Biotechnology Advances 34 (2016) 362–379

Contents lists available at ScienceDirect

Biotechnology Advances

journal homepage: www.elsevier.com/locate/biotechadv

Research review paper

Stem cells and injectable hydrogels: Synergistic therapeutics in myocardial repair☆

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ARTICLE INFO

Article history:
Received 15 July 2015
Received in revised form 27 February 2016
Accepted 7 March 2016
Available online 11 March 2016

Keywords:
Myocardial tissue engineering
Injectable hydrogel
Stem cell transplantation
Infarction
Regeneration

ABSTRACT

One of the major problems in the treatment of cardiovascular diseases is the inability of myocardium to self-regenerate. Current therapies are unable to restore the heart's function after myocardial infarction. Myocardial tissue engineering is potentially a key approach to regenerate damaged heart muscle. Myocardial patches are applied surgically, whereas injectable hydrogels provide effective minimally invasive approaches to recover functional myocardium. These hydrogels are easily administered and can be either cell free or loaded with bioactive agents and/or cardiac stem cells, which may apply paracrine effects. The aim of this review is to investigate the advantages and disadvantages of injectable stem cell-laden hydrogels and highlight their potential applications for myocardium repair.

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☆ Competing financial interests: The authors declare that they have no competing financial interests.

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http://dx.doi.org/10.1016/j.biotechadv.2016.03.003
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1. Introduction

Myocardial infarction (MI) leads to heart-wall thinning, myocyte slippage, and ventricular dilation, with progressive damage to the heart-wall muscle. MI occurs when the source of oxygen and nutrients to the cardiac muscle is impaired due to blocked coronary arteries. Damage to muscle tissue in the left ventricle (LV) can cause progressive dilation and structural changes to the myocardium. As a result, the contractile efficacy of the ventricles impressively decreases (Fig. 1). After injury, myocardial tissue lacks the inherent ability to regenerate itself (Baig et al., 1998).

Current therapeutic treatments for heart failure focus on inhibition of ventricular remodeling and are not expected to correct the underlying pathophysiology of normally organized functional cardiomyocytes (CMs). In addition, cell transplantation is limited by restricted cellular proliferation and inability to form new functional cardiac tissues. Therefore, cell-based tissue engineering (TE) approaches have attracted significant attention as a therapeutic treatment for heart failure (Buikema et al., 2013; Radhakrishnan et al., 2014).

Recent studies have resulted in the development of TE platforms based on two key factors: cells and/or biomaterial scaffolds for the regeneration of the infarcted myocardium. The cellular element, as an intricate part of the engineered cell-based platforms, should contract, remodel and finally regenerate a defective myocardium. The ideal cell source should be easily obtainable and cultivatable in great numbers because the native myocardium is densely populated, with approximately 5×10^8 cells/cm^3 (Gerecht-Nir et al., 2006).

Several evolving technologies have been recently reported to improve cell survival, differentiation, spatial organization and/or biomechanical integration with the host myocardium following transplantation for TE purposes. These include the use of injectable materials and surgical patches as scaffolds (Li and Weisel, 2014), in addition to application of various stimulants that include mechanical (Zimmermann et al., 2002), perfusion (Radisic et al., 2004), electrical (Pahnke et al., 2015), and biochemical (hypoxic pre-conditioning stimulation) techniques (Wang et al., 2009a). Among these, injectable biomaterials (generally made of hydrogels) are easily administered through minimally invasive procedures (Radhakrishnan et al., 2014), which provide patient convenience as well as site-specific release. The goal of myocardial tissue engineering (MTE) is to produce biocompatible heart muscles with morphological, mechanical and functional properties comparable to the innate myocardium. However, the poor mechanical properties of the injectable hydrogels may limit their clinical applications (Li and Weisel, 2014). Thus, we will firstly discuss hydrogel parameters and prominent cell source and finally will investigate the advantages and limitations of free and cell-based injectable systems for MTE.

2. Architecture and components of the myocardium

The heart muscle is exceedingly vascular with contractile tissue surrounded by the pericardium, as a double-walled sac that protects the heart. The outer wall of the human heart is comprised of three layers — an outer layer or epicardium, a muscular myocardium, and an endothelial-lined endocardium (Kennedy, 2012).

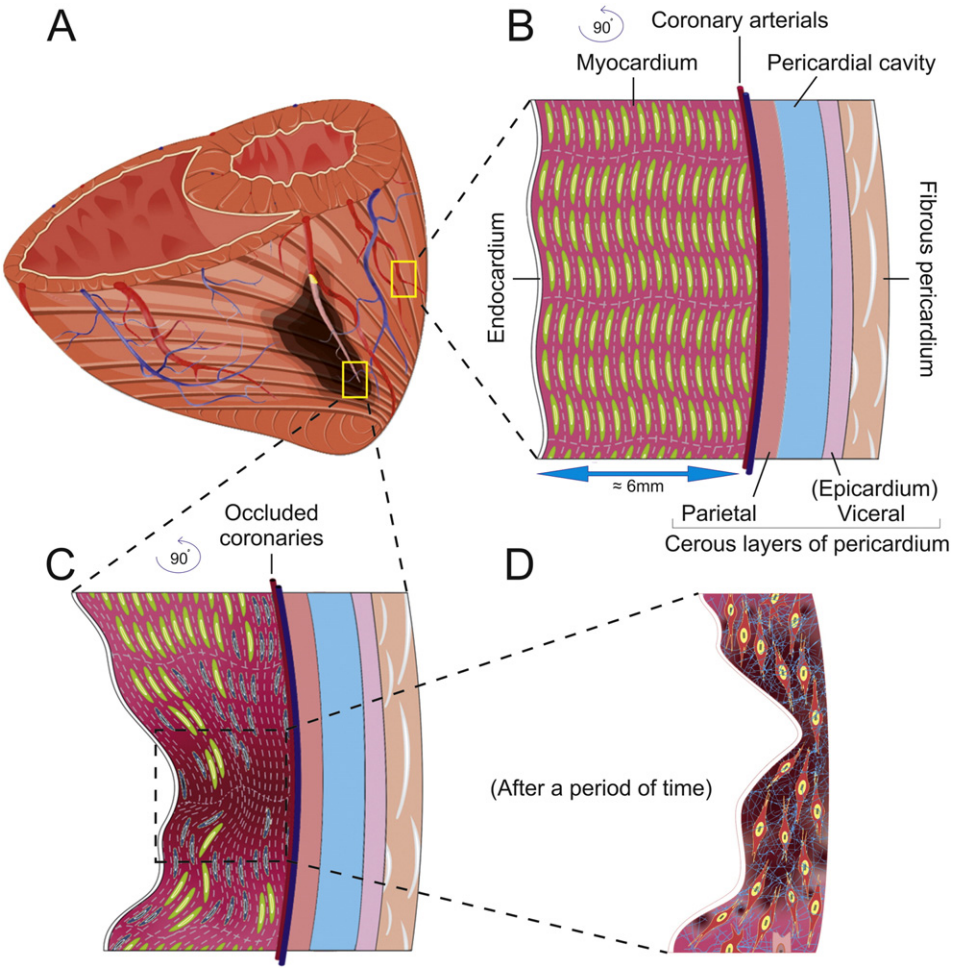

Fig. 1. Myocardial infarction (MI). (A) Ischemia, coronary occlusion, reduced nutrition and oxygen, and cell death. (B) Healthy myocardium. (C) Infarcted myocardium. Rupture of the extracellular matrix (ECM), cell apoptosis, and reduction in wall thickness. (D) After a period of time fibrosis and scar tissue form, and wall thickness decreases in the infarcted region.

Myocytes are surrounded within an extracellular matrix (ECM) network that is produced by cardiac fibroblasts (Eghbali et al., 1989). ECM constituents, which include protein and non-protein components, are classified into structural [e.g., collagens, elastin, proteoglycans, glycosaminoglycans (GAGs)] and functional [e.g., growth factors (GFs) and cytokines] components (Hynes, 2009).

Collagen is the most plentiful protein in myocardium. In turn, clusters of CMs are encircled by weaves of collagen. The major ECM proteins in the myocardial ECM are collagen type I (approximately 85%, depending on the species) and collagen type III (approximately 11%) (Bosman and Stamenkovic, 2003; Caulfield and Borg, 1979). Various amounts of simultaneous collagens are responsible for the anisotropic mechanical properties in different regions of the heart (Robinson et al., 1988). The interstitial and perivascular matrix is mostly comprised of collagen types I and III. Collagen types I, II, III and V have rod-like shaped structures because of the distinctive assembly of three helical structures. This unique assembly of helical structures is predominantly responsible for retaining the mechanical integrity of tissues and organs. Collagen type IV is typically found in the basement membrane of tissues or blood vessels. It has also been shown that collagen type VI plays a key role in maintaining tissue structure (Singelyn and Christman, 2011; Zern and Reid, 1993).

There are important proteins such as fibronectin and laminin inside the ECM that do not have a direct influence on mechanical integrity. Fibronectin is a glycoprotein secreted through the resident cells helping to guide cell adhesion, spreading and migration. Laminin is another glycoprotein present in the basal lamina. According to reports, the ability of stem cells (SCs) to differentiate into beating CMs is increased by laminin. Similar to fibronectin, it contributes to cell binding and migration. Laminin is comprised of cell-binding amino acid sequences such as Arg-Gly-Asp (RGD), Tyr-Ile-Gly-Ser-Arg (YIGSR) and Ile-Lys-Val-Ala-Val (IKVAV) with chemotactic characteristics (Badylak et al., 2009; Singelyn and Christman, 2011).

Naturally occurring polysaccharides in the ECM are called GAGs. These polysaccharides render specific viscoelastic properties to tissues and anchor essential GFs (Fomovsky et al., 2010; Freytes et al., 2014). For instance, heparin is a negatively charged polysaccharide that interacts with positively charged proteins such as basic fibroblast growth factor (bFGF) and vein endothelial growth factor (VEGF) (Zieris et al., 2011, 2010).

A high degree of compliance is required for myocardium and other elastic tissues. The myocardium contains elastin in the walls of the arteries and in the interstitium, however it is currently unknown how it contributes to myocardial mechanics. Nevertheless, research has shown that acute ischemia, overloaded pressure and subsequent heart failure can disrupt the interstitial elastin fibers and impact myocardial action (Cheng et al., 2006; Sato et al., 1983).

More importantly, the microstructure of aligned collagen fibers in the ECM induces the intrinsic anisotropy of the native myocardium. CMs are strongly organized into muscle bands that accumulate to shape the heart as an elliptical chamber with a highly asymmetrical and anisotropic architecture. This organization enables a 35%–40% increase in left ventricular wall thickness in systole which is only an 8% thickness increase of a single myofiber. Thus, the LV can generate an ejection fraction (EF) of 60% with only a 15% fiber shortening. If muscle bands are collected spherically, an EF of only 30% can be produced. Furthermore, the anisotropy of the heart is functionally reflected in the heterogeneity of both myocardial blood flow and contractile function (Buckberg, 2002; Heusch and Schulz, 1999).

The ECM composition provides a bioactive substrate for cell recruitment, attachment, orientation, maintenance, proliferation, differentiation and maturation. It also provides mechanical support and transmits the mechanical forces to resident cells by structural proteins. The ECM also functions as a local GF reservoir and delivery for the cells. Besides, it relays physical stimuli and administers them into the cells through specific integrins (Badylak et al., 2009; Barczyk et al., 2010; Corda et al., 2000). In an infarct, the myocardial ECM will rupture; hence, it must be assisted in order to achieve appropriate regeneration or redevelopment. A suitable scaffold for heart regeneration should mimic the compositions and properties of native myocardial ECM (Badylak, 2007; Badylak et al., 2009; Freytes et al., 2014).

3. Essential cell source for myocardium regeneration

The myocardium consists of at least four basic cell types: 20%–40% CMs, 60%–80% cardiac fibroblasts, smooth muscle cells (SMCs), and endothelial cells (ECs) (Gerecht-Nir et al., 2006; LeGrice et al., 1995). It has been shown that at the age of 25 years, no more than 1% of these cells are annually substituted by progenitor cells, with the percentage falling to less than 0.5% at the age of 75 years. Totally, less than 50% of all CMs are renewed throughout a normal lifespan (Bergmann et al., 2009). A high-level of cell slippage occurs during MI and post-MI reperfusion, and it is necessary to recruit the cardiac progenitor cells to compensate this cell losing (Baig et al., 1998; Leri et al., 2005). Therefore, the treatment technique should have the capability to provide adequate cell populations to support CMs that are electromechanically coupled to the host tissue, as well as provide an appropriate vascular source and connective tissue for functionality (Caspi et al., 2007; Li and Weisel, 2014). CMs solely contribute to functional contraction with limited ability for ex vivo expansion. Therefore, several sources have been used to obtain CMs for regeneration of an infarcted region. These sources comprise embryonic stem cells (ESCs) (Caspi et al., 2007; Xi et al., 2010), induced pluripotent stem cells (iPSCs) (Nelson et al., 2009; Zwi et al., 2009), bone marrow mesenchymal stem cells (BMSCs), bone marrow-derived mononuclear cells (BMMNCs) (Chen et al., 2014; Labovsky et al., 2010), cardiac stem cells (CSCs), endothelial progenitor cells (EPCs), cardiac progenitor stem cells (CPCs) (Atluri et al., 2014; Beltrami et al., 2003; Garbern and Lee, 2013), and adipose-derived stem cells (ADSCs) (Wang et al., 2014).

SCs are the optimal source for myocardial regeneration. In general, the contribution of SCs to myocardial restoration is believed to occur by two independent mechanisms of action: direct differentiation and paracrine effects. Restoration is mainly mediated by the paracrine effect, whereas direct differentiation only plays a minor role (Fig. 2) (Chimenti et al., 2010; Fisher et al., 2014; Yang et al., 2013).

In vitro investigations have demonstrated that SCs effectively differentiate into ECs, SMCs and CMs (Kajstura et al., 2005; Segers and Lee, 2008). However in vivo tests show that the rate of cardiomyogenesis and vasculogenesis as well as the frequency of SC engraftment are very slow to support myocardium regeneration. Recently, transplanted SC released soluble factors which acted in a paracrine fashion have been observed to contribute to myocardial repair and regeneration. In fact, cytokines and GFs possibly motivate cytoprotection and neovascularization. Endogenous regeneration through recruitment and activation of resident EPCs and CPCs may be mediated by paracrine factors (Burchfield and Dimmeler, 2008; Leri et al., 2005).

There are three types of injection routes for SCs within the heart: intravenous infusion, intracoronary delivery, and intramyocardial injection. Each strategy offers its own advantages and disadvantages. The intravenous infusion strategy is simple with potential systemic benefits. This delivery system has been used in autologous BMSCs in stroke patients (Lee et al., 2010; Segers and Lee, 2008). In the intracoronary infusion strategy, the infarcted-related region is accessible. Nonetheless, a microvascular obstruction may be induced by this strategy (Segers and Lee, 2008; Wollert et al., 2004). Intramyocardial delivery allows for targeted treatment, which focuses on the injured site with limited systemic effects. This method includes epicardial and endocardial injection techniques; in both approaches it requires calibrated equipment and expert operators (Hou et al., 2005; Krause et al., 2009; Segers and Lee, 2008; Wollert and Drexler, 2005).

Despite promising results in the use of SCs for heart regeneration, development has been modest. Thus far, a number of controlled clinical

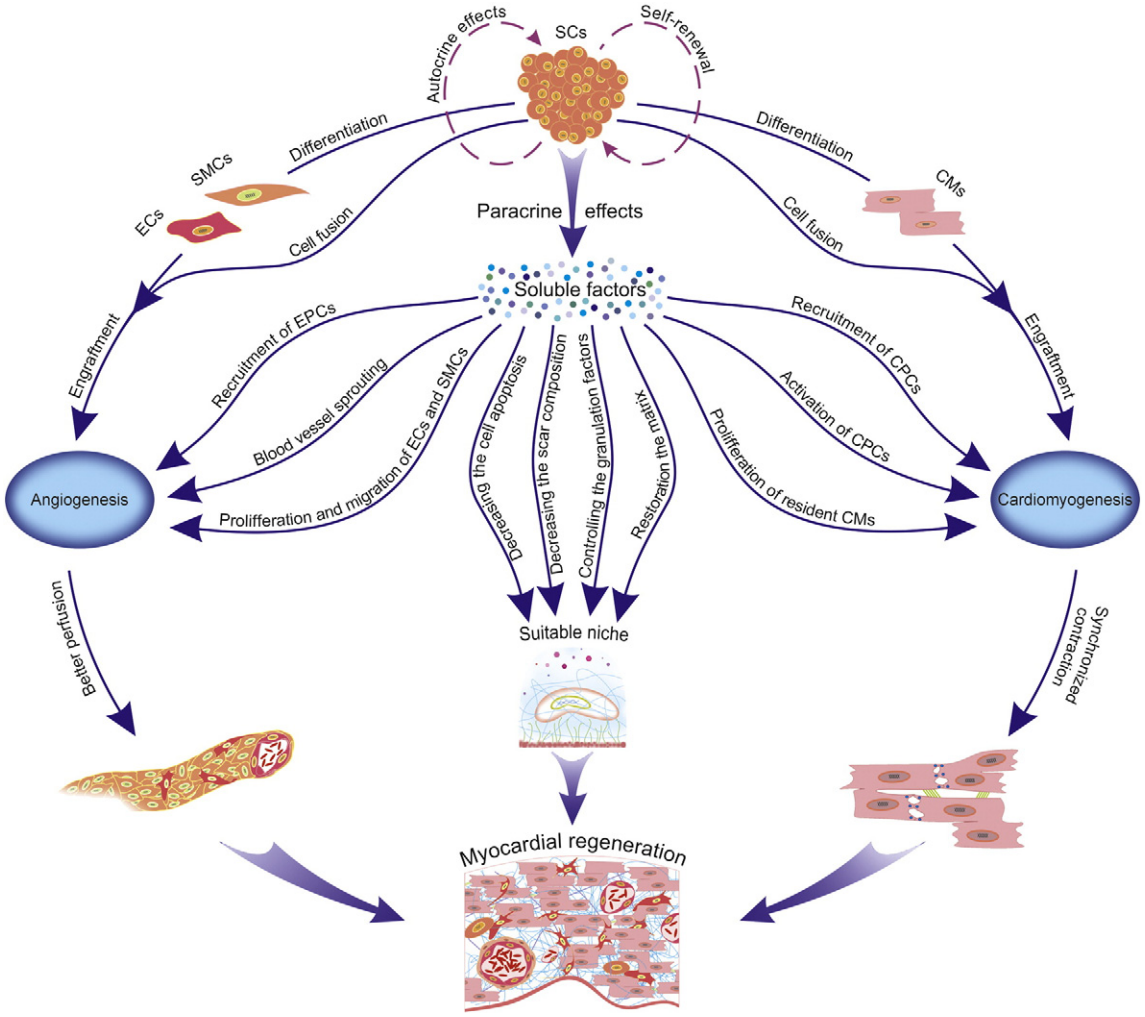

Fig. 2. Mechanisms involved in myocardial stem cell (SC) therapy. Stem cells (SCs) differentiate into endothelial cells (ECs), smooth muscle cells (SMCs), and cardiomyocytes (CMs) (Yang et al., 2013). Cell fusion between transplanted and host cells (Kajstura et al., 2005). Paracrine effects include recruitment and activation of resident endothelial progenitor cells (EPCs) and cardiac progenitor cells (CPCs) [and/or cardiac stem cells (CSCs)] (Burchfield and Dimmeler, 2008; Segers et al., 2007), along with proliferation of CMs, ECs and SMCs. In addition, they impact contractility of the CMs (Leri et al., 2005); stimulate ECs sprouting from pre-existing blood vessels; reduce cell apoptosis (Burchfield and Dimmeler, 2008; Gnecchi et al., 2008); prevent extracellular matrix (ECM) degradation; and inhibit granulation factors and scar composition in the matrix (Gnecchi et al., 2008).

studies showed contradictory results. It has been reported that only an approximately 6%–7% increase in EF can be expected approximately 6 months after cell transplantation (Chen et al., 2004; Fischer-Rasokat et al., 2009; Wollert et al., 2004). The infarcted myocardium is considered a harsh hypoxic environment inappropriate for cell survival and renewal. Further, significant apoptosis occurs soon after implantation inside the infarcted myocardium (Robey et al., 2008). Most injected cells do not incorporate with the host myocardium and die within the first few days after injection (Murry et al., 2002). The use of adult SCs may be also compromised through either aging or disease, both of which reduce their therapeutic applications (Passier et al., 2008; Segers and Lee, 2008). After the intramyocardial injection numerous cells have been shown to migrate to other organs outside of the cardiac region such as the spleen, lungs and liver (Zhang et al., 2007).

4. Myocardial tissue engineering

Myocardial cell transplantation may be a suboptimal method for myocardium repair. Lack of mechanical support and vascular washout may be responsible for a large amount of cell loss upon intramyocardial injection (Teng et al., 2006). Transplanted SCs probably require the physical support of a biomaterial scaffold in order to maintain their placement in the injury zone, protect the cells from host inflammation, and enable functional integration with the injured myocardium. Of note, any engineered heart tissue must form functional and electrical syncytia, endure diastolic load, develop systolic force, and include a blood-supply system; in turn, this tissue should rhythmically generate electrical signals to the myocardium (Leor et al., 2005; Zimmermann et al., 2006).

For TE purposes, a biocompatible material that provides suitable cell–biomaterial interactions for cell adhesion, proliferation, differentiation, and maturation is essential (Leor et al., 2005; Lutolf and Hubbell, 2005). Here, we summarize various natural and synthetic biomaterials used for heart regeneration with a specific direct focus on the injectable hydrogel-based systems for MTE.

4.1. Classification of applied biomaterials in myocardial tissue engineering

Biomaterials used for myocardial tissue regeneration are derived from either synthetic or natural sources. Natural materials possess high bioactivity, biocompatibility and biodegradability. However, there is a high inconsistency related to their production with limited control over their compositions and physical features. Therefore, standardization and quality control in their mass production is difficult. In contrast, synthetic materials have a stronger, more vigorous manufacturing ability, which permits control of their biochemical compositions and characteristics. However their bioactivity and biocompatibility are less

satisfactory compared to naturally derived scaffolds (Sarig and Machluf, 2011).

The biological properties of the materials affect cell–biomaterial interactions. Natural polymers such as collagen and gelatin have intrinsic peptide sequences easily identifiable by cell-surface receptors. Thus, cell–biomaterial interactions in the case of natural polymers are more predominant, resulting in favorable cell proliferation and differentiation. Previous studies have indicated that collagen scaffold increased the wall thickness with viable tissue while limiting remodeling and normalizing cardiac wall stress in the injured regions (Chachques et al., 2007). ECM-derived hydrogels as prominent natural biomaterials show wonderful efficiency on the angiogenesis and myocardial regeneration because of their critical components (such as GFs, fibronectin, and GAGs) (Blatchley and Gerecht, 2015; Okada et al., 2010; Seif-Naraghi et al., 2012; Singelyn et al., 2009; Williams et al., 2015).

On the other hand, synthetic biomaterials show enhanced cardiac remodeling and contractile function of the heart (Burdick and Dorsey, 2015; Pascual-Gil et al., 2015). Different forms of poly(N-isopropylacrylamide) (PNIPAm) are currently under research as injectable materials for cardiac tissue regeneration. Injection of hydrogels into hearts has led to an increase in the left ventricle end diastolic diameters (LVEDD) and decreased EF compared to sham. LVEDD was considerably lower for hydrogel treated hearts compared with those treated by saline (Fujimoto et al., 2009; Wang et al., 2009d). Similarly, researchers demonstrated that injection of poly(ethylene glycol) (PEG) hydrogel decreased dilatation observed in saline-injected hearts by inhibition of an increase in LVEDD (Dobner et al., 2009). Researchers reported that short poly(glycerol sebacate) (PGS) nanofibers increased cell-transplant maintenance and survival within the infarcted region compared to a standard cell-injection system. These nanofibers prevented cell loss and offered a more site-directed myocardial repair mechanism (Ravichandran et al., 2012b).

Modified biomaterials may be advantageous to improve the efficacy of myocardial cell transplantation. Pure synthetic polymers do not have cell-recognition moieties, which are a feature of natural materials. On the other hand, pure natural materials suffer from uncontrolled compositions and lack suitable mechanical properties. Thus, in order to overcome a deficit of pure materials, synthetic polymers are frequently used in combination with natural polymers or small peptide sequences to promote cell–biomaterial interactions while the structural support is maintained for tissue regeneration (Krupnick et al., 2002; Ravichandran et al., 2012a). For example, collagen or gelatin blended with polyurethane (Stankus et al., 2004), nanofibers of poly(caprolactone) (PCL) (Shin et al., 2004), poly(L-lactide acid) (PLLA) (Krupnick et al., 2002) or PGS (Ravichandran et al., 2011) improved cellular adhesion and behavior. Comparably, the combination of collagen and poly(lactide-co-glycolide acid) (PLGA) as well as poly(lactide-co-caprolactone) enhanced cardiac cell function and promoted cardiac maker expressions (Park et al., 2005). Similarly, fibrin with poly(ether)urethane–polydimethylsiloxane improved metabolic activities, proliferation and differentiation of a human MSC cardiac lineage (Lisi et al., 2012). PGS and fibrinogen core/shell substrate improved neonatal CMs function to form a gap junction and express cardiac pace makers (Ravichandran et al., 2013). An injection of collagen combined with fibrin and alginate prevented expansion of an infarct (Mukherjee et al., 2008).

In another approach, cell recognition motifs such as small immobilized peptides were used instead of entire proteins. Because many polymers do not have functional groups such as hydroxyls, aminos or carboxyls on their surfaces, these functional groups must be introduced through blending, copolymerization, chemical or physical treatments. Peptides have a number of advantages that include higher stability toward sterilization conditions, heat treatment and the variation of pH, storage and conformational shifting, easy characterization and cost-effectiveness. In addition, they require a lower space and can be packed with higher density on the surface. These characteristics have been used to promote cell adhesion properties of biomaterials (Rowley and Mooney, 2002; Shu et al., 2015). Hydrogels that incorporated small integrin binding peptides, such as RGD, Gly-Arg-Gly-Asp-Ser (GRGDS) (Lee et al., 2008), Asp-Gly-Glu-Ala (DGEA) (Alsberg et al., 2001) and YIGSR (Dhoot et al., 2004) were investigated in previous reports. However, the majority of investigations mainly focused on RGD conjugated hydrogels. Early studies reported the presence of RGD in fibronectin. In later reports it was associated with cell–matrix binding through $\alpha_5\beta_1$ integrin and commonly employed as a coating molecule to improve cell attachment to synthetic surfaces (Bökel and Brown, 2002; Pierschbacher and Ruoslahti, 1984). RGD modified alginate hydrogels improved cardiac function, EC proliferation, and increased arteriole density (Yu et al., 2009). When QHREDGS, as the integrin binding site of Ang-1 was covalently immobilized on a photocrosslinkable chitosan hydrogel, it promoted CMs attachment and survival, induced cell maturation and assembly contractile structure assembly, and recruitment of myofibroblasts (Rask et al., 2010a, b). It has been reported that addition of peptide did not considerably affect the mechanical properties or porous structure of QHREDGS-modified thermo-gel chitosan–collagen hydrogels. The in vitro cell viability and functional properties of the engineered construct improved. In addition, QHREDGS-modified gel was favorable to myofibroblasts and enhanced the presence of CMs in a subcutaneous model (Reis et al., 2012).

A number of reports evaluated the appropriate or favorable effects of combining nanomaterials with polymers to improve CM function. For example, carbon nanotubes (CNTs) were employed as reinforcement in methacrylated gelatin (GelMA) hydrogels (Fig. 3B). This patch showed enhanced mechanical integrity as well as improved electrophysiological performances (Fig. 3C). This composite showed that electrically conductive and nanofibrillar structures might provide mechanical reinforcement, cell adhesion and electrical coupling (Fig. 3D) (Shin et al., 2013). Gold, an excellent electrically conductive material, could be combined with polymers to form conductive scaffolds. In a report, PEG-based hydrogels that contained gold nanoparticles were developed for cardiac tissue regeneration and showed enhanced expression of Connexin 43 (You et al., 2011).

4.2. Engineering methods

The development of an engineered myocardium is a complicated process. These constructs should have nanofibrous and anisotropic structural properties comparable to the myocardial ECM (Bosman and Stamenkovic, 2003; Sarig and Machluf, 2011).

Patch-based systems are widely studied for cardiac regeneration. These systems are comprised of biomaterial with or without cells, which can be transplanted on the epicardial surface of the infarct. Patch-based systems are employed as carriers comprised of biological therapeutics and GFs required for the support of the transplanted cells until the time of angiogenesis, cell remodeling of the natural ECM, and replacement of the artificial patch platform (Rajabi-Zeleti et al., 2014; Ye et al., 2014).

Although these scaffolds have some advantages (Sarig and Machluf, 2011), challenges in their design include: i) cell–biomaterial interactions, ii) electro-stimulation, and iii) hypoxia (Berthiaume et al., 2011; Lanza et al., 2011). Furthermore, patch deposition needs a more invasive surgical intervention compared to the relatively ease of injection-based platforms. Therefore, it is believed that injection of biomaterials with or without cells may somewhat overcome the above-mentioned drawbacks (Stevens and George, 2005). An injectable material system offers a key solution for regeneration of an injured myocardial ECM. The fundamental principle of the injectable biomaterial approach for myocardium repair is that the injected agent will form a physical scaffold in situ to reduce LV wall stresses and stabilize LV remodeling following recovery from an MI. The injectable biomaterials can be designed to provide biomimetic ECM architecture. The biomaterials should also have proper gelation properties and kinetics to remain in a liquid state inside the catheter during the delivery and form a solid gel within the myocardium after injection (Radhakrishnan et al., 2014; Sarig and Machluf, 2011).

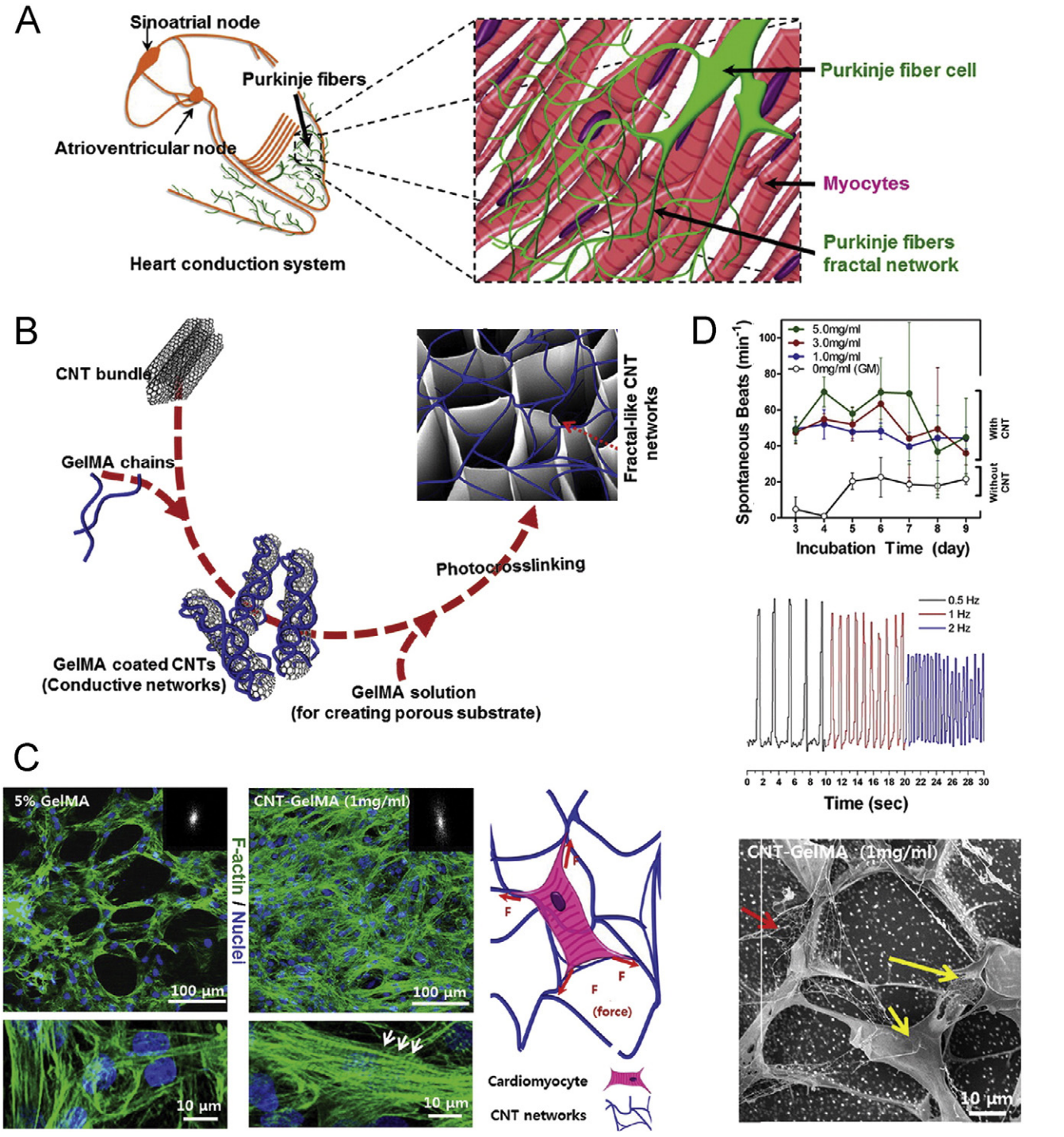

Fig. 3. CNT-GelMA as an electroconductive myocardial hydrogel. (A) Schematic diagram illustrating the isolated heart conduction systems that include Purkinje fibers, located in the inner ventricular walls of the heart. Heart muscle with Purkinje fiber networks on the surface of the heart muscle fibers. (B) Preparation process of fractal-like carbon nanotube (CNT) networks embedded in a methacrylated gelatin (GelMA) hydrogel. (C) Confocal images of CMs obtained after 5 days of culture on pristine GelMA and 1 mg/mL CNT-GelMA indicated more uniform cell distribution and partial cell alignment on CNT-GelMA. F-actin and cell nuclei were labeled fluorescent green and blue, respectively. Higher magnification images showed well-elongated cardiac cells and well-developed F-actin cross-striations (bottom right, white arrows) on CNT-GelMA. However, this was not observed on pristine GelMA (bottom left). The resultant stretching force from strong cell-CNT interactions might affect CM organization and stimulate myotube striation. (D) Spontaneous beating rates of cardiac tissues were recorded daily from days 3 to 9. Recording of synchronous beating signal of a tissue sample cultured on 1 mg/mL CNT-GelMA after the application of an external electric field at 0.5, 1, and 2 Hz. SEM image shows the morphology of cardiac cells cultured on CNT-GelMA. Red arrow: Cytoplasmic prolongations adhered to CNT fibers. Yellow arrows: Flat cell bodies. Reproduced with permission (Shin et al., 2013), copyright 2013, ACS nano.

The injection can be achieved either directly into the infarct zone or by the coronary circulation if the cell size allows. Intracoronary injection of cells has limited achievement in terms of delivery efficacy into the myocardium and survival of transplanted cells compared to direct intramyocardial injections (Bonaros et al., 2008).

One class of injectable biomaterials for MTE are hydrogels, reviewed in depth elsewhere (Li and Guan, 2011; Radhakrishnan et al., 2014). Hydrogels are hydrophilic polymer networks with high water content and diffusivity. They are widely used for TE applications (Annabi et al., 2013b; Annabi et al., 2014; Babavalian et al., 2014). Injectable hydrogel-based biomaterials for cardiac regeneration include fibrin (Christman et al., 2004), self-assembled nanopeptide (Davis et al., 2005c), alginate (Hao et al., 2007; Landa et al., 2008), PNIPAm (Fujimoto et al., 2009), PEG (Dobner et al., 2009), collagen and other ECM-derived hydrogels (Dai et al., 2005; Seif-Naraghi et al., 2012; Singelyn et al., 2009; Toeg et al., 2013).

4.2.1. Mechanism of in situ gelation

Several approaches employed for in situ gelling of polymers include photocrosslinking, chemical crosslinking, ionic interaction, enzymatic crosslinking, temperature induced gelation, pH-induced gelation, electric field, magnetic field, hydrophobic interactions, and the presence of antigen, glucose and their combinations (Jeong et al., 2002; Singelyn and Christman, 2010). Ideally, these approaches should integrate with the host tissue in order to provide mechanical support to the infarcted heart, reduce wall stress, compensate for contraction function, and inhibit ventricular remodeling (Wang et al., 2010).

pH-responsive hydrogels are one of the most widely used physiologically responsive hydrogels (Li et al., 2012). Researchers have prepared a GF reversible gel that used a copolymer based on PNIPAm, propyl acrylic acid and butyl acrylate, which was subsequently incorporated with bFGF and heparin (to stabilize bFGF) and subsequently injected into infarcted rats. Their results showed that the polymer gelled at 37 °C and pH 6.8. Under conditions of intermediate acidity, this system released antigenic GFs. Upon the tissue's return to normal physiological conditions (37 °C, pH 7.4), the hydrogel returned to its liquid phase and was removed from the tissue (Garbern et al., 2011).

Thermoresponsive gels show temperature-sensitive swelling behavior due to a change in the polymer/swelling agent compatibility over the desired temperature range. One important characteristic of temperature-sensitive polymers is their lower critical solution temperature (LCST). Below the LCST, the polymer is soluble and the crosslinked gel significantly swells to higher degrees due to increased compatibility with water. However, upon increasing the temperature above the LCST, the polymer is typically hydrophobic and does not considerably swell in water (Rimmer, 2011).

Some polymers show sol–gel transition above the critical gel concentration in response to temperature change. They remain in the sol state at lower temperature and above their LCST, these polymers convert to the gel state due to increased hydrophobicity. PNIPAm is a typical member of this family. This polymer and its copolymers are thermoresponsive and show a phase transition from the sol to gel phase at a temperature of approximately 32 °C. This phase transition occurs when the water molecules bound to its isopropyl groups are released, leading to increased inter and intra-molecular hydrophobic interactions above the LCST of PNIPAm (Jeong et al., 2002). The injection of thermosensitive PNIPAm-based hydrogels is an effective approach to inhibit adverse cardiac remodeling and dysfunction in MI induced rabbits (Wang et al., 2009d).

Photopolymerization at physiological pH and temperature can lead to in situ formation of crosslinked hydrogels that have the ability to encapsulate cells within their 3D structures without affecting viability. The ease of incorporation of a variety of chemistries by derivatization of macromers is an advantage of this system (Hoffman, 2012). A typical example is PEG-dimethacrylate and PEG-diacrylate that carry unsaturated C—C groups. Other polymers such as chitosan, alginate, tropoelastin, chondroitin sulfate, and hyaluronic acid (HA) have been methacrylated which makes them light activated for photocrosslinking (Annabi et al., 2013a, b; Camci-Unal et al., 2013).

The Michael addition reaction based on acrylate and thiol precursors is ideally employed to engineer in situ polymerizable hydrogels because of atunable gelation process (Mather et al., 2006). In the Michael addition reaction, a nucleophile (Michael donor) with an activated electrophilic olefin (Michael acceptor) is added which results in the formation of a 'Michael adduct'. An injectable hydrogel based on HA fabricated by reaction between PEG-thiol and acrylated HA has been shown to improve the function of the heart until it resembled a normal heart (Yoon et al., 2009). Calcium crosslinked alginate was used as an effective injectable implant for cardiac remodeling and function restoration (Landa et al., 2008). Alginate scaffold has been shown to be practical, effective and safe for intracoronary injection in the swine model. In this case, the alginate gel crossed the damaged permeable coronary vessel, deposited on the infarct tissue, and remodeled the LV (Leor et al., 2009).

Shear thinning injectable hydrogels have been also developed for TE applications. These polymers in high viscous or in relatively crosslinked gel forms are inclined to deform and flow upon being under shear stress. Gelation occurs after removal of the shearing force. This phenomenon is used in injectable hydrogels. Self-assembly is the chief criterion for crosslinking in these gels, which can be formed from proteins, peptides, colloidal systems and polymer blends (Guvendiren et al., 2012; Lu et al., 2012). As an example, alginate based shear-thinning hydrogel has been shown to enhance the viability of human umbilical vein endothelial cells (HUVECs) (Aguado et al., 2011).

Triblock and hybrid copolymers show a number of promising results in myocardial regeneration (Chen et al., 2014; Jiang et al., 2009; Xu et al., 2015). Triblock copolymers that include PEG–PCL–PEG, PCL–PEG–PCL, PLGA–PEG–PLGA, PCLA–PEG–PCLA (PCLA is poly[chitosan-g-lactic acid]), PEG–PLLA–PEG and PEO–PPO–PEO (PPO and PEO are poly[propylene oxide] and poly[propylene oxide], respectively) are reversible thermogelling polymers that undergo sol–gel transitions based on bridged micelle formation (Tan and Marra, 2010). Previous studies have reported that PEG–PCL–PEG hydrogel could serve as an injectable biomaterial that prevented LV remodeling and dilation for the treatment of MI (Jiang et al., 2009). Also, hybrid hydrogels comprised of thiolated collagen (Col-SH) and multiple acrylate that contained OAC–PEG–OAC copolymers (OAC is oligo[acryloyl carbonate]) were formed in situ through Michael-type addition between Col-SH and OAC–PEG–OAC in phosphate-buffered saline (PBS) solution (at 37 °C, pH 7.4) and no catalyst. In a rat infarction model, echocardiography confirmed that both hybrid hydrogel and BMSC-encapsulating hybrid hydrogel treatments might improve EF after 28 days of post-MI injection (Xu et al., 2015).

When self-assembling peptides are incorporated into the physiological medium, stable nanofibrillar hydrogels can be formed (Joshi and Kothapalli, 2015). For example, injectable self-assembling RAD16-II peptide (AcN-RARADADARARADADA-CONH2) provides an appropriate microenvironment in the myocardium and promotes vascular cell recruitment of ECs, SMCs, and some nonvascular cells in the injected area. This hydrogel has been employed as a cell-carrying capsulate (Davis et al., 2005b; Lin et al., 2010).

4.2.2. Mechanical and biological properties of injectable biomaterials

The biological and mechanical properties of biomaterials are of great importance in TE applications. Engineered biomaterials should be biodegradable and have the capability to degrade in vivo over a period of time after implantation and upon functional regeneration of the infarcted myocardium. Injection of biomaterial into the myocardium may increase stiffness as well as obstruct the heart's relaxation (diastolic) and elastic properties. Although weak hydrogels may be forced through a needle, the material's property should be insufficient for the mechanically robust environment of the myocardium. Therefore, the formation of mechanically stable biomaterial is desired for their applications as injectable gels for heart regeneration (Chi et al., 2012).

Most currently available hydrogels possess unmatched mechanical properties to the infarcted myocardium, e.g., they are considerably softer than both normal and failed human cardiac muscle at the end of diastole (Table 1). Thus, they possibly cannot provide adequate mechanical support to the infarcted myocardium. Therefore, it is

mandatory to engineer a suitable biomaterial system with mechanical properties comparable to that of the native myocardium (Barocas et al., 1995; Kim and Healy, 2003; Omens, 1998; Rizzi et al., 2006; Semler et al., 2000; Stokke et al., 2000; Urech et al., 2005).

The mechanical properties of polymer-based hydrogels can be tuned through modifying the degree of polymer crosslinking. The degree of crosslinking polymers is defined as the establishment of covalent bonds between polymer chains. Another important advantage of hydrogels is tunable elasticity that allows them to imitate the stiffness of different natural tissues such as the elasticity of myocardium that ranges from 30 to 80 kPa. Further, cell mobility and infiltration can be modulated inside the scaffolds and support in vivo neovascularization using recruited host cells (Williams et al., 2015). Also, matrix elasticity may play a key role in cell fate. Recently, adult SCs such as MSCs seem to have a very sensitive capacity to commit to a specific lineage, which is dependent on the elasticity of the matrix. Soft matrices with an elastic modulus that imitate brain tissues seem to be rather neurogenic. In contrast, soft matrices with an elastic modulus comparable to muscle tissue are more suitable for myogenic cellular commitment. However, rigid matrices that imitate collagenous bone tissues are more osteogenic (Engler et al., 2004). Finally, it is possible to tune the physical state of hydrogels, after which two forms of hydrogel-based myocardial can be taken into consideration: a viscous-liquid injectable form which is mostly favored for cell delivery and viscous elastic gel form which is mostly employed to make 3D constructs (Li and Guan, 2011).

The gelation and degradation rate of hydrogels are important parameters for their therapeutic applications. When hydrogels are used for myocardial regeneration, the gelation rate can affect their mechanical properties and biological performance. Biomaterials with slow gelation rates may raise the possibility of a blockage to the blood's flow, leading to tissue necrosis in vivo (Eschenhagen et al., 2002). The degradation rate of hydrogel must be in line with myocardium remodeling (Fujimoto et al., 2009; Wu et al., 2008).

According to results, the use of stiff biomaterials maintains the geometry and is beneficial in short-term applications. However, for long-term applications, more studies must be performed to develop materials with the required characteristics. A tremendous understanding of the degree of stress relief is required to halt pathological remodeling (Dobner et al., 2009). Furthermore, a material must be degraded within a determined time period and the degradation products must be biocompatible, non-toxic, non-immunogenic, easily absorbed and removed from the body. Most natural ECM-based biomaterials meet these criteria because they are inherently comprised of biocompatible compositions, which are necessary for cell attachment and renewal. In addition, they easily degrade within days to months both in vitro and in vivo by means of enzymes secreted by the cells that form a natural ECM turnover. This enables cells to readily remodel their surrounding environment and establish a suitable ECM (Li and Guan, 2011).

ECM-based hydrogels generally possess a relatively low storage modulus (Singelyn and Christman, 2010, 2011). According to previous reports, the use of chemical and physical crosslinking of hydrogels may improve their mechanical properties (Cha et al., 2014; Robb et al., 2007). For instance, restrictions exist with the use of physical and chemical crosslinked polyphosphazene derivatives to improve mechanical properties. Chemical crosslinking of polymers may also limit their potential clinical use. On the other hand, crosslinking of thiol groups with acrylate groups improve the mechanical properties of injectable hydrogels (Potta et al., 2009, 2010). Several other approaches increase stiffness and elasticity of biomaterials used for myocardial repair. For example, in one study a double interpenetrated network has been created with an ECM hydrogel combined with a biocompatible injectable material with a high storage modulus (Duan et al., 2011; Williams et al., 2015). Another study employed a mild chemical crosslinker to produce a ten-fold increase in storage modulus (Singelyn and Christman, 2011).

Another important challenge, the characterization of materials (e.g., distribution, chemical composition) after injection is usually problematic and relies on invasive and destructive procedures. To overcome this problem, scientists have recently utilized a new magnetic resonance imaging (MRI) acquisition technique based on chemical exchange saturation transfer (CEST) where the signal relies on the exchange of protons in specific molecules with bulk water protons. CEST MRI can become an important tool for following injectable hydrogel properties (Dorsey et al., 2015).

Table 1
Stiffness of injectable cardiac biomaterials.

Material	Stiffness (Pa)	References
Normal human cardiac muscle	5×10^4	Omens (1998)
Failed human cardiac muscle	2×10^5–3×10^5	Omens (1998)
Fibrin	50	Urech et al. (2005)
Matrigel™	30–120	Semler et al. (2000)
Collagen (type I)	20–80	Barocas et al. (1995)
PNIPAm	10^2–4×10^2	Kim and Healy (2003)
Alginate	10^2–6×10^3	Stokke et al. (2000)
PEG	10^3–3×10^3	Rizzi et al. (2006)

PNIPAm: poly(N-isopropyl acryl amide); PEG: poly(ethylene glycol).

4.2.3. Growth factor-containing injectable systems

GFs play a key role in angiogenesis and cardiomyogenesis. It has been reported that the administration of bFGF in different animal models of MI stimulated cardiac angiogenesis (Landau et al., 1995; Yanagisawa-Miwa et al., 1992). Also, VEGF is proven to play an important role in angiogenesis initiation and the formation of new capillaries (Zieris et al., 2010). Transforming growth factor-beta (TGF-β) induces stable and functional vessel networks and efficient differentiation into functional CMs (Goumans et al., 2008). In addition, hepatocyte growth factor (HGF) is pro-angiogenic and anti-fibrotic (Wang et al., 2004); insulin-like growth factor-1 (IGF-1) promotes survival and cardiac action of CMs (Suleiman et al., 2007). Platelet-derived growth factor (PDGF) activates signaling pathways in CMs and promotes the maturation of the resultant capillaries into larger, more stable vessels. Other reports have demonstrated that delivered PDGF activated the PDGF signaling pathway in CMs and, in turn, attenuated CMs apoptosis after infarction. However this sustained delivery of PDGF did not improve arterial and capillary densities, nor did it increase regional blood flow (Hao et al., 2007; Hsieh et al., 2006).

GF loading for hydrogels is achieved via physical entrapment, absorption, encapsulation, and ligands with specific affinity for the active agent. Controlled delivery of GFs in myocardium is also dependent on the physicochemical properties of the polymer structure, molecular weight and 3D structure of GFs, type and density of crosslinks, and target release kinetics. Such system should provide protected delivery and regulated time- and dose-dependent release and supportive scaffolding for cell migration and proliferation that will lead to the generation of ECM and vascular networks for enhanced tissue integration and repair (Babavalian et al., 2014). It can restore myocardial functions by diminishing wall stress via increasing wall thickness and stabilizing chamber size. In addition, injectable biomaterials can create an improved environment for myocardial repair as well as a platform for controlled delivery of therapeutic proteins (Nguyen et al., 2015; Ruvinov et al., 2011). Researchers have reported that injectable biopolymers which contain GFs improved angiogenesis and LV contractility after MI (Hsieh et al., 2006). Injection of IGF-1/HGF modified alginate hydrogel in the infarct site increased angiogenesis and blood vessel formation (Ruvinov et al., 2011). The commonly used delivery approach for bFGF to the infarcted myocardium was through incorporation into a gelatin hydrogel after which bFGF was released as the hydrogel degrades (Yamamoto et al., 2001). Recently, bFGF-bounded ECM-derived hydrogels significantly enhanced neovascularization in the MI region compared to non-modified injected hydrogel (Seif-Naraghi et al., 2012). The sequential delivery of VEGF and PDGF from alginate

hydrogels showed effective angiogenesis in the MI model (Hao et al., 2007).

Other studies designed a novel modified self-assembling peptide for GF delivery. The heparinized self-assemble peptide was incorporated into nanofibrillar scaffolds, which resulted in sustained delivery of VEGF, significantly improved cardiac function, reduced scar size and collagen deposition, and enhanced microvessel formation (Guo et al., 2012). A self-assembling peptide employed to obtain a sustained release of PDGF to the myocardium showed a decrease in CMs death and maintained systolic function after MI (Hsieh et al., 2006).

Aside from GFs, cytokines and other proteins have also been delivered to the infarcted myocardium using hydrogel injections. Cytokines release by cells and affects the behavior of other cells. They can also be involved in autocrine signaling (Starr et al., 1997). Researchers designed a form of stromal cell-derived factor-1immune to matrix metalloproteinase (MMP)-2, which has the capability to retain chemotactic activity (Segers et al., 2007). The sustained local release of erythropoietin is achieved using PEG hydrogel. Erythropoietin has cardioprotective effects, but if administered systematically, it induces polycythemia and following thromboembolic results (Wang et al., 2009b).

4.2.4. Cell-laden hydrogel-based injectable systems

It is suggested that myocardial cell therapy can be complemented with novel in situ polymerizable and physicochemically controllable biomaterials. The identification of a suitable biomaterial for CMs transplantation is a significant area of research in cardiac TE. Several recent reports based on combining CMs with biomaterials have shown the feasibility of creating contracting tissue with myocardial features (Shin et al., 2004; Zimmermann et al., 2002). However, some of the materials used for scaffold formation are difficult to handle and highly vulnerable to proteolysis and premature degradation (Shapira-Schweitzer and Seliktar, 2007). According to the above-mentioned limitations, it is reasonable to have a cell-laden scaffold created from an injectable biomaterial with proper handling features and controllable physicochemical properties that does not impede CMs contractions (Bonaros et al., 2008; Komeri et al., 2015).

An injectable biomaterial must undergo in situ liquid-to-solid transition with CMs in suspension without harming the cells or the surrounding host myocardium. After polymerization, the cells should be able to easily remodel the polymer such that exact engraftment is possible through natural, cell-mediated pathways. In this regard, a biomaterial susceptible to tissue remodeling enzymes is of benefit (Lutolf and Hubbell, 2005). Simultaneously, the injectable polymer must not hinder cellular remodeling or distort myocardial geometry (Davis et al., 2005a; Kofidis et al., 2005). Therefore, it is vital to consider the influence of material compliance on CMs phenotype. The biomaterial should be an appropriate growth environment for myocardial cells to survive and express a contracting cardiac phenotype for functional integration upon implantation (McDevitt et al., 2003). However, it is partially unknown how the composition and structure of injectable biomaterials affect CMs remodeling and functional integration of the cell graft (Seliktar, 2005). Of note, an increased density of biologically active motifs in the hydrogel may influence cell behavior, resulting in different contraction patterns in the constructs. The relationship between matrix composition and cell density also provides some understanding into the mechanism of cellular reorganization (Shapira-Schweitzer and Seliktar, 2007).

A few studies compared injectable biomaterial therapy and/or cell transplantation with their incorporation for repair of a damaged myocardium. These studies investigated Matrigel™ versus mouse ESCs (mESCs), fibrin versus skeletal myoblasts (SMs), chitosan versus ESCs, small intestinal submucosa (SIS) versus circulatory angiogenic cells (CACs), and oligo[poly(ethylene glycol) fumarate] (OPF) versus mESCs (Table 2) (Christman et al., 2004; Kofidis et al., 2005; Landa et al., 2008; Toeg et al., 2013; Wang et al., 2012).

The vast majority of these studies demonstrated that injectable biomaterials improved the therapeutic benefit of cell transplantation in MI models. The injection of a CM-laden hybrid hydrogel (a combination of collagen with alginate) showed better cardiac function compared to individual hydrogels or only CMs (Zhang et al., 2006). Similarly, SM-laden fibrin gel improved fractional shortening (FS) and wall thickness compared with either fibrin or SMs. Five weeks after the injection of SMs into the infarcted zone, the cells predominantly localized in the border zone. However, the injection of SM-laden fibrin into the infarcted zone led to localization of these cells both in the border zone and within the infarct (Christman et al., 2004). An injection of BMMNC-laden fibrin gel also led to both an increase in microvessel density and internal diameter which showed that BMMNC-laden fibrin provided more effective vessels compared to only BMMNCs (Ryu et al., 2005). The injection of marrow-derived CSC-laden fibrin obtained comparable results. Better cardiac function and decreased scar area were reported (Guo et al., 2010b). EPC-laden fibrin also resulted in better angiogenesis with more EF and less scar formation compared to either fibrin or EPCs (Atluri et al., 2014). Also, after an intramyocardial injection of BMSC-laden fibrin hydrogel, fewer migrated cells were detected in the organs outside the myocardium, in particular the spleen, kidneys and liver, compared to an injection of only BMSCs (Martens et al., 2009).

In another study, an injection of Matrigel™ showed enhanced FS and wall thickness compared to injected mESCs. However the combined injection of Matrigel™ with mESCs in the MI heart showed improved functionality (Kofidis et al., 2004). Additionally, the graft/infarct area (G/I) ratio in the group that received Matrigel™ with mESCs was relatively higher compared to mESCs alone (Kofidis et al., 2005). A number of researchers reported that the combination of mESCs and OPF led to an increased G/I ratio. There was better heart function with mESCs combined with OPF compared to just OPF. Additionally, infarct size and the fibrotic area were reduced, along with reductions in the levels of MMP-2 and MMP-9. Although injected mESCs induced better angiogenesis than OPF, the combination of mESCs and OPF showed superior angiogenesis (Wang et al., 2012). In contrast, an injection of cell-laden SIS did not considerably improve the results obtained from an SIS only injection. EF, infarct size, angiogenesis and cardiomyogenesis showed no meaningful differences between the two groups, however the results of the two groups were better than an injection of only cells (Toeg et al., 2013).

The combination of mESCs with chitosan showed an acceptable G/I ratio compared to mESCs injected with PBS. There was a significant difference in FS, EF, infarct size, wall thickness and microvessel density between the mESC-laden chitosan group compared to either chitosan or mESCs; however, the authors detected no considerable difference between only chitosan or mESC groups (Lu et al., 2008). In another research, bioluminescent signals showed increased numbers of ADSCs that survived when encapsulated with chitosan hydrogel. The researchers observed improved EF and FS, more wall thickness with less infarct size, and a significant decrease in apoptosis. The vessel density in the ADSC combined chitosan injection was meaningfully more than either ADSCs or chitosan injections (Liu et al., 2012).

Lately, bioluminescence imaging results of the first four weeks in a study showed that an injection of BADSC incorporated chitosan hydrogel induced higher cell viability compared to BADSCs alone. In addition, the authors observed a significant difference in FS, EF, infarct size and fibrosis area between the BADSC-laden chitosan group compared to groups of either chitosan or BADSCs. There was no significant difference between the chitosan or BADSC groups. Evaluation of the expressions of cardiac troponin I+ and T+ had evidence of higher myocardial differentiation in the BADSCs and chitosan group compared with only BADSCs. Evaluation of Connexin 43 showed proper cell–cell interactions in cell-laden chitosan. Immunofluorescence staining for von Willebrand factor and α-smooth muscle actin showed increased microvessels in the ischemic zone, from group BADSCs to BADSCs and chitosan (Wang et al., 2014).

Table 2
Brief summary of injectable cell-laden hydrogel outcomes.

Material sources	Cell source	Outcome	Ref
Fibrin	SMs	Better result in FS and wall thickness.	Christman et al. (2004)
Fibrin	BMMNCs	More extensive tissue regeneration and enhanced neovascularization.	Ryu et al. (2005)
Fibrin	EPCs	Reduced myocardial scar formation and increased angiogenesis.	Atluri et al. (2014)
Fibrin	CSCs	Improved cell viability, cardiac differentiation, angiogenesis, cardiac function and decreased scar area.	Guo et al. (2010b)
Matrigel™	mESCs	Enhanced results in FS and wall thickness.	Kofidis et al. (2004)
Matrigel™	mESCs	Better G/I ratio and wall thinning.	Kofidis et al. (2005)
Chitosan	mESCs	Better results in G/I ratio, FS, EF, wall thickness, infarct size and angiogenesis.	Lu et al. (2008)
Chitosan	ADSCs	Improvements in the viability of engrafted cells. Smaller infarct size with improved heart function, wall thickness and angiogenesis.	Liu et al. (2012)
Chitosan	BADSCs	Enhancements in the survival and differentiation of engrafted ADSCs. Decreased infarct size and fibrotic area. Improved heart function, wall thickness and angiogenesis.	Wang et al. (2014)
SIS-ECM	CACs	Non-significant difference between SIS-only and cell-SIS injections reported. Whereas, appropriate with cell transplantation, the results significantly improved.	Toeg et al. (2013)
OPF	mESCs	Better G/I ratio, reduced infarct size and collagen deposition, improved heart function, and decreased MMP-2 and MMP-9 expressions.	Wang et al. (2012)
PEG–PCL–PEG	BMSCs	LV remodeling and dilation prevention, improvement in local systolic and diastolic functions.	Chen et al. (2014)
Self-assembling peptide nanofibers	BMMNCs	Reduced scar formation and improved cell retention, angiogenesis and cardiac function.	Lin et al. (2010)
RGD modified self-assembling peptide	mESC–CMs	Reduced fibrosis ratio and improved cell engraftment, EF and FS.	Ban et al. (2014)
Col-SH and OAC–PEG–OAC	BMSCs	Improved wall thickness, EF, vessel density and less infarct size.	Xu et al. (2015)
Collagen–alginate	CMs	A perceptibly improved cardiac function.	Zhang et al. (2006)

FS: fractional shortening; G/I: graft/infarct; EF: ejection fraction; LV: left ventricle; SIS: small intestinal submucosa; SMs: skeletal myoblasts; BMMNCs: bone marrow derived mononuclear cells; EPCs: endothelial stem cells; mESCs: mouse embryonic stem cells; ADSCs: adipose derived stem cells; BADSCs: brown adipose derived stem cells; CACs: circulatory angiogenic cells; BMSCs: bone marrow mesenchymal stem cells; OPF: oligo[poly(ethylene glycol) fumarate]; PEG–PCL–PEG: poly(ethylene glycol)–poly(caprolactone)–poly(ethylene glycol); Col-SH: thiolated collagen; OAC: oligo(acryloyl carbonate).

As such, researches demonstrated that BMMNC-laden self-assembling peptide nanofibers reduced scar formation and improved cell retention, angiogenesis, and cardiac function compared with both BMMNCs and nanofibers groups in a pig model of MI. However, the differentiation into CMs was not reported in the groups (Lin et al., 2010). A novel biomimetic self-assembling peptide was constructed by the attachment of a cell-adhesion motif with Arg-Gly-Asp-Ser-Pro (RGDSP) to the self-assembling peptide Acn-RADARADARADARADA-CONH2 (RAD16-I). The combination of this hydrogel with CSCs resulted increased cell retention and survival, along with better cardiac function and collagen deposition, and enhanced cardiac differentiation when compared with cell transplantation (Guo et al., 2010a). Similarly, CM-laden RGD modified self-assembling peptide amphiphiles showed a higher reduction in fibrosis ratio and increased improvement in EF and FS compared with both CMs and hydrogel groups. The CM-laden hydrogel showed more cell engraftment than CMs alone (Fig. 4) (Ban et al., 2014).

Intramyocardial injection of BMSCs with PEG–PCL–PEG hydrogel into infarcted myocardia of rabbits showed that the survival and retention of transplanted cells increased, and more angiogenesis and better cardiac function were observed compared to BMSC implantation alone (Wang et al., 2009c). Recently, a collagen–fibrin–dextran sulfate microcapsule has promised to overcome the shortcoming of limited cell retention in cell-based therapies of MI and improve the therapeutic effects of MSCs (Blocki et al., 2015). Lately, the combination of BMSCs and PEG–PCL–PEG hydrogel showed no significant differences four weeks after an EF injection compared with either BMSCs or hydrogel. However, the authors observed a higher quantitative tissue imaging curve and wall thickness for the combination of BMSCs and hydrogel compared to either BMSCs alone or hydrogel alone (Chen et al., 2014). Similarly, BMSC-encapsulating hybrid hydrogels led to a significant increase in wall thickness, EF, vessel density and less infarct size compared with the BMSCs and hybrid hydrogel groups (Xu et al., 2015).

In summary, compared to cell transplantation and injected hydrogel, cell-laden hydrogel systems show decreased infarct size and fibrotic area along with increased wall thickness and heart functionality with improved EF, FS, differentiation and angiogenesis. In this method, the combination of a paracrine effect with hydrogel's physical support provides a suitable niche and induces recruitment, proliferation and differentiation of vital cells which may lead to better myocardial regeneration.

5. The activation of cell signaling pathways by injectable systems

The cell–cell interactions are vital for several cellular activities. Thereby, close vicinity between cells often plays the key role on cell fates. Also, the cells communicate with each other through paracrine signaling that regulate cell fates in response to environmental stimuli (Gnecchi et al., 2008; Hui and Bhatia, 2007).

Moreover, a variety of signaling pathways are regulated by ECM, which leads to cellular responses such as differentiation and contractility. It is likely that these pathways are undergone significant temporal regulation throughout development as cells are secreted and assembled ECM, resulting in mature tissues (Engler et al., 2006; Sheldahl et al., 2003). Those that are more related to the cardiac maturation are probably due to the spatiotemporal heart patterning pathways (Wagner and Siddiqui, 2007) and non-canonical Wnt/Ca (Sheldahl et al., 2003) and Wnt/polarity pathways (Schneider and Mercola, 2001). The Wnt/β-catenin signaling plays important roles during heart development and it is re-activated in response to cardiac injury. It is suggested that the main roles of Wnt/β-catenin signaling in cardiac tissue development is the protection of CMs from apoptosis and regulation of CM hypertrophy. Similarly, GATA4 which is a regulator of cardiac development and plays essential roles cardiac hypertrophy, activating the expression of angiotensin II and beta-myosin heavy chain in response to pressure overload applied to the LV (Suzuki, 2011). Toeg et al. have shown that the expression of β-catenin and GATA4 are both increased either via cell therapy or hydrogel injection, nevertheless cell-laden hydrogel injection revealed higher level of efficiency (Toeg et al., 2013).

Also, hydrogel properties could be effective on Notch1 and mechanotransduction pathways (Boopathy et al., 2014; Martin et al., 2004). An important signaling pathway in cardiac regeneration is Notch1 playing an important role in cardiac regeneration. In this pathway, Notch1 receptors and their ligands are presented on the surface of a signal sending and signal receiving cell respectively, thereby, physical contact between the two cells are required for activation. In addition, an external force is needed for Notch activation, which is yielded

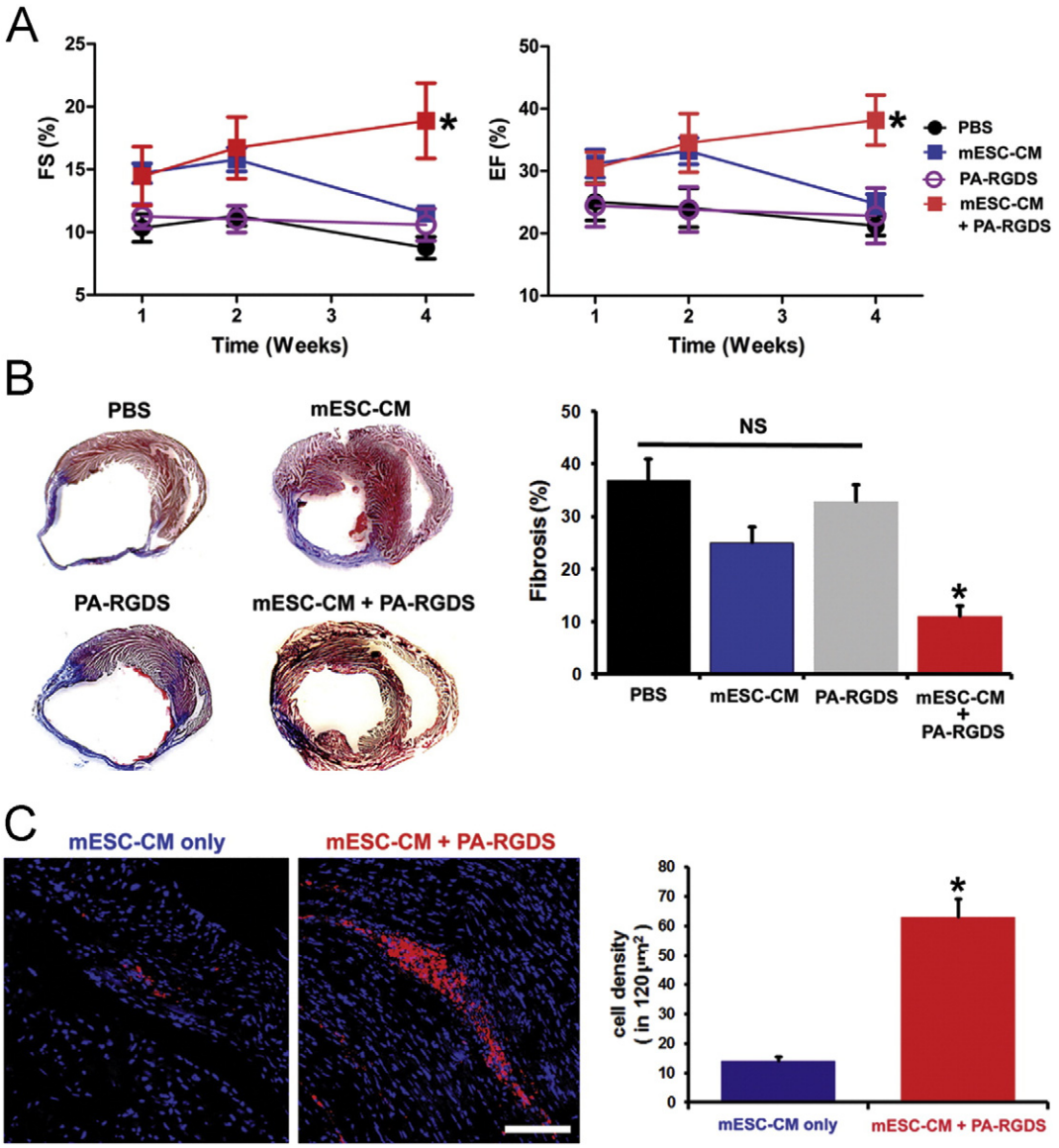

Fig. 4. Favorable effects of mESC–CMs with PA-RGDS on a myocardial infarction (MI) mouse model. (A) As measured by echocardiography, improvement of cardiac function was observed in mice treated with mESC-derived CMs with PA-RGDS. The mESC–CM laden RGD modified peptide amphiphile (PA-RGDS) group showed considerably higher fractional shortening (FS) and ejection fraction (EF) compared to the three other groups. (B) Representative images of the four treated groups revealed that cardiac fibrosis occurred after staining with Masson's trichrome in hearts harvested 4 weeks after MI. (C) Confocal microscopic images of heart sections obtained 4 weeks after MI and cell injection indicated a noticeably higher engraftment of mESC–CMs when cells were encapsulated. *$p < 0.05$.
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by ligand endocytosis on binding to the Notch1 receptor (Bray, 2006; Melody-Kapella et al., 2012). In this cell signaling cascade, the hydrogel may activate the Notch1 by ligand Notched Jagged1, and promote CPCs proliferation. It is important note that the concentration-dependent physical properties of the hydrogel including stiffness and hydrogel porosity can affect Notch1 activation in 3D structure. However, high concentration hydrogels could increase the stiffness, which saturates Notch1 signaling (Boopathy et al., 2014). Therefore, the effective parameters on stiffness must be taken into consideration to adjust the injectable hydrogel's properties. Similarly, literatures have shown that the complex cellular signaling and transcriptional responses are elicited by the unique link between the substrate modulus and intracellular biochemical signaling driving by the PI3K pathway and matrix elasticity. These data postulated that P13K/AKT pathway is potentially able to transmit external biomechanical cues of elasticity to intracellular cytoskeleton remodeling, gene regulation, and cell fate determination. For example stiff substrates would inhibit PI3K/AKT pathway and block myofibroblast activation (Wang et al., 2013).

Furthermore, the biochemical factors within the hydrogel could have influence on activation of mechanotransduction pathways. High molecular weight HA, which is a ubiquitous ECM component, drives mechanotransduction through CD44 that is most probably conveyed by activation of the Rho and Rac small GTPase pathways (Eriksson et al., 2003; Seidlits et al., 2010). Rho/Rho-kinase pathway plays a critical role in myocardial ischemia/reperfusion injury. It plays an important role in the signal transduction initiated by several agonists including angiotensin II, thrombin, endothelin-1, norepinephrine and urotensin, which have been observed in myocardial infarction (Shimokawa, 2002). It is also known that Rho/Rho-kinase acts as a molecular switch to exert various cellular activities such as smooth muscle contraction, cell–cell surface adhesion, motility and cytokinesis (Narumiya, 1996).

On the other hand, the inflammatory reaction has been known as a hallmark of myocardial reperfusion injury. Inflammatory cells and pro-inflammatory cytokines are two important constituents implicating in reperfusion injury (Bao et al., 2004). GTP-binding protein Rac is a key component, which mediates capillary assembly and vascular permeability (Eriksson et al., 2003). Another important parameter is post-ischemic control of MMP activation and expression (Spinale et al., 2000). Researchers have shown that although stem cell transplantation and hydrogel injection both could independently reduce the MMP-2 and MMP-9 protein levels in ischemic region, the cell-laden hydrogel would be more effective (Wang et al., 2012).

Hydrogels, which have essential ECM proteins inside themselves, ECM-derived hydrogel, and hydrogels modified with such proteins, could have suitable effects on cell signaling cascade. For example, the modification of synthesized hydrogels with RGD and fibronectin would provide suitable conditions for cell homing and adhesion as well as laminin modification would have proper influence on cell binding and communications.

6. Present issues

Although the positive effects of SC transplantation have been demonstrated in in vitro, their regeneration capability is not adequate for acceptable myocardial regeneration in the clinical applications. Infarcted myocardium is a relatively acidic environment in which the strand of its ECM is severely insufficient. In addition, reduction in ruptured ECM's mechanical properties cause ventricular applied tension that is higher than that of the threshold of defective tissue. If the action of endogenesis pathways is insufficient or assisted therapies are not performed, this process progressively causes the perforation of myocardium. In this environment, a significant volume of cells will undergo apoptosis immediately after cell transplantation. However, results show that even the small remaining numbers of transplanted SCs may lead to restoration of the infarcted myocardium. In this improvement, it appears that the paracrine effects contribute more than cell differentiation, particularly in the restoration of CMs. The majority of researchers believe that transplanted SCs lead to recruitment of CSCs from the host tissue. The total or at least a significant amount of restored CMs in the injured region are the consequences of the differentiation of the host CSCs. Although neovascularization, cell recruitment, proliferation and differentiation are reported in cell therapies, their outcomes remain unsatisfactory (Fig. 5) (Leri et al., 2005; Nakanishi et al., 2008; Segers and Lee, 2008; Wollert and Drexler, 2005).

In situ injection of hydrogels shows promising results in prevention of post-ischemic damages. The viscoelastic properties of these biomaterials directly affect the inhibition of applied tensions on the injured region and prevent progressive fibrous and scar tissue formation. These biomaterials facilitate the environment for migration of CSCs to the injured site, as well their survival and performance (Fig. 5). Although the majority of studies indicate that the amounts of dilatation of myocardial wall are low and more repetitive compared with the injection of cells, the effective vessel density in the cell therapy seems more favorable. This is probably due to the differentiation of cells to vessel lines as well as the efficient paracrine effects in vascularization (Kofidis et al., 2004, 2005; Li and Guan, 2011; Radhakrishnan et al., 2014; Singelyn and Christman, 2010; Wang et al., 2012).

The results indicated, in all cases in situ SC-laden hydrogels showed better (or at least equal) effects compared to SCs either transplantation or hydrogel injection alone. In the majority of cases the synergistic effects of the two approaches led to a significantly acceptable progress in recovery of the infarcted myocardium (Fig. 5) (Atluri et al., 2014; Chen et al., 2014; Guo et al., 2010b; Kofidis et al., 2004, 2005; Lin et al., 2010; Liu et al., 2012; Lu et al., 2008; Martens et al., 2009; Wang et al., 2012, 2014; Xu et al., 2015).

The variable parameters limit the statistical evaluations. The time interval of MI induction to treatment, and MI induction to echocardiography, animal species, injection volume, cell density, injection site, surgery and post surgery procedure, MI method as well as cell and material sources, separately affect the whole process. Thus, two groups (ESC-laden chitosan (Lu et al., 2008), ESC-laden OPF (Wang et al., 2012)) of high similarities (the animal species, time interval of MI induction to treatment, and MI induction to echocardiography, injection volume as well as cell density) have been studied in order to have statistical comparison. As it can be seen in Fig. 6, A–C, chitosan injection not only showed better effects on infarct size but also obtained better cardiac outputs and angiogenesis compared to that of OPF injection. However, ESC-laden OPF showed better results in all parameters as compared with ESC-laden chitosan. Nevertheless, the though-provoking note about ESC-laden OPF is that although the cell density and injection volume were equal to that of ESC-laden chitosan, the results were significantly different. Similarly, the slight difference of LVESD and LVEDd data is questionable because if so, a very low cardiac output is expected for the PBS group as Wang et al. reported (Wang et al., 2012).

In order for more statistical analysis on hydrogel the other parameters should be simplified. To achieve this, by more concentrating on time interval of MI induction to treatment, and MI induction to echocardiography, there are four groups include injection of SIS to mouse (Toeg et al., 2013), chitosan (Lu et al., 2008) and OPF (Wang et al., 2012) to rat and MPEG–PCL–MPEG to rabbit (Chen et al., 2014), immediately 1 week after MI. The infarct size in these groups 4th week after the injection revealed that SIS hydrogel significantly showed better results compared to other groups (Fig. 6D). It is worth noting that the infarct size reduction requires modulation of applied physical tension on LV, and chemical integration of hydrogel with tissue. Nevertheless, it must be noted that SIS injection was performed on mouse, which requires repetition and confirmation in big animals. These promising results require more investigations on ECM-derived hydrogel injection for myocardial repair. The other thought-provoking note is the poor effects of PEG–PCL–PEG compared to other groups, which may be attributed to its structural difference, viscoelastic properties and mechanical resistance.

Similar results were obtained regarding to cardiac outputs by injection of cell-free SIS (Toeg et al., 2013) and chitosan (Lu et al., 2008) hydrogels, but in angiogenesis evaluation SIS hydrogel was poor (Fig. 6, E–F). However, previous studies indicate that bFGF level of SIS significantly affected its angiogenic efficiency (Okada et al., 2010), but unfortunately there is no data about active bFGF's level in SIS used by Toeg et al. Despite all the superiority of SIS to chitosan, SIS + CACs showed frustrating results as compared to chitosan + ESCs and this indicates the importance of selecting appropriate cell source to provide suitable autocrine and paracrine effects. It should not be overlooked that the cell density might also have been effective.

The suitable time for cell implantation to generate new tissues and secrete their own matrix is about 4 to 6 weeks, so most of previous in vivo studies followed at least for 4 weeks (Fig. 7A) (Atluri et al., 2014; Chen et al., 2014; Christman et al., 2004; Guo et al., 2010b; Kofidis et al., 2004, 2005; Lin et al., 2010; Liu et al., 2012; Lu et al., 2008; Ryu et al., 2005; Toeg et al., 2013; Wang et al., 2012, 2014). The literatures have shown that the degradation time of chitosan hydrogel in myocardium is about 4 to 6 weeks (Lu et al., 2008), due to the glycosidic hydrolase enzyme effects (Han et al., 2012). According to previous report, the degree of deacetylation of chitosan was the important factor affecting the degradation rate. Also, chitosan concentration in the primary solution have influence on hydrogel degradation; the higher concentration the lower degradation rate (Ganji et al., 2007). Similarly Matrigel™ degrades through Proteinase-3 (Pezzato et al., 2003). The OPF hydrogel would degrade completely up to 6 weeks, by the hydrolysis of ester bonds (Wang et al., 2012), and The PEG–PCL–PEG degrades through hydrolytic process (Chen et al., 2014). Also, while considering fibrin glue it could be said that although, it could improve cell survival and preserve cardiac function post-MI, its degradation rate is too short to support adequate ECM secretion, due to cell-associated enzymatic activity (Ye et al., 2000).

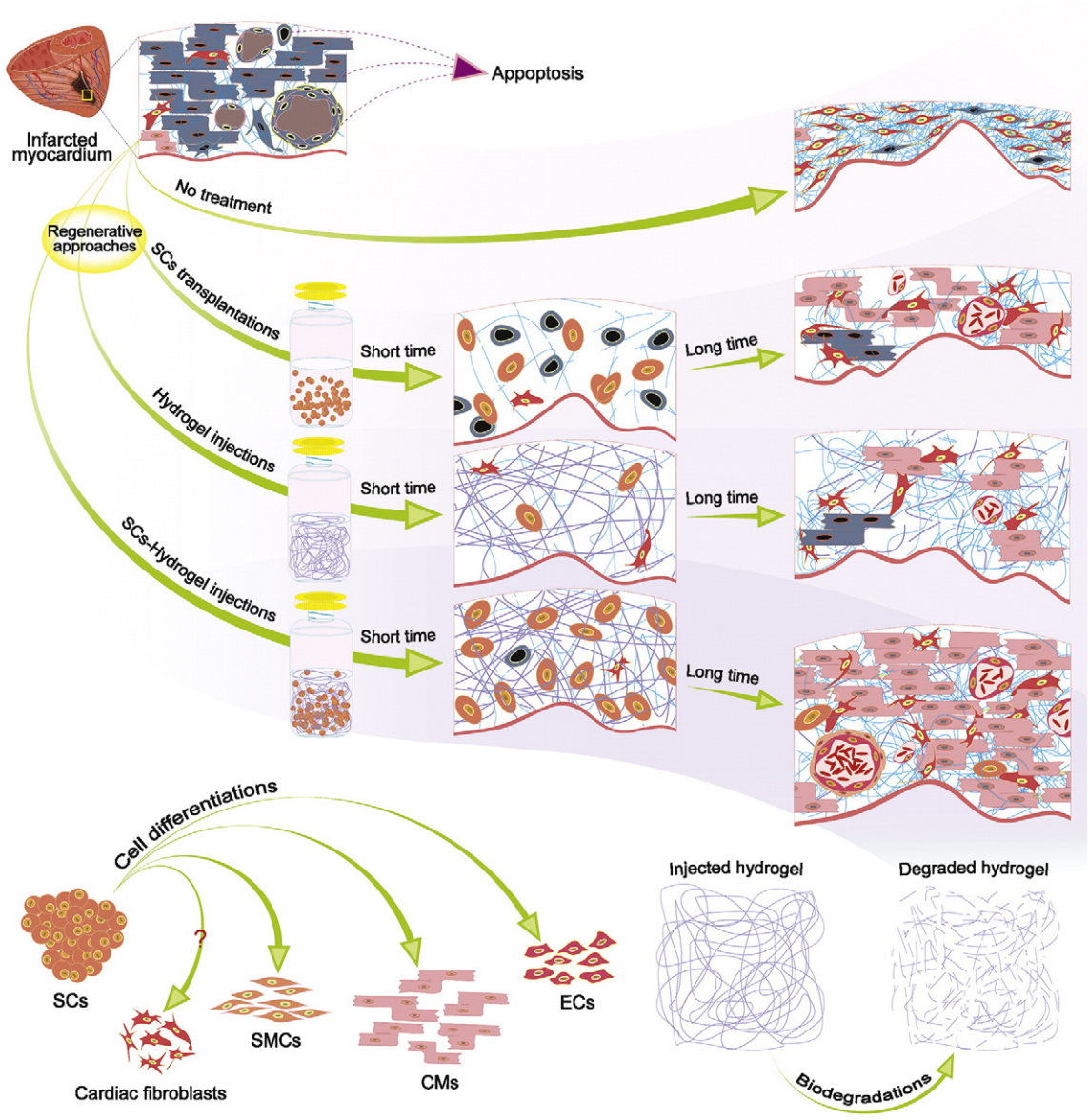

Fig. 5. Regenerative approaches for the heart. (A) Regeneration methods. In the first hour after cell transplantation, large numbers of transplanted cells died due to an inappropriate environment. However, the remaining cells began to secrete soluble factors that led to autocrine and, more importantly, paracrine effects. In this method, the present initial tension killed a broad volume of cells and decreased myocardial wall thickness. Injection of hydrogels inhibited myocardial wall thickness reduction, retained cardiac function, prevented formation of fibrous tissue, and provided a suitable environment for cell survival. The output of these hydrogels could be improved by the addition of stem cells (SCs) and/or growth factors (GFs). SC-laden hydrogels inhibit reductions in wall thickness, by damping the physical tensions, provide a suitable environment, and significantly improve the efficacy of SC therapies. (B) Cell differentiation. The natural and grafted SCs can supply all necessary cell lines for the myocardium — cardiomyocytes (CMs), endothelial cells (ECs) and smooth muscle cells (SMCs). There is doubt regarding the resource for fibroblasts, as they are frequently regarded as recruited cells from the endocardium (Krenning et al., 2010). (C) Hydrogel degradation. Hydrogels can be degraded via several processes (hydrolysis, enzymatic, pH, etc.) in the reverse pathway of myocardial extracellular matrix (ECM) restoration (Li et al., 2012; Li and Guan, 2011; Wu et al., 2008).

7. Conclusion and future prospective

It is clear that biomaterials combined with adult SCs are necessary for optimal and effective myocardial recovery, and usually shown synergistic effects that play an important role in recovery progress of the infarcted myocardium. However, biomaterials and cells possess their own drawbacks, which limit their clinical applications. This approach is promising by which, the efficiency of current therapeutic methods may be shifted for further recovery of myocardium in ischemic patients. However, more comprehensive and precisely studies are required to increase our basic knowledge on how this synergic affect occur and to gain a deep knowledge about the appropriate cell line and other cell parameters, hydrogel type and other related parameters as well as the synergistic effects of these tow on each other.

Also there are some suggestions for next researches. Cells can be encapsulated within hydrogels, and release while receiving signals. The degradation of hydrogels and kinetic of release could be specific-agent (e.g. MMP) sensitive, which is better not to be inflammatory agents sensitive since there are various inflammatory agents in the infarcted myocardium that can make the equation of degradation more complex and out of control. Of course, the loading of anti-inflammatory agents within inflammatory-sensitive hydrogel, which injects prior to reperfusion, may be effective to reduce the injuries related to post-ischemic reperfusion.

In addition, injectable hydrogels can be used as smart hydrogels for individual biomedical applications (Cha et al., 2014; Hoffman, 2012; Rimmer, 2011; Tan and Marra, 2010). For example, hydrogel degradation products can be designed in such a way that positively affects cell

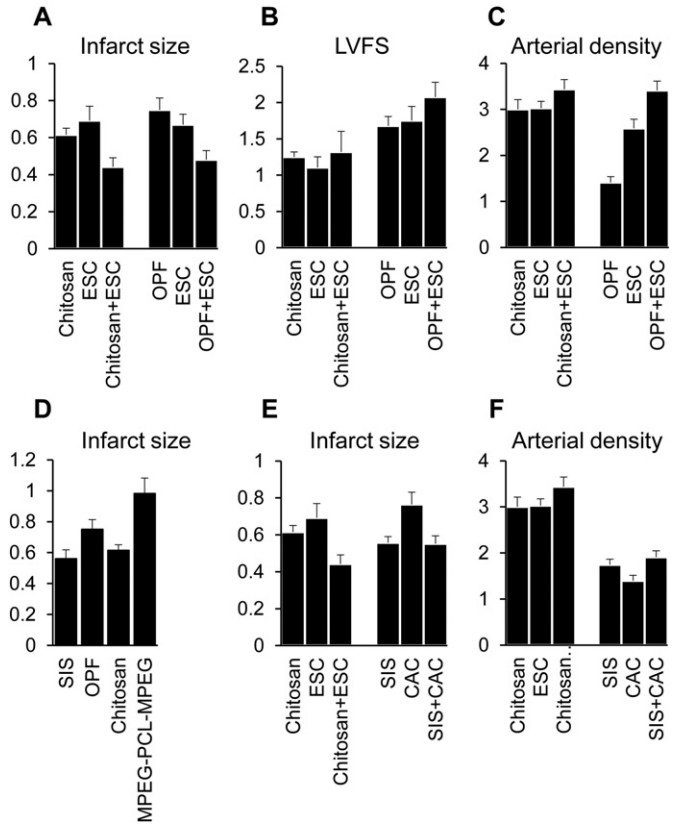

Fig. 6. Statistical analysis of some myocardial injected hydrogels. (A) The infarct size, (B) left ventricular fractional shortening and (C) arterial density, 4 weeks after the injection of chitosan, embryonic stem cell (ESC), ESC-laden chitosan, oligo[poly(ethylene glycol) fumarate] (OPF) and ESC-laden OPF (Lu et al., 2008; Wang et al., 2012). (D) The infarct size 4 weeks after the injection of small intestinal submocusa-derived (SIS), OPF, chitosan and poly(ethylene glycol)–poly(caprolactone)–poly(ethylene glycol) (PEG–PCL–PEG) hydrogels respectively to mouse, rat, rat and rabbit (Chen et al., 2014; Lu et al., 2008; Toeg et al., 2013; Wang et al., 2012). (E) The infarct size and (F) arterial density of infarct site 4 weeks after the injection of chitosan, embryonic stem cell (ESC), ESC-laden chitosan, SIS, circulatory angiogenic cells (CACs) and CAC-laden SIS (Lu et al., 2008; Toeg et al., 2013).

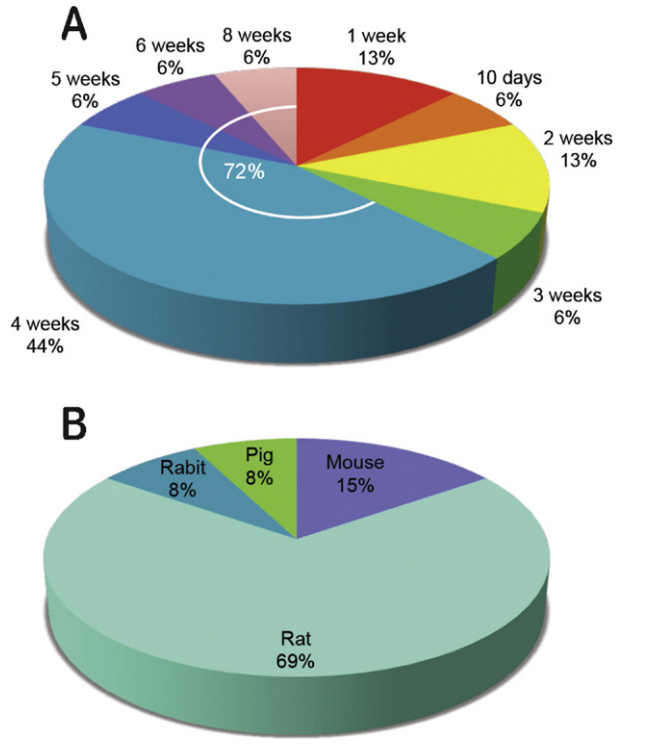

Fig. 7. Quantitative analysis of animal species and the time followed after injection. (A) Abundance of the time interval between the treatment and the histological evaluation. (B) Abundance of the evaluations implemented on different animal species in vivo (Atluri et al., 2014; Chen et al., 2014; Christman et al., 2004; Guo et al., 2010b; Kofidis et al., 2004, 2005; Lin et al., 2010; Liu et al., 2012; Lu et al., 2008; Ryu et al., 2005; Toeg et al., 2013; Wang et al., 2012, 2014).

performance and myocardial regeneration. The modulation of cell-laden hydrogel degradation with pH may be another interesting approach.

Although it is clear that hydrogel's stiffness and viscoelasticity will be effective on recovery of myocardium, there is lack of advanced studies. Research on the designing injectable hydrogel with tunable properties into myocardium may be valuable. In addition, the engineering of biocompatible, biodegradable and conductive injectable hydrogel may be a potential suggestion. Also, it is worth considering that although heart undergoes constantly cyclic loading, in none of the present studies the material evaluated in terms of cyclic fatigue. The optimization of properties and improvement of resistance to cyclic loading of these materials may be a valuable step in this approach. If the mechanical properties and degradation of hydrogels are improved, and injectable hydrogels can have anisotropic characteristics of a myocardium, they may simultaneously obtain the advantages of physical support, paracrine effects and cell differentiation.

Also, in none of the previous studies, the effects of CPCs loading have compared with pluripotent SCs and SC-derived beating cells. It seems that such study may clarify the uncertainties about differentiation stage of loaded cells. Furthermore, there is no precious investigation on ideal cell density loaded within hydrogel. This study may be matter since the cell communication is important to several intercellular interactions.

Most of the previous studies have performed on rodents so far and there is a severe lack of valuable investigations on large animal models (Fig. 7B). In addition, it is worth investigating the effect of time interval between the cell-laden hydrogel injection and MI induction. The combination of cells and hydrogels may considerably affect myocardia and warrants additional research.

Acknowledgments

We express our appreciation to Mohammad Kazemi-Ashtiani, Dr. Mahmoud Talkahbi, Hamed Daemi and Mohammadhossein Ghanian and other co-workers in Cell Engineering group at Royan Institute for their kind comments. This study was funded by grants provided by the Royan Institute, the Iranian Council of Stem Cell Research and Technology, the Iran National Science Foundation (INSF), and the Iran Science Elites Federation to HB.

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