cell proliferation and differentiation, guiding cell migration and modulating cellular responses, ECM is recognised as an attractive biomaterial for tissue engineering-based regenerative medicine [96]. In this context, scaffolds derived from ECM can be generated by decellularisation of biological tissue samples. This procedure removes cellular and nuclear content by physical (*i.e.* agitation, sonication, direct pressure, and freeze / thaw cycles), chemical (detergent and salts) and enzymatic treatments (*i.e.* trypsin) [97], producing 3D acellular matrices with identically retained anatomical geometry and vascular architecture [98,99]. In order to select the optimal tissue-specific decellularisation protocol, cell removal efficiency and the adequacy of ECM retention are crucial parameters to be considered [100]. In line, decellularisation affects not only ECM composition but also scaffold structure and mechanics, depending on a variety of factors such as detergent, concentration and, exposure time [101]. Importantly, collagen and laminin retention after the decellularisation process could facilitate subsequent scaffold recellularisation with regenerative cells by providing spatial orientation [100], while glycosaminoglycans and adhesive proteins removal might slow cell migration onto the scaffold and its bioactivity [101]. Before cell reseeding and surgical application, ECM-derived scaffolds need further processing, *i.e.* disinfection, lyophilisation and sterilisation, which adjust matrix integrity and architecture [98]. Preliminary results in both pre-clinical and clinical contexts show that management of allogeneic and xenogeneic ECM is safe and beneficial for cardiac tissue engineering [102].

In this context, different decellularised scaffolds from animal and human origins have been used (Figure 1) [103].

- Small intestinal submucosa: In brief, a commercial ECM patch derived from porcine small intestinal submucosa (SIS) (CorMatrix-ECM®) attenuates myocardial remodelling and improves cardiac performance in rats after MI [104]. Currently, a clinical trial is enrolling participants to evaluate its safety and functional benefit in subjects with ischemic myocardium (NCT02139189).
- Urinary bladder matrix: On the other hand, application of cell-free urinary bladder matrix (UBM) in the MI dog model increases myofibroblast recruitment, reduces inflammatory response and limits thrombus expansion, although no improvements in cardiac function, left ventricular dilation or contractility were observed [105]. Moreover, a similar study has shown increased contraction and myocyte recruitment and proliferation 8 weeks after treatment [106].

- Pericardium: Pericardial tissue is routinely resected or even removed during cardiothoracic surgery without adverse consequences [107] and shows similar properties within human individuals, presenting a porous ECM, which facilitates cell retention and vascularisation [108]. *In vitro* studies demonstrate that pericardial-derived ECM allows cell survival, growth and attachment induced pluripotent stem cells (iPSCs)-derived cardiomyocytes and MSCs [109], and also facilitates differentiation of progenitor cells derived from adipose [110] and cardiac [108] tissue towards endothelial or cardiac cell lineage, respectively. Furthermore, decellularised human pericardium embedded with self-assembling peptide RAD16-I and adipose tissue--derived progenitor cells (ATDPCs) increases scaffold vascularisation and reduces infarct size one month after implantation in the swine MI model (Figure 2 E-G) [110]. Hence, although the pericardial matrix is not an exact match to myocardial ECM, it favours cell retention and cardiac differentiation, offering an additional autologous / allogeneic therapeutic option for cardiac repair. Most recently, it has been demonstrated that an acellular decellularised pericardium implanted over infarction in swine is neovascularisated and neoinnervated by host cells and improves ventricular function (Figure 2 H,I) [111].
- Myocardium: Decellularised myocardial ECM shows better preservation of the original composition, 3D-architecture and ECM microenvironment [99] than natural matrices and conserves similar native myocardium stiffness [112]. *In vitro* studies using myocardial ECM as a scaffold demonstrate cell engraftment and differentiation towards cardiac [109,112-115] and endothelial lineage [113-115], beside spontaneous recellularised scaffold contraction [112,114]. These promising findings may promote *in vivo* testing of cell reseeded decellularised myocardial ECM scaffolds for MI repair. The first challenge is combining decellularised myocardial ECM with fibrin embedded with MSCs, already released to encourage pre-clinical outcomes in the near future [116].

20.4. BIOARTIFICIAL HEARTS

In 2008, a decellularisation protocol was adapted to generate a 3D whole-heart scaffold, using sodium dodecyl sulfate (SDS) by antegrade coronary perfusion in cadaveric rat hearts [117]. Acellular generated heart preserved main matrix proteins, vascular architecture, valves and chambers and, importantly, the recellularised heart exhibited contractile activity when reseeded with cardiomyocytes and endothelial cells [117]. In recent times, further studies have reported decellularisation of porcine hearts, which is a model scalable to human proportions [118-120]. The perfusion-decellularisation procedure is particularly efficient for whole-organ decellularisation, since it reduces the diffusion distance required for decellularising agents to reach cells [121], and makes use of convective forces facilitating tissue removal of cellular components [122]. In order to improve decellularisation of whole hearts, attempts integrating a software-controlled automatic coronary perfusion system have been developed, obtaining reproducible scaffolds [123,124].

Recellularisation is a final mandatory step that involves seeding cells onto the decellularised scaffold, which could be placed in a bioreactor to support tissue growth [7]. The heart has a high cellular density, with around 10^8 cardiomyocytes/cm^3, and its 3D scaffold has to be fully recellularised at the time of implantation in order to be completely functional [125]. Therefore, seeding such large numbers of cells would require several weeks of organ culturing in the bioreactor before implantation [117].

Despite whole-organ providing a promising platform for cardiac tissue engineering techniques, many unresolved issues and challenges need to be addressed before engineered hearts can be a clinical reality.

20.5. PITFALLS, CONCLUSIONS AND PERSPECTIVES

Researchers and clinicians agree in the commitment to supply new and better treatments for patients, including those suffering heart diseases. In line, the most immediate benefit of the converging research areas (*i.e*. those dedicated to stem cells, tissue engineering and nanotechnology) is essentially in regenerative medicine.

However, there are hurdles on the road ahead, regulatory as well as technical, and those related to deleterious side-effects for treated patients. For instance, although the engineering of nanomaterials holds great promise for development of innovative immunomodulatory approaches [126,127], the potential risk of their accelerating use has become relevant. Respiratory exposure to manufactured magnetic iron oxide nanoparticles generates a great number of extracellularly secreted membrane vesicles (exosomes) capable of transferring activation signals to the immune system [128]. In particular, in those individuals who already have pre-existing allergic conditions (known as

sensitised individuals), the generated exosomes may result in a delayed type of hypersensitivity reaction and subsequent severe allergic responses; whereas in unsensitised individuals, the resulting immune activation response may be much lower. Furthermore, a key role of nanoparticle-induced exosomes as signalling mediators in the induction of immune activation *via* T helper cell type 1 has been reported [129]. Taken together, these studies suggest that the generated exosomes aggravate the immune activation and inflammatory responses induced by exposure to nanoparticles. Alternatively, in order to regulate potential harmful immune response *in vivo* further, nanoparticles could be co-administered with immunosuppressive cells or cell-free immunomodulatory agents [130,131].

In sum, cell- and tissue engineering-based therapies involving nanostructure materials are exciting exploration areas, and have shown both intriguing and instructive preliminary results in the treatment of cardiac diseases. However, as clinical trials proceed, our incomplete understanding of the behaviour and functions of regenerative cells and associated nanoparticles is made evident by numerous unresolved concerns, including the optimal therapeutic strategy that can be readily translated to patients.

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