Multimodal biomaterial strategies for regeneration of infarcted myocardium†

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Myocardial tissue engineering (MTE) is an exciting front of research which is both highly competitive and extremely challenging for researchers. MTE aims to attenuate the functional set back in terms of cardiac output faced by the heart undergoing myocardial infarction (MI). MI results in substantial death of cardiomyocytes in the infarct zone followed by a strong inflammatory response and heart transplantation is the most common corrective measure for cardiac tissue engineering. Researchers are continuously striving to develop a better alternative to this highly invasive technique. Although numerous cell-based and cell-free strategies have been employed to bring about the repair of myocardium in recent times, the quest for optimal biomaterial still continues, owing to hurdles in design and fabrication of fully functional and efficiently engineered construct. In order to fabricate the constructs for myocardial repair, several biodegradable and non-biodegradable polymeric biomaterials have been analyzed over the years for their mechanical properties, biocompatibility with various cell types and functionality upon implantation. A hallmark of functional myocardium is its ability to propagate electrical impulses and respond to these impulses by synchronized contractions that generate forces for pumping blood for all metabolic activities of the body. For biomaterials to influence the myocardium microenvironment, suitable designs for cell recruitment and formation of functional conductive bundles are expected. The unique tissue structure and functioning of heart have prevented constructs from being proficient enough to be taken to clinical trials. Nonetheless, various tissue engineering strategies have evolved such as 3D implants, 2D patches and injectables, whose positive indications render optimism to investigators, that the tissue engineered regimen, would bring new treatments for patients who have suffered from agonizing MI. Focusing on biomaterials, this review provides an insight into such multi-modal research strategies, major advances and promising paradigm shifts in the field of myocardial tissue engineering.

1. Introduction

Cardiovascular diseases (CVD) are the single leading cause of deaths globally. In 2004, 17.1 million people died of CVD which represents 29% of global deaths out of which 42% deaths were solely due to coronary heart diseases (US Census Bureau 2004). CVD eventually lead many serious complications including congestive heart failure (CHF) that remains a significant problem for the global medical community. Currently, more than 10 million people suffer from CHF in the USA, UK and southeastern Asia. Acute myocardial infarction (MI) is the leading cause of CHF.[1] MI is caused when supply of oxygen and nutrients to the cardiac muscle is impaired, usually due to occluded coronary arteries. As a result, massive cell death occurs in the affected heart region.[2] Besides life threatening arrhythmia, damage of muscle tissue in the left ventricle can cause dysfunction and remodeling in terms of progressive dilation imparting structural changes which culminate in the formation of non-contractile fibrotic scar tissue.[3,4] Hence, the damage incurred to the heart wall is beyond recall as the myocardial tissue has limited regeneration capacity.[5,6] Although the body compensates for LV remodeling initially, mismatch of the mechanical and electrical properties of the scar with native myocardium ultimately affects functioning of the heart leading to chronic heart failure, whereby the heart cannot pump adequate blood for all metabolic activities of the body.[7] About 30% of the people are unable to survive the acute shock of MI.[8] The survivors are required to endure pharmacological therapy in the form of catecholamine, beta-blockers, aldosterone or acetylcholine esterase (ACE) inhibitors to pacify peaked immunological activities.[9] However, drugs alone cannot control disease progression competently.[10] As a result, the patients depend upon two life saving options: heart transplantation or the use of left ventricular assist devices (LVADs). Limitations such as the availability of a donor organ for transplantation and the cost of LVADs encourage engineering of cardiac aiding constructs that represent a prospective source of advanced therapy in combating CHF. Many intriguing modes of regenerating injured myocardium have emerged over time with pioneering research in a variety of technologies including, cell therapy using various cell

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types, injection of biomaterials, bioengineered patches, 3D construct implantation and even bioreactor treated implants.[11,12,13] However, at present, there are no successful models of bioengineered cardiac implant that can completely replicate anatomy, physiology and biological stability of a healthy heart. It is in quest of this ideal construct that tissue engineering as a discipline has aggressively moved towards designing artificial tissues using cells and specifically designed biomaterials. In order to resolve the conundrums of tissue engineering of the heart, deep understanding of material chemistry, cardiac anatomy and cellular biology is required. This review aims to provide an in-depth look at heart anatomy, biomaterial advances and novel methodologies involved in the development of multimodal strategies in myocardial tissue engineering.

2. Anatomy of heart muscle

2.1. Structure, function and extracellular matrix

Heart muscle is highly vascular and contractile with three major layers namely an outer covering pericardium, a muscular myocardium and an endothelial lined endocardium. The muscular myocardium consists of cardiac muscle cells, cardiomyocytes and cardiac fibroblast.[14,15]

Although replete in number, cardiac fibroblast account for only one third of the total volume of cells in the muscular myocardium.[16] Cardiomyocytes are the functional units of the heart whose contractile properties make the heart a unique organ. Each cardiomyocyte is efficiently fueled by a large number of capillaries.[17] These bi-nucleated cells lose their ability to divide after birth. Cardiomyocytes have distinctive structural features such as striations, similar to those of voluntary skeletal muscles and involuntary function, like those of smooth muscles.[18] After birth, cardiac cell growth is associated with increase in size of myocytes without substantial increment in the cell number.[19,20] Limited proliferative capacity of adult cardiomyocytes implies that compensation of cell loss is effectuated by increased workload on remaining cells.[21] Despite these considerations, cardiac regeneration during the ageing process is now stipulated.[22–24] Adult hearts have been shown to contain resident stem cells which make the idea of cardiac regeneration through ageing and post pathological trauma seem conceivable.[25,26] The myocytes are held up by a three dimensional extracellular matrix (ECM) network produced by cardiac fibroblasts.[27] Essentially, the ECM is made up of 80% and 10% collagen types I and III, respectively.[28] The other, less abundant, matrix molecules are collagen types IV, V and VI, elastin and laminins.[29] Differential quantitative values of coexisting collagens account for altered stiffness and mechanics in different regions within the heart.[30] In the healthy heart, collagen serves to maintain normal cardiac architecture by surrounding and bridging myocytes, which consistently assist in coordinating the contractility and maintenance of cardiac shape and size as well as the function of the cardiomyocytes.[31] Circumferential forces from stretching of helically wound elastin about shortened, thickened myocytes in systole promote elongation in tandem with intra-myocyte forces of elongation.[32] The interconnected myocytes form unique muscular bands which are typically folded to make up the heart chambers, imparting an apical shape to the heart.[33]

The primary function of the heart is to pump blood by orchestrated contraction and relaxation cycles of cardiomyocytes. The regulation of contractility of individual cardiomyocytes is achieved by spatially defined ion exchange channels present in its membrane coupled with electrical stimulations. Changes in the rate and force of contraction in stressful situations are outlined by calcium ion mediated responses.[34–36] During systole, alignment of myocytes is maintained by surrounding collagen that is responsible for transmission of force, although most of the wall stress is borne by myocytes themselves. During diastole, lengthening of myocytes uncoils collagen fibers and suction of blood occurs. Appropriate lengthening protects the myocytes from overstretching while subsequent shortening causes contraction of heart and ejection of blood.[37,38] Intimate associations of ECM with cardiomyocytes allow cardiac fibroblasts to recognize mechanical, electrical and chemical cues within the myocardium, thereby facilitating cardiac workload responses.[39]

The structure of the heart largely affects its functional efficiency. The pumping activity occurs in the form of a twist around the apex of the heart by virtue of augmenting tensile strength due to twisting collagen. The suction and ejection of blood in the left ventricle is brought about by precise shortening and lengthening of the cardiac muscles. Essentially, in a healthy heart 15% fiber shortening during contraction causes ejection of 60% of the blood off the left ventricle. Due to MI, dilation of damaged left ventricle occurs and the apical contour is replaced by an atypical spherical contour curbing ejection to a mere 30%.[20]

2.2. Cardiac failure and remodeling

The partly damaged heart tries to sustain its functional activities following an agonizing infarction of the heart. The dead tissue is replaced by a rather non-contractile and non-functional collagen scar.[40] As a result, the pumping efficiency of the ventricles is greatly reduced. The injured heart conducts impulses slowly that may result in chaotic and irregular beating rhythms. Hereafter, cardiac output and blood pressure are dangerously affected posing the risk of infarct extension.[41] These proceedings leading initially to a downfall of cardiac activity and eventually to the clinical syndrome of end stage heart failure (HF). At the end stage, survival options are limited to artificial devices and transplanted heart.[2,27]

Since myocardial collagen is not a static protein, ECM homeostasis requires equilibrium between synthesis and degradation that is maintained by co-ordinated activity of stimulators and inhibitors.[27] However, in the failing heart, activation of a number of humoral, autocrine and paracrine pathways determine how the ECM metabolism is regulated and ultimately dictates the extent of myocardial remodeling.[42] In this respect, changes in the balance of ECM synthesis and degradation may eventually lead to disruption of the composition of collagen network in the heart.[43]

Remodeling of LV is characterized by increased stress on its wall.[2] The ability to generate external work is dependent upon the developed tension also its radius of curvature. These

functional aspects to LV remodeling demonstrate the significance of the shape and size of heart. Taking these into account, Laplace's law explains anatomical design affecting functional capacities in terms of the relationship between stress (T) and pressure difference across the inner and outer wall (ΔP). If 't' is wall thickness, and R is radius of the wall, stress is mathematically calculated as $T = (R\Delta P)/t$. The mechanical properties of the construct should be such that it has the potential to take the stress off the heart walls. This can be done by decreasing the ΔP to such a level that the stress on the heart wall is naturalized.[44]

2.3. Current treatments for heart failure

At end stage HF, the incapacitated myocardium deteriorates the pumping efficiency of the heart to an extent that it is unable to meet the metabolic requirements of the body.[2] At this stage, the patient is left with the option of either heart transplantation or use of LVAD for survival. Currently, other than heart transplantation, no standard clinical procedure is available to restore damaged myocardium.[45] Heart transplantation is plagued with problem of donor shortage and majority of patients die while waiting for organ transplantation.[46,47] It is a highly invasive surgery and includes major risks involving immune-rejection by host. The patient must be given immunosuppressants all through their life, which might lead to damaged kidney and also poses risk of cancer development.[48] With advances in medicine, there are now devices available which decrease the load on the heart such as LVADs.[49,50] These can be surgically implanted for short time, to either bridge the time gap while patients wait for a transplant[51] or as destination therapy[52] for those who are unsuitable for transplantation. The few devices are currently used in clinical practice, have proved prohibitive as they are highly expensive.[33] Existing limitations call for newer and more feasible alternatives such as MTE.

3. Myocardial tissue engineering

MTE is an inter-disciplinary field of research whereby diverse cell based and cell free strategies are being investigated in the quest for a sustainable therapeutic for refurbishment of cardiac functionality. Essentially, tissue engineering is an attempt at bringing about repair by mimicking nature. It is aimed at boosting the low regenerative capacity of the damaged myocardium by applying principles of engineering, material chemistry and cell biology. The classical strategy employed in tissue engineering is the provision of external help in the form of biomaterials and biomolecules with properties bearing close resemblance to their natural counterparts. However, owing to the uniqueness of heart tissue, the quest for optimal biomaterials and an efficient strategy of MTE remains persistent. This review provides an insight into the biomaterial advances and rationale behind multimodal strategies towards treatment of an infarcted myocardium.

A bioengineered construct is desired to possess certain essential characteristics, such as appropriate physical and mechanical properties, ready adherence, nontoxicity, non-antigenicity, non-invasive applicability and ability for complete integration with host. It is highly advantageous to have an artificial ECM that promotes cell adhesion as well as can be assimilated by the body as the new tissue regenerates. For myocardial tissues, endothelial and cardiomyocyte adhesion have been proven beneficial and can be achieved by suitable modifications of biomaterial surface chemistry such as addition of RGD moieties or growth factors for cell attachment or chemotactic recruitment.[53,54] Optimally, myocardial scaffolds should persist long enough to guide the integration of applied cells with native tissue without interfering with eventual physiological coupling in the myocardium. Techniques such as varying macromer concentrations and cross-linking of groups have shown to manipulate degradation rates while maintaining mechanical integrity. Procuring control over mechanical properties such as porosity and stiffness of biomaterial, while maintaining their bioactivity is critical.[55] Porosity is shown to influence cell trafficking of cardiomyocytes, which may have a cell dimensions exceeding 100 μm. Large interconnected pores allow integrity *via* colonization of cells, but excessively large pores may impair vascularization.[56,57] On the other hand, smaller pores may cause failure of implant due to poor diffusion of oxygen and nutrients.[58] The stiffness of native heart tissue ranges from 10–20 kPa at early diastole and increases to 50 kPa at the end of diastole, which may shoot up to 200 kPa or more in infarcted hearts.[59,60] It is indeed challenging to design a biomaterial capable of working in synchronization with its nonlinear elastic behavior upon integration. Successful integration also portends proper spatial vascularization since it has far reaching impacts beyond oxygen and nutrient supply. Angiogenesis may be achieved by addition of function specific growth factors. However, disappointments of therapeutic angiogenesis in clinical trial necessitates development of better strategies for controlled delivery of angiogenic promoters such as fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), platelet derived growth factor (PDGF), and hepatocyte growth factor (HGF).[61] Because angiogenesis arises from a series of cellular events in response to these factors, controlled release of multiple factor using biomaterials is extensively studied.[62,63,64] Design of such bioactive biomaterials that can respond to their environment seems to be a promising approach towards myocardial angiogenesis and regeneration of myocardium using exogenous cells (Fig. 1).

3.1. Cellular-strategies

MI is mainly associated with ventricular dysfunction due to death and irreversible loss of cardiomyocyte cell mass. Traditionally, a cardiomyocyte has been considered terminally differentiated in response to injury. However, recent evidence raises the possibility that a natural system of myocyte repair exists. According to this study, less than 50% of cardiomyocytes are exchanged during a normal life span. This system appears to be inadequate in face of an ischemic or heart failure insult and its treatment.[12] Nonetheless, the capacity of the adult human heart to generate myocytes suggests that it is therefore rational to work towards the development of therapeutic strategies aimed at stimulating the regenerative process. Cellular therapeutic approaches involve delivery of an adequate cell dose to the area of interest in the injured region. It is estimated that a favorable microenvironment would aid homing of cells and eventually improve the functioning of the myocardium. Currently, routes of cell administration include intravenous,[65] intracoronary,[66] transmyocardial (by direct epicardial injection),[67] catheter-based

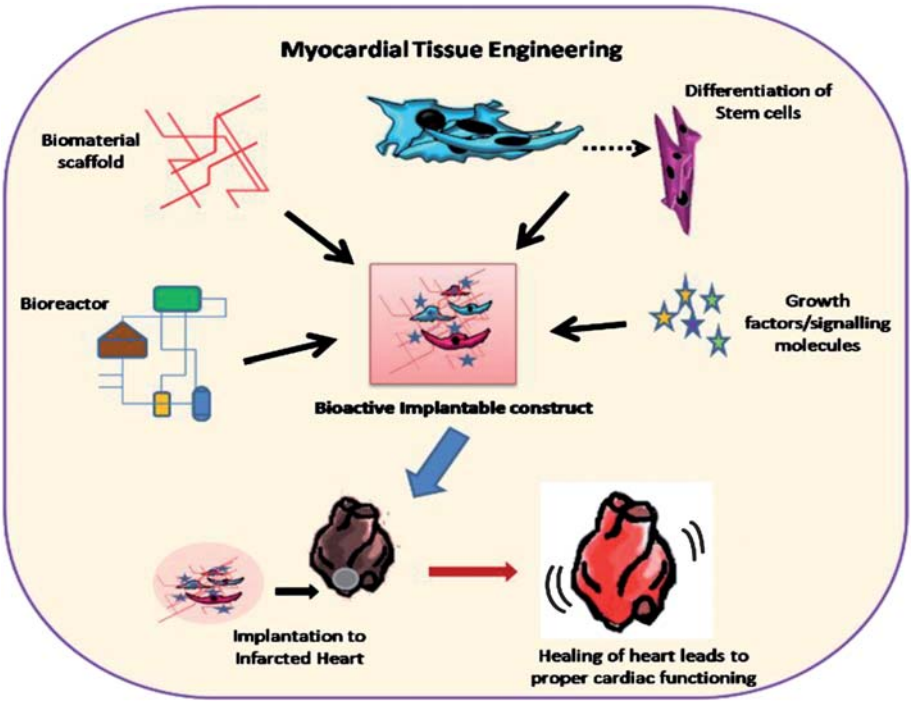

Fig. 1 An overview of MTE.

transendocardial injection using electromechanical voltage mapping,[68,69] and a recently implemented approach of intravenous injection into coronary veins.[70,71] No single stratagem has emerged as the preferred technique and perfect timing and area of administration is yet to be determined. Despite several advantages such as safety and improved functioning, the efficiency of delivery and retention is lower than expected, and retention and survival of cells at sites of delivery have been limited. Furthermore, underlying mechanism of repair without sufficient retention is still not fully understood. Nonetheless, it is now apparent that a large number of cell types are potential in bringing about physiological benefits to infarcted heart such as bone marrow cells (BMCs),[72,73] mesenchymal stem cells (MSCs),[74,75,76] endothelial progenitor cells (EPCs),[77] resident cardiac stem cells (CSCs),[78,79] Skeletal myoblast cells (SMCs),[80,81] Embryonic stem cells (ESCs), and induced pluripotent stem cells (iPS).[82,83] Contractile properties of iPS-derived cardiomyocytes and ESC derived cardiomyocytes have been reported to be similar although significantly different from ventricular tissue of comparable age. ESC-cardiomyocytes exhibit fairly mature electrophysiological properties, suggesting that mature CMs could be obtained from hESCs. Transplantation of hESC-CMs after extensive MI result in the formation of stable cardiomyocyte grafts, attenuation of the remodeling process, and functional benefit.[84,85] Eccentrically, the significance of cardiac output improvement of ESC injection diminishes over long term making them unsuitable for clinical therapy.[86] An important consideration in cell sourcing can be whether the cells are autologous (self) or allogenic (non-self). ESC derived cells fail to escape immune surveillance in the xenograft and elicited a rejection phenomenon in immune competent rats.[87] In contrast, MSCs have the therapeutic advantage considering their easy availability, expansion, differentiation potential and immunosuppressive characteristics in many fields of tissue engineering including MTE.[88,89,90,91,92,93] Currently, 25 new clinical trials are in progress in the United States, and a similar number are ongoing in Europe using different cell types; however, no agreement has been reached regarding the standardization of methods, especially cell harvest, isolation, and preparation.[94,95]

3.2. Biomaterial strategies

3.2.1. Natural biomaterials. Natural polymers are extremely diverse and have evolved to perform specific biochemical, structural and functional roles in the body. In an attempt to make natural microenvironment mimicking construct, the preference of such biomaterials is inevitable owing to the presence of multifunctional groups and high bioactivity.[96] Natural biomaterials used for developing scaffolds may consist of components found in the ECM, such as collagen, fibrinogen, hyaluronic acid, glycosaminoglycans (GAGs), hydroxyapatite (HA) *etc.*, that account for its high bioactivity, biocompatibility, and similar mechanical properties as native tissue.[97,98] Collagen is the most abundant protein in animals. Due to its high mechanical strength and good resistance to degradation, it has been utilized in a wide range of products in industry[99] while its low antigenicity has resulted in its widespread use in medicine.[100] Characteristics of collagen as a biomaterial are distinct from those of synthetic polymers mainly because of its mode of interaction in the body. It is a good surface-active agent and demonstrates an ability to penetrate a lipid-free interface.[101] Collagen exhibits biodegradability, weak antigenecity[102] and superior biocompatibility as compared with other natural polymers, such as albumin and

gelatin. In fact, collagen gel was reported to be the first engineered heart tissue (EHT).[103] Later on, similar cellular and cell-free collagenous constructs were fabricated using different strategies by other research groups.[104,105,106] Another commonly used natural polymer which is derived from collagen is gelatin. Gelatin is a denatured protein and the potential pathogens are eliminated during its denaturing hydrolysis process. The isoelectric point of gelatin can be modified during the fabrication process to yield either a negatively charged acidic gelatin, or a positively charged basic gelatin at physiological pH.[107] Gelfoam®, commercial gelatin foam, has been shown to be supportive of rat ventricular muscle into grafts.[108] Other commercially available natural biomaterials such as Matrigel™, comprised of a variety of ECM components including laminin, collagen IV, and heparan sulfate proteoglycans, has also been shown to contribute to the improvement of neovascularization in ischemic mouse model.[109,110] Natural biomaterials fibrin and alginate have been in the limelight for a while, given to their injectable properties. Fibrin glue is an already Food and Drug Administration (FDA) approved biomaterial that is routinely used as a surgical adhesive and sealant. Fibrin is formed by the addition of thrombin to fibrinogen. Thrombin enzymatically cleaves fibrinogen, which then forms the polymer fibrin.[111] It can be delivered to myocardium easily with minimal inflammatory responses and is reported to prevent the deterioration of cardiac function and infarct wall thinning after MI in rats.[112,113] Alginate is a linear polysaccharide copolymer of (1–4)-linked β-mannuronic acid (M) and α-guluronic acid (G) monomers, derived primarily from brown seaweed. Alginate is being extensively studied at clinical level for different biomedical applications.[114,115,116,117] Hydrogels of alginate are produced when divalent cations, such as Ca^{++}, cooperatively interact with blocks of G monomers to form ionic bridges between different polymer chains.[118] The absolute value of the ultimate stress and modulus of alginate gels are lower than collagen and elastin although the ultimate strain lies in the same range.[119,120] It is true that all natural gels are significantly softer than native heart tissue and therefore unlikely; to provide sufficient mechanical support to stressed ventricular walls. In such a case, ECM proteins synthesized and assembled by cells are expected to provide the mechanical integrity of the tissue overtime. The ultrastructure and 3-D architecture of decellularized scaffolds can be largely preserved using mild decellularizing ionic solutions. Decellularized organ ECM are clear of immunogenic material can serve as excellent scaffolds as they contain the structural and functional molecules secreted by previously residing cells.[121] Very recently (2009), Singelyn and colleagues have demonstrated the feasibility of a myocardial matrix as an injectable MTE scaffold (Fig. 2).[122] Diverse polysaccharides and proteins such as silk fibroin and chitosan have been recognized to have significant roles in the organization of living cells and tissue growth. Interactions of these biopolymers in the ECM can lead to the formation of macromolecular structure. Additionally, the structure of chitosan resembles that of glycosaminoglycan in an ECM and can ultimately support the growth and differentiation of stem cells into cardiomyocytes.[123] It is generally accepted that the degradation of silk materials should match the function needs and ensure optimum mechanical and physiological integration of the device. Control over the rate is an important feature of function tissue design as the rate of scaffold degradation should match the rate of tissue growth.[124,125] The degradation behaviour also ranges from purely bulk to entirely surface degrading, based on the nature of the backbone chemistry and type of degradable units. The mechanical properties of these polymers are primarily based on factors such as the network cross linking density and polymer concentration. As we better understand the biological features necessary to control cellular behavior, smarter materials are being developed that can incorporate and mimic many of these factors.[126]

3.2.2. Synthetic biomaterials. Successful tissue engineering requires development of compliant biodegradable scaffolds that can sustain and recover from multiple deformations without disturbing the surrounding tissues. The development of synthetic elastomeric scaffolds for MTE is desirable as their mechanical conditioning regimens have shown to promote tissue formation along with gradual stress transfer from the degrading synthetic matrix to the newly formed tissue.[127,128] Biomaterials such as poly(glycolic acid) (PGA), poly(lactic acid) (PLA) and their copolymer PLGA have been studied for their tissue engineering application.[129–131] The first engineered beating construct on synthetic polymers was, in fact, achieved using PGA scaffolds.[132] The beatings were, however, no way comparable to that of native ventricular contractions. Poly(ε-caprolactone) (PCL) is used in many FDA approved medical devices. PCL is a flexible semi-crystalline linear aliphatic polyester whose degradation products are either metabolized by the body or excreted out.[133,134] To provide better control over its mechanical and degradation properties, it is often copolymerized with lactide or glycolide.[135] For example, epsilon-caprolactone-*co*-L-lactide has shown better *in vitro* response with alpha smooth muscle cells compared to PGA sponge reinforced with knitted poly-L-lactide fabric (PCLA) patches with respect to provoking the immune system response.[136] Despite superior biocompatibility, applicability of PLA and PGA based materials is limited in MTE due to their extremely high stiffness.[137] Contemporary research aims at fabricating scaffolds of biomaterials with mechanical properties comparable to native heart tissue such as poly glycerol sebacate (PGS) with stiffness as low as 0.05–1.2 MPa.[138–140] Quality elastomeric properties are impossible to achieve with thermoplastic polymers as they undergo plastic deformation and failure upon long-term cyclic strain.[141] PGS scaffolds and fiber mats are biocompatible with cardiomyocyte as well as a variety of stem cells.[142] Despite fragility, patches carrying ESC sutured over the left ventricle of rats *in vivo* remained intact over a 2 week period without any deleterious effects on ventricular function.[143] Suitable tailoring of PGS in terms of chemical, structural, mechanical, and degradation properties are potentially useful for the soft tissue engineering application.[144] Other polymers suitable for implantable devices include polyurethane (PU), polyester urethane (PEU) and polyester urethane urea (PEUU) owing to their broad range of mechanical properties.[145–147] PEUU permits cellular integration and endocardial endothelialization with minimal inflammation (Fig. 3).[148,149] Nevertheless proven biocompatibility, its clinical application is questionable due to its toxic degradation end products.[150,151] The mechanical characteristics are exhibited by poly (1,3-trimethylene carbonate) (PTMC), a rubbery amorphous polymer,

Table 1 An overview of biomaterials used in MTE

Biomaterial	sheet	3D	injectable	Other (knitted/bioreactor treated)	References
Natural biomaterials					
Collagen		✓		✓	103–105
Gelatin		✓			108
Decellularized ECM		✓	✓	✓	122,173
Chitosan	✓				123
Silk fibroin	✓				88
Alginate		✓	✓		155,156,163
Fibrin			✓		159–161
Hydroxyapatite			✓		165
Peptide nanofiber			✓		182
Matrigel			✓		109,110
Synthetic biomaterials					
PLLA		✓			131
PTMC		✓			152
PLLA with PGA		✓			135
PCL				✓	178
PGS		✓			137,138,139
PU	✓				146
PEUU	✓				147,148
PEG			✓		168
NIPAAm	✓		✓		169
Poly propylene	✓			✓	175,183,184

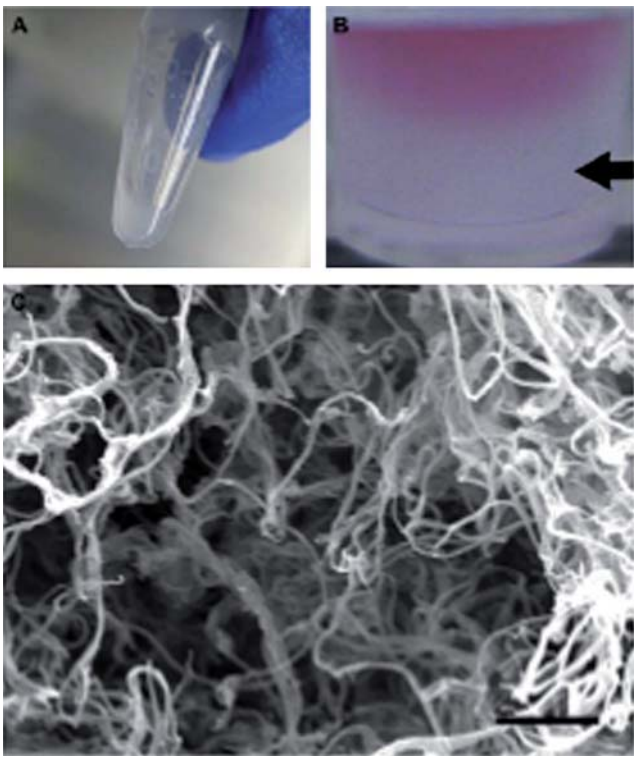

Fig. 2 Myocardial matrix gelation and characterization. (A) At room temperature the solubilized matrix was a liquid. (B) At 37 °C, the myocardial matrix self-assembled into a hydrogel, as indicated by the arrow. The pink media is shown on top as a contrast to the solidified gel. (C) A scanning electron micrograph of a cross-section of the myocardial matrix gel with nanofibers approximately 40–100 nm. The scale bar is 1 mm. Reproduced with permission.[122]

is unable to recover from deformation unless cross-linked.[152,153] Its copolymerization with lactide and caprolactone imparts an interesting range of properties for MTE.[154] (See Table 1.)

4. Biomaterial fabrication strategies employed to repair the infarcted myocardium

4.1. Injectables

Current treatments for myocardial infarction use highly invasive methods such as open heart surgery. Given the patient morbidity and complications involved with current procedures, it is not surprising to witness that the most actively pursued strategy for treating CHF post MI is the development of minimally invasive techniques such as injectable therapies. Computational models suggest that injection of biomaterials, with or without cells, contribute to fractional changes in the myocardial wall that greatly alter the ventricular performance. Also, major mechanical changes are associated with minor wall thickening due to injection. As a result, significant reductions in the ventricular wall stress and minimize stress-induced remodeling of the wall takes place.[155] Many biomaterials have supported this theory demonstrating attenuation in loss of cardiac function post MI upon injection into the sensitive areas of the heart. In a recent investigation, Landa and colleagues (2008) studied the effect of injectable alginate implant on cardiac remodeling and normal functioning in infarcted rats. An easy to inject, low-viscosity calcium cross-linked alginate solution was prepared which displayed phase transition into hydrogel upon injection due to increased calcium ion concentration in infarct zone. Interference of biomaterial in cardiac environment was marked by high myofibroblast infiltration resulting in increased scar thickness and diminution of infarct expansion.[156] Alginate hydrogel is also an effective delivery system for the precise dosage of angiogenic growth factors into myocardium and hence a promising biomaterial for the development of angiogenesis therapeutics.[157] Alginate manipulates mechanical properties of conducting polymers such as polypyrrol converting it into a brittle polymer for easily injectable blend. The resultant modified biopolymers possess improved cell attachment and arteriole formation properties.[158]

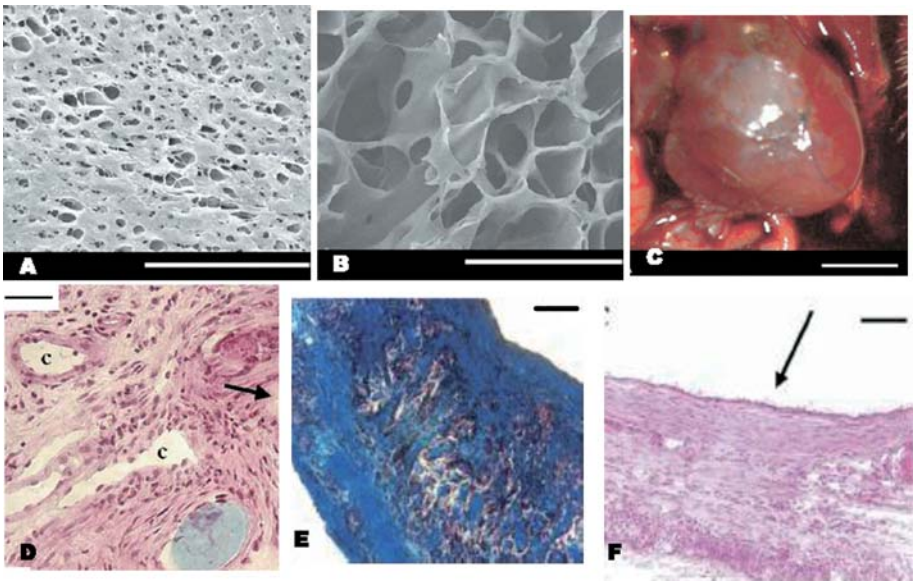

Fig. 3 Electron micrographs of polyester urethane urea (PEUU). (A) The scaffold surface (scale bar, 50 μm) and (B) cross-section (scale bar 100 μm). (C) Representative images at 12 week explantation for PEUU (scale bar, 5 mm). (D) Fibrous tissue transition region between the implanted PEUU material and the native right ventricular muscle, microvasculature (Scale 20 μm). (E) Masson trichrome staining of sections indicating collagen (blue), fibrous cells (red), and nuclei (black) for PEUU 12 weeks (all scale bars, 100 μm). (F) Completely endothelialized PEUU at 4 weeks. Reproduced with permission.[148]

Vascularization of a construct is crucial for its functional success *in vivo*. The myocardial microenvironment is pivotal in new blood vessel formation upon implantation. Many commercially available biomaterials such as collagen, matrigel and fibrin glue, display potential to enhance capillary formation by endothelial cells in the infarct area.[83] Fibrin is a critical blood component responsible for homeostasis and a versatile biopolymer scaffold in tissue engineering. Fibrin alone or in combination with other materials has been used as a biological scaffold for stem or primary cells to regenerate various tissue systems.[159] Temperature sensitive chitosan hydrogels have been shown to provide a favorable microenvironment along with ESC by promoting angiogenesis and therefore better cell survival. Delivery of stem cells along with hydrogels can reduce the frustrations in the field of stem cell transplantation.[160–163] Biomaterials are known to preserve cardiac function by reversing collagen fibrosis. Recently, Mukherjee and colleagues (2008) studied the targeted myocardial microinjection of alginate-fibrin biocomposite within the MI regions in pigs. Intramyocardial injection of novel biocomposite was seen to be associated with reduction of soluble collagen in the MI region, a suggested mechanism for attenuation of LV remodeling. Paradoxically, the biocomposite did not affect the infarct size.[164] Therefore, experts feel the need to furnish details of inflammation, interval of degradation of collagen and the possibility of its migration before deciding its safety in clinical use.[165] An example of innovative biomaterial strategy to address early infarct expansion is the technique adopted by Ryan and colleagues in 2009.[166] In a simple way, Radisse (Bioform Medical Inc., CA), a commercially available hydroxyapatite dermal filler, was injected below the epicardial surface of the heart in their well established MI ovine model. The acute effects were characterized by significant increase in LV ejection accompanied by decrease in LV volume.

Synthetic polymeric hydrogels can absorb a large volume of liquids which makes them similar to soft tissues.[167] Owing to their fluidity, these hydrogels have enormous clinical applications as they can be easily injected into injured sites without causing morbidity to patients.[168] For example, intramyocardial injection of α-cyclodextrin and poly(ethylene glycol)-*b*-polycaprolactone-(dodecanedioic acid)-polycaprolactone-poly(ethylene glycol) (MPEG-PCL-MPEG) was shown to improve LV dilation without neovascularization.[169] Similar results have been furnished with co-polymer of *N*-isopropylacrylamide (NIPAAm), acrylic acid (AAc) and hydroxyethyl methacrylate-poly (trimethylene carbonate) (HEMAPTMC). The resultant biodegradable thermosensitive hydrogel poly (NIPAAm-*co*-AAc-*co*-HEMAPTMC) supports finite element model simulations and its use as potential biomaterial in ischemic cardiomyopathy appears to be warranted.[170] Injectable biomaterials are emerging therapeutics which can provide a bypass for traumatic open heart surgeries and largely revolutionize CHF treatment to save patients with injured hearts. Certain self assembly peptides have also been detected to produce nanofibers-like microenvironment within the myocardium. These self assembling peptide nanofibers can be modified in variety of ways to promote recruitment of vascular cells and injected noninvasively into the myocardium. Davis and colleagues (2005) have demonstrated the efficient delivery of injectable self-assembling peptide nanofibers for therapeutic intervention of myocardial repair.[171] However, recent studies point to the inability of self assembling peptide nanofibers to support myoblast survival, despite significant angiogenesis. This draws attention to the fact that certain biomaterials may be cell-specific and thus, highlights the need for investigating different biomaterial designs for different types of transplanted cells.[172]

4.2. Cardiac patch

Recently, it has been noticed that cell transplantation in recipient myocardium by direct myocardial injection or *via* the coronary artery shows insufficient prevention of progressive left ventricular dilation due to low efficiency in cell survival. In order to overcome this deficiency, tissue engineered cardiac patches have been extensively studied as an alternative therapeutic strategy. A cardiac patch is expected to cover the damaged area and aid the pumping efficiency of the heart. Badylak and colleagues (2003) developed a decellularized ECM patch from porcine urinary bladder's latent membrane which could colonize cardiomyocytes.[173] Later on, it was shown to be a better alternative in terms of mechanical support, immunogenicity and stiffness to Dacron, a widely used myocardial patch for a variety of diseases.[174] ECM components, such as collagen, tether cardiomyocyte in native heart tissue. Collagen fibres extracted using microbial methods from skeletal tissue are proposed to be suitable patch material since the novel method saves structural deformation of collagen.[175] Many such innovative biomaterials are under investigation for applicability as cardiac patches. Biomaterial patches provide mechanical support and adhesion sites for cells and allow them to spread and proliferate. Fujimoto and colleagues (2007) designed a degradable porous PEUU patch to facilitate cellular ingrowths upon cardiac implantation. In a recent study, silk fibroin (SF) and silk fibroin/chitosan, silk fibroin/chitosan hyaluronic acid hybrid patches were fabricated to investigate their effects on the growth of rat MSCs, and cardiomyogenic differentiation using 5-azacitidin *in vitro*. These hybrid patches were suggested as potential biomaterials for MTE given their pronounced effect on growth, differentiation and cardiac protein expression on MSC.[88]

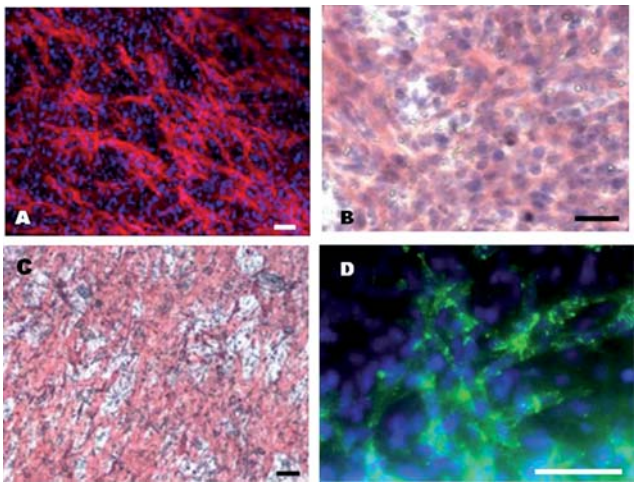

Fig. 4 Rat cardiomyocytes grown on PCL nanofibers. (A) Immunohistochemical staining for F-actin. Actin filaments cover the entire surface of the nanofibers mesh. (B) Immunohistochemical staining for tropomyosin. The surface is covered with tropomyosin-positive cells. Some fiber segments are also visible. (C) Immunohistochemical staining for cardiac troponin-I. The surface is covered with cardiac troponin-I-positive cells. The nuclei are also shown. (D) Immunofluorescent staining for connexin43. Diffuse gap junctions between the cells can be seen. All scale bars = 50 μm. Reproduced with permission.[179]

In native tissue, cells growth and structural development is supported by the ECM. Lack of an appropriate microenvironment in scarred myocardium might be a plausible reason for colossal loss and ineffective homing of injected cells. To enhance cell attachment, proliferation and differentiation, it is necessary to mimic some of the nanostructure of the natural ECM. Scaffolds with nano-scaled architecture provide larger surface area to adsorb proteins and provide more binding site to cell membrane receptors, unlike micro-scale and flat surfaces.[176] This makes nanofabrication of biomaterials for MTE is an attractive strategy. The ultrafine woven nanofibers having ECM like topography can be achieved by electrospinning of biomaterial or self-assembly of certain peptides *via* non-covalent interactions.[177,178] A versatile, biodegradable *in vitro* construct made of PCL nanofibers and cardiomyocytes was reported by Shin and colleagues (2004). Being able to foster cellular in-growth, it was proposed to be more desirable than 3D construct in patch application (Fig. 4).[179] The bioengineered cardiac tissue structure and function, chemistry and geometry of the provided nano- and micro-textured using PLGA nanofibers were later demonstrated. Thereafter, nanofibers of blended and conductive polymers were shown to be potential choices in MTE.[180,181] Very recently, coaxial electrospun PGS nanofibers were fabricated opening up new horizons in MTE owing to its resemblance to elastin fibers.[182]

4.3. Ventricular constraints

In order to control dilation of LV post MI, strategies to apply biomaterial as mechanical restraints have been investigated extensively. The goal of such investigations have been to preserve the geometry of the heart by physically wrapping it and therefore preventing the decline of cardiac function that results from altered spherical structure of post MI heart. Ventricular restraint Marlex, first of its kind, was shown to prevent infarct expansion by Kelley and colleagues.[183] The LV geometry preservation potential of poly(propylene) mesh was evaluated upon suturing onto infarct location. Despite molecular examination of ECM around the border zone of the myocardium, matrix components within the infarct remained unchanged and LV volume treatment rendered unsatisfactory.[184] Rather complete wrapping of LV using Marselene, a knitted polyester mesh, was reported to relatively enhance at combating LV remodeling.[185] Acorn Cardiovascular Inc. developed another polyester mesh Cardiac support device (CSD), CorCap™, in view of providing passive diastolic support. This device principally follows Laplace's law, by sharing LV pressure and maintaining normal myocardial wall pressure, for preventing LV remodeling.[186] Benefits of wrapping both ventricles with CSD were demonstrated not only by LV volume decrement but also by reversal of remodeling.[187–189] Many preclinical[190,191] and clinical studies[192–194] have repeatedly proven the efficacy of restraints in improving patient quality of life with the most pivotal being Acorn's clinical trial encompassing 300 patients.

4.4. Bioreactors

A bioreactor is a dynamic cell culture apparatus that attempts to encourage tissue formation by mimicking biological conditions.

The advances in bioreactor research have been rather fuelled by frustrations faced by *in vitro* tissue engineering. Despite encouraging reports, static culture of cell mass often suffers from limited thickness of new muscle and hence displays little effect on the mechanical properties of the infarcted heart. In the body, cells are continuously bombarded with mechanical, chemical and electrical cues which monitor their behavior. Whereas, a static culture prevents adequate diffusion of nutrients and oxygen due to increasing cell mass and decreasing implant porosity due to ECM deposition. Famished cells in a construct tend to dedifferentiate, become disorganized and eventually die. This largely affects the construct properties namely number and density within the graft, cells orientation and their electromechanical connections. A bioreactor is particularly crucial for maintenance of *in vivo* conditions to facilitate regeneration of complex constructs such as myocardial substitute. Dynamic cultures of cardiac constructs tend to reorganize cellular and morphological architecture with preconditioned cyclic strains stimulations.[195] The earliest reports of bioreactor cultivated constructs, from over a decade ago, used mixed bioreactors associated with poor mass transportation in central areas of construct. Later, discoveries using rotating bioreactor facilitating laminar flow improved cell distribution, quality and metabolism. However, substantial improvements of oxygen diffusion magnitudes were achieved using perfusion bioreactors.[196–198] Novel perfusion systems have enabled superior properties such as equal medium flow and adequate shear stresses corresponding to average blood velocity within native myocardium. Adequate shear stress that encourages growth differentiation of myocytes is around 0.001 Pa, and an increase to even 0.1 Pa can damage these sensitive cells.[199] Contact of cells allow functional assembly using low shear stress due to better oxygen diffusion using perfusion bioreactors. Direct perfusion also enables uniform seeding of hypoxia sensitive cells such as cardiomyocytes with high spatial density and maintained viability.[200] Recent developments of perfusion systems facilitating bidirectional flow suggest better response to growth factors and increased contractility of constructs, in such reactors, highlighted by lowered lactate production, a measure of aerobic metabolism. Pulsatile perfusion bioreactors also enable cultivation of electrically stimulated constructs which exhibit remarkable ultra-structural differentiation comparable with native tissue in several respects such as mitochondria and glycogen contents, induction of well aligned registers of sarcomere, elevated levels of intercalated discs and high expression of cardiac markers.[201] Moreover, bioreactor stimulations are associated with differential expression of morphogenetic and angiogenic pathways similar to ones seen during cardiac development.[202] Although, it is worthwhile to study bioreactor based strategies for functional assembly of constructs, their applicability from a clinical point of view is still unclear considering the cost and invasive surgery techniques.

4.6. Growth factors for design of smart biomaterials

Regeneration of tissue is indeterminate without reproduction of serial signals that compromise normal developmental processes. The future of biomaterials is in the design of smart and bioactive materials that respond to their environment by means of predetermined protein release responses, initiated by microenvironmental conditions.[203] Although design for such smart biomaterials is still in their infancy, the potential for engineering them has been repeatedly demonstrated in recent studies. Biomaterials can be designed to respond to mechanical stimuli since a mechanical stimulus induces expression of VEGF gene and proteins in many cells.[204] For example, Lee *et al.* (1999) demonstrated mechanically-induced release rate of VEGF by implanted hydrogel in mice to increase collateral vessel formation. Targeted delivery of VEGF using encapsulating immunoliposomes into the MI zone has been shown to enhance morphology and function of infarcted heart significantly.[205] Over expression of chemotactic factors such as stromal derived factor 1 (SDF1), after acute MI, have urged investigators to study stem cell recruitment by controlled release of SDF-1 by way of conjugated PEG fibrin patches.[206] In this study, Zhang *et al.* (2007) demonstrated that such novel methods improve c-kit (+) cell homing which eventually improve cardiac performance. Further investigations elucidated that stem cell transplantation by PEGylated fibrin biomatrix covalently bound to HGF enhances cell engraftment.[207] Various other growth factors such as transforming growth factor (TGF β1), bone morphogenic factor -2 (BMP-2), insulin growth factor (IGF) and thyroxin have also been shown to influence myocardial regeneration in view of *in vitro* growth and differentiation of stem cells,[208] modulation of electrical properties of cardiomyocytes[209] and functional properties of bioengineered heart muscles.[210] Thus, biomaterials with controlled bioactivity could be potentially designed to respond and enhance regenerative capability of myocytes or exogenous cells to adjust the myocardial mechanical load for MTE.

5. Key issues and challenges

The heart is an engineering marvel with structural complexities for pivotal functional efficacy. Therefore, regenerating the heart tissue poses large scientific challenges apart from scale-up and ethical challenges in its therapy. Despite reaching impressive milestones, it seems that there is little indication of a contractile bioartificial construct for clinical trial in next 5 years. The current concepts of random stem cell injection into the highly differentiated environment are not very promising.[207] Although many cell types have shown potential by mechanisms of regeneration, the best choice of cells, their dosage and timings remain unknown. Recent experimental studies have addressed, but only partially answered many important aspects of cell therapy. However, retention and survival of delivered cells for therapy are poor. Nevertheless, positive results have been reported, including increased healing, vascular density, increased regional circulation and cardiac function. The current problematical situation of intense clinical activity without a comprehensive foundation in basic science appears to be driven by an innate human trait for pursuit. If effective and tumour free mammalian myocardial regeneration is ever to be achieved, this accomplishment is going to require a more clear understanding of myocardial biology than currently available. Also, novel imaging methods are required to track the fate of therapeutically administered cells. It is an immense technological challenge to mimic the entire milieu of ECM without evoking immune response. From a tissue engineering perspective, applying physiological stress on

immature construct could be a way of mimicking the natural environment. Notably, stiffness of heart changes considerably within a single cardiac cycle. Novel tissue forms such as a bio-engineered construct which have anti-remodeling capacity to render repair with minimally invasive techniques seems to more realistic approach for a failing heart.[211] However, lack of precision, even to trivial extent, can produce amplified insulating effects in signal transduction in the already malfunctioning organ. A scenario for implantation of an engineered tissue graft needs to provide a means for effective and timely connection with the host blood supply. A poorly organized construct will lead to attenuation of electrical impulses and adversely affect the cardiac functioning. A hallmark of functional myocardium is its ability to propagate electrical impulses and respond to these impulses by synchronized contractions that generate forces for pumping blood. Finally, advanced culture platforms for appropriate mass transportation and mechanical conditioning are required for functionality of construct. Apart from these scientific demands, research strategies require facilities of suitable imaging systems and animal models for achieving novel cardiac patch for MTE.

6. Summary

Various MTE strategies have been demonstrated to be promising for cardiac repair. However, safety and efficacy issues related to these potential therapies are yet to be answered before the technologies are taken to clinical trials. The use of biomaterials for *in situ* MTE is being appreciated either as acellular or as hybrid therapy with cells and growth factors. Although each of this technique brings about myocardial functional enhancement, the mechanisms of repair pathways are still unclear. It would be worth considering whether it is the functional activity of cells injected or structural changes brought about by biomaterial volume that can be actually held responsible for repair of the infarcted heart. Long term studies are required to understand these crossroads of biomaterials, myocardial repair, stem cells and growth factors. Furthermore, for biomaterials to influence the myocardium microenvironment, suitable designs for cell recruitment and formation of functional conductive bundles are expected. Also, the reaction of a biomaterial construct at implantation site must be given further consideration as immune response such as inflammation and hypersensitivity may highly affect the regeneration.

Nonetheless, various strategies have evolved to provide insights into MTE and many investigators are optimistic that multi modal strategies, similar to ones in native heart, will bring new treatments for patients with myocardial infarction.

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