

## Review

# Application of injectable hydrogels for cardiac stem cell therapy and tissue engineering

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Cardiovascular diseases are responsible for approximately one-third of deaths around the world. Among cardiovascular diseases, the largest single cause of death is ischemic heart disease. Ischemic heart disease typically manifests as progressive constriction of the coronary arteries, which obstructs blood flow to the heart and can ultimately lead to myocardial infarction. This adversely affects the structure and function of the heart. Conventional treatments lack the ability to treat the myocardium lost during an acute myocardial infarction. Stem cell therapy offers an excellent solution for myocardial regeneration. Stem cell sources such as adult stem cells, embryonic and induced pluripotent stem cells have been the focal point of research in cardiac tissue engineering. However, cell survival and engraftment post-transplantation are major limitations that must be addressed prior to widespread use of this technology. Recently, biomaterials have been introduced as 3D vehicles to facilitate stem cell transplantation into infarct sites. This has shown significant promise with improved cell survival after transplantation. In this review, we discuss the various injectable hydrogels that have been tried in cardiac tissue engineering. Exploring and optimizing these cell-material interactions will guide cardiac tissue engineering towards developing stem cell based functional 3D constructs for cardiac regeneration.

## Keywords

Cardiac tissue engineering; cardiac stem cell therapy; biomaterials; injectable hydrogels; decellularized tissues

## 1. Introduction

Cardiovascular diseases (CVD) are the leading causes of death around the world (Pagidipati and Gaziano, 2013). In 2015, deaths related to CVD globally were estimated to be around 18 million. Amongst different forms of CVD, deaths related to ischemic heart

disease (IHD) were top on the list (Roth et al., 2017). Some of the risk factors associated with IHD include hypertension, dyslipidemia, diabetes, smoking, family history, and physical inactivity (Hajar, 2017; Kannel et al., 1976). In IHD, the coronary artery in the heart gets progressively narrowed, which limits the blood flow to the heart, ultimately resulting in myocardial infarction and systolic dysfunction (Vlodaver et al., 2017; Hood, 1971). There is no permanent cure for heart failure with reduced ejection function (HFrEF) and the condition often requires urgent medical attention. The gold standard treatment for end-stage HFrEF is heart transplantation. However, there are several problems associated with this, including a limited supply of donor hearts and acute or chronic rejection by the host immune system (Blanche et al., 2002; Lund et al., 2017). Long-term use of immunosuppressants is also associated with cumulative risks of infection, malignancy and bone marrow suppression (Birati and Rame, 2014). Therefore, a regenerative therapy is urgently required to treat the patients suffering from HFrEF secondary to IHD. In this regard, stem cells and biomaterials-based approaches have shown promise for repair and regeneration of damaged cardiac tissue after an injury (Cutts et al., 2015). In this comprehensive review, we discuss the different stem cell and biomaterial-based approaches currently being studied for application in cardiac regeneration and tissue engineering.

## 2. Cell therapies for the treatment of heart diseases

The adult human heart has a limited regenerative capacity. Heart failure occurs through multiple mechanisms centered on the loss of functional cardiomyocytes (Doppler et al., 2017). Despite the plethora of available medical and surgical therapies, the body's inability to regenerate myocardium poses a significant ongoing risk to heart failure patients (Chiong et al., 2011). Stem cell therapy is a promising approach to myocardial regeneration that has been extensively studied by researchers. The concept of replacing cells lost in myocardial infarction with new stem cell-derived

cardiomyocytes has captivated many researchers and research organizations (Nunes et al., 2011). Many cell types ranging from adult stem or progenitor cells to embryonic or induced pluripotent stem cells (iPSCs) are currently being investigated for treatment purposes (Haraguchi et al., 2012). Cell attrition due to poor cell engraftment of the transplanted cells into the host native myocardium is a major obstacle that needs to be addressed in cardiac tissue engineering (CTE).

### 3. Biomaterial mediated cardiac regeneration in heart diseases

The advent of cell therapies has garnered significant interest in the field of cardiac regeneration. However, challenges including poor cell survival upon implantation and immunologic rejection cannot be addressed easily using conventional cell therapy methods (Sun et al., 2014). To accelerate translation, CTE was formed as an interdisciplinary field that combines the use of biomaterials, cells and growth factors to fabricate and/or regenerate damaged myocardium. The ultimate goal of CTE is to improve the survival and quality of life for patients through reversal of their HFrEF (Hasan et al., 2015; Hirt et al., 2014). Biomaterials, such as scaffolds, can augment cell therapy by bulking the scarred myocardium and improving cell survival after injection into the infarct site (Chen et al., 2008). Typical scaffolds are three-dimensionally cross-linked polymer networks that can act as artificial extracellular matrix (ECM) for cellular attachment. An ideal scaffold should provide enough mechanical strength to house the cells and disintegrate once mature tissues are formed. Their degradation products should be nontoxic and safely eliminated from the body. In addition, scaffolds can be engineered to facilitate neovascularization and nutrient diffusion to the cells housed within them (Huang et al., 2018; Novakovic et al., 2014).

Hydrogels are the most commonly used cellular scaffolds. These 3D hydrophilic polymer networks are formed through molecular interactions between the different functional groups present on the base polymers. Fig. 1 represents the different molecular structures of the hydrogels which will be discussed in detail below in this section. They will readily swell upon absorption of biological fluids without a change in their underlying molecular structure. This characteristic allows them to serve as a soft and elastic scaffold, which can mimic the tissue microenvironment. Hydrogels have been generated using both natural and synthetic polymer sources (Drury and Mooney, 2003; Lee and Kim, 2018). Fig. 2 represents an overview of injectable hydrogels loaded with stem cells for myocardial repair.

#### 3.1 Naturally derived polymer-based Hydrogels

Naturally derived polymers are typically considered prime candidates for regenerative hydrogels given their derivation from native ECM or ECM-like components. They have excellent biocompatibility, biodegradability and have been extensively studied in bone, cartilage, skin, nerve and cardiac regeneration (Asghari et al., 2017; Boni et al., 2018; Brovold et al., 2018; Fakoya et al., 2018; LogithKumar et al., 2016; Malafaya et al., 2007; Van Vlierberghe et al., 2011). Some of the commonly employed natural polymers in CTE discussed in this review include chitosan, collagen, gelatin, fibrin, alginate and other 3D decellularized tissues. These polymers can be used independently or in combination with each other

to synthesize an ideal injectable hydrogel for cardiac regeneration (Hasan et al., 2015; Peña et al., 2018).

##### 3.1.1 Chitosan

The use of chitosan (CS) for biomedical application dates back to the 1960s (Periyah et al., 2016). It is a linear cationic polysaccharide derived from chitin and is composed of glucosamine and N-acetylglucosamine linked by  $\beta$ -(1-4) glycosidic linkages. In humans, chitosan is degraded by hydrolytic enzymes into nontoxic chitosan oligosaccharides. Importantly, chitosan can be cross-linked using a variety of methods including light, temperature or chemical cross linkers (Tormos and Madhally, 2017; Jayakumar et al., 2010; Kim and Rajapakse, 2005). In cardiac regeneration, this feature is very desirable as it permits one to fabricate a material that is liquid at 4 °C and quickly gels at human body temperature after injection into the heart. This was the technique used in a study by Lu and colleagues, who fabricated a chitosan hydrogel loaded with ESCs. To study the regenerative potential of this hydrogel, they injected it into the hearts of post-MI rats. The chitosan material rapidly formed a gel at body temperature and significantly improved infarct wall thickness and ventricular function compared to animals injected with saline. Interestingly, the authors also demonstrated that incorporation of ESCs to the gel further improved the reparative nature of the gel; wall thickness, ventricular function and microvascular density were all improved with the ESC-loaded hydrogel when compared to chitosan alone (Lu et al., 2008). This study demonstrates the use of CS as injectable hydrogels to deliver stem cells to the infarct region.

Additionally, CS hydrogels can also be easily modified through encapsulation of growth factors and nanoparticles. This can provide the hydrogel with additional conductive or pro-reparative properties. For example, Baei and colleagues have published the synthesis of a thermosensitive CS hydrogel mixed with gold nanoparticles (GNP). This CS-GNP hydrogel had enhanced conductivity over the base CS hydrogel, and when loaded with bone marrow (BM)-derived mesenchymal stem cells (MSCs), enhanced the cardiomyogenic differentiation of MSCs (Baei et al., 2016). These versatile features make CS hydrogels an excellent candidate for cardiac regeneration and there is considerable ongoing research examining different types of CS and CS-derivative hydrogels (Cui et al., 2018).

##### 3.1.2 Collagen

Collagen is the major structural ECM protein in mammals and functions to provide tensile strength, support cell development and facilitate cell migration (Rozario and DeSimone, 2010). Its cell binding domains can facilitate attachment of both endogenous and transplanted cells (Antoine et al., 2014; Li and Guan, 2011). The effect of collagen hydrogels as bulking agents was evaluated by the direct injection of these hydrogels into the hearts of post-myocardial infarct (MI) rats. Animals that received a hydrogel injection had thicker scars, higher left ventricular stroke volume and higher left ventricular ejection fraction (LVEF) (Dai et al., 2005). However, the function of collagen hydrogels is limited by its electrical insulation and poor mechanical strength (Yu et al., 2017). Incorporation of conductive nanoparticles can offer a solution to address these shortcomings (Ashtari et al., 2019). Increased elastic modulus and electrical conductivity can be achieved through

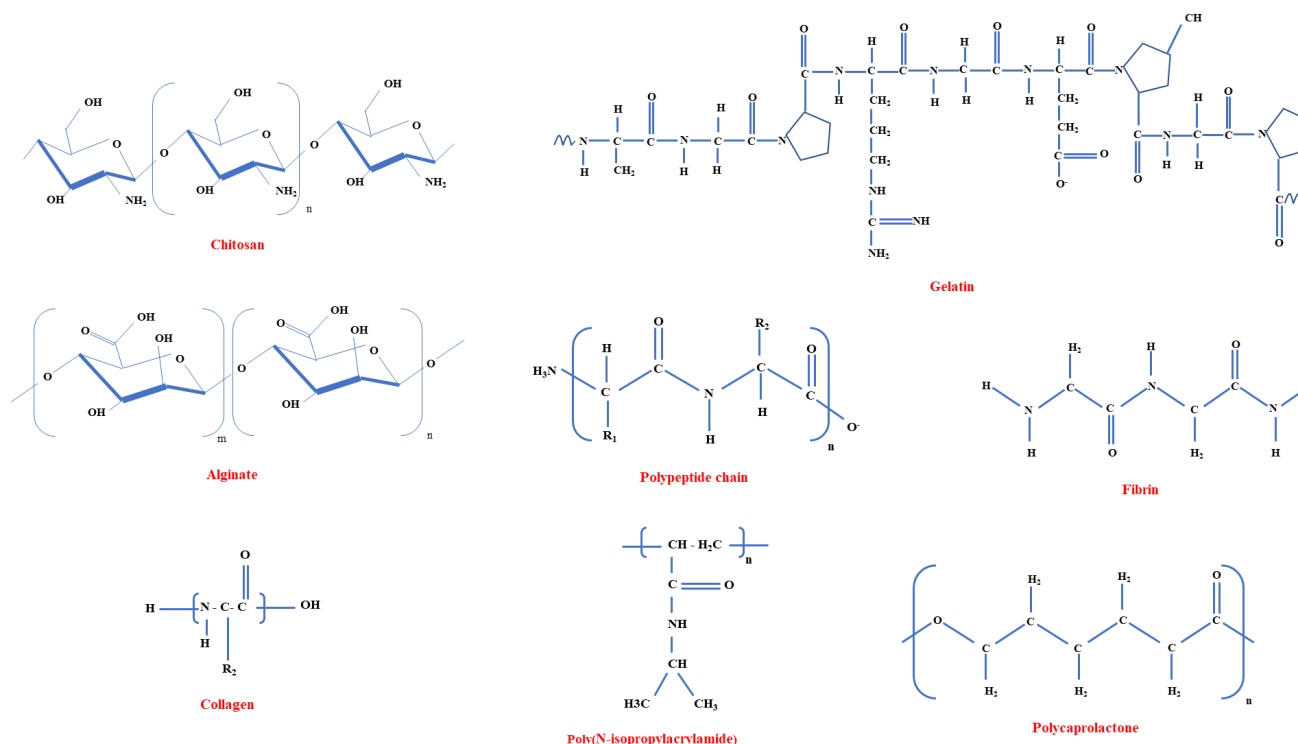


Figure 1. The molecular structures of different injectable hydrogels employed in cardiac tissue engineering.

insertion of carbon nanotubes into the collagen hydrogel. Collagen/Carbon nanotube (CNT) hydrogels also improved the cell to cell alignment of neonatal rat ventricular cardiomyocytes (NRVM) and contributed to improved mechanical contraction when compared to collagen group alone (Sun et al., 2017). These studies suggest that injected collagen hydrogels have favorable property for application in cardiac regeneration.

### 3.1.3 Gelatin

Gelatin is composed of peptides derived from collagen and offers improved hydrophilicity and reduced immunogenicity when compared to collagen. It can also be chemically modified to provide a specific environment for different cell types (Nikkhah, 2016; Lynn et al., 2004). The efficacy of gelatin hydrogels in cardiac cell transplantation was explored by Nakajima et al. (2015) in 2015. Fetal rat cardiomyocytes were encapsulated in gelatin hydrogels and subsequently injected into the infarct site of post-MI rats. Significant engraftment of the injected cardiomyocytes (CMs) was observed at one month along with an improvement in LVEF when compared with animals injected with saline. To further improve the regenerative potential of these hydrogels, graphene oxide (GO) was added to the gels to improve its mechanical strength and electrical signal propagation for enhanced cell-cell coupling and synchronous contractile activity (Amezcuca et al., 2016; Jiang et al., 2019; Stoppel et al., 2016). In a study published in 2019, Zhang et al. (2019a) incorporated GO into gelatin hydrogels, which improved the Young's modulus of the hydrogel, helping it mimic the natural stiffness of the heart. The study also showed that the gelatin/GO hydrogel improved the sarcomeric alignment and increased beating velocity of NRVMs when compared to gelatin hydrogels. The above-mentioned properties of gelatin such as low

immunogenicity and bioactive sequences similar to that of collagen hydrogels makes it a suitable injectable hydrogel for cardiac tissue engineering.

### 3.1.4 Alginate

Alginate is a linear polysaccharide derived from brown algae and is composed of different ratios of glucuronic acid and mannuronic acids. It is a natural polymer of high interest in CTE due to its tunable properties, facile hydrogel synthesis, biodegradability, high biocompatible nature and can be scaled up due to their low cost (Augst et al., 2006). The bulking property of the alginate hydrogel was evaluated in CTE by Landa et al. (2008). In this study, it was observed that direct injection of alginate hydrogels alone improved left ventricular ejection fraction in post-MI rats by decreasing the wall stress and reducing adverse ventricular remodeling. The most important shortcoming in employing alginate hydrogels in cardiac cell therapy is the lack of cell binding domains for the prospective cells to attach and proliferate. Hence, to address this issue, Shachar et al. (2011) modified alginate hydrogels with specific peptide sequences to provide an attachment surface. Alginate/Arginylglycylaspartic acid (RGD) hydrogels improved cell adherence and survival when compared to alginate hydrogels group (Shachar et al., 2011). This study demonstrates the influence of an external factor on the improvement of the physicochemical properties associated with alginate hydrogels for better cell attachment.

Reactive oxygen species (ROS) are released during the ischemia-reperfusion process and can worsen myocardial damage. Excess ROS can trigger injury and death in cells introduced to the infarct region. Antioxidant hydrogels are introduced in CTE to reduce the oxidative stress induced by ROS thereby preventing cel-

lular damage (Peña et al., 2018). Hao et al. (2017) incorporated antioxidant fullerene nanoparticles to alginate hydrogels and assessed its antioxidant properties. The fullerene/alginate hydrogels significantly improved the survival of encapsulated adipose-derived stem cells when injected into the post-MI heart of rats. This directly translated to improved cell retention when compared to the alginate hydrogel group (Hao et al., 2017). It is evident from the above-mentioned studies that alginate hydrogel is a potential candidate for CTE.

### 3.1.5 Fibrin

Fibrin similar to collagen is a protein based natural polymer, which has been extensively studied in myocardial cell therapy and CTE approaches (Li et al., 2015). Fibrin is formed by rapid polymerization of fibrinogen monomers catalyzed by enzymatic activity of thrombin (Weisel, 2007). The physiochemical properties of fibrin hydrogels such as length of the fibers, pore size, thickness can be tuned through the change in factors such as pH, fibrin concentration and its hydrophobicity. Fibrin hydrogels have ligands by which the cells and various cell mediators attach on them thereby recapitulating the native extracellular matrix microenvironment (Barsotti et al., 2011). In many studies, injection of fibrin hydrogels into post MI rats improved the survival of the transplanted cells, reduced the infarct size and increased angiogenesis by blood vessel formation (Christman et al., 2004a,b). Fibrin hydrogels have high elastic modulus, which can mimic the native myocardium. In addition to the above advantageous properties low inflammatory response towards the body's immune system adds up to the reliability of fibrin hydrogels as suitable scaffolds for cardiac tissue engineering applications (Wang et al., 2010).

### 3.1.6 Other naturally occurring 3D scaffolds

Other naturally occurring 3D scaffolds, which are commonly utilized in cardiac tissue engineering includes Matrigel, intestine-derived patches and pericardium which will be discussed in this section. Matrigel is a commercial product which is derived from the ECM of Engelbreth-Holm-Swarm (EHS) mouse sarcoma. It is majorly composed of collagen and other ECM proteins such as laminin, entactin and heparin sulfate proteoglycan (Hughes et al., 2010). Studies showed intramyocardial injection of Matrigel hydrogel alone improved the cardiac functions and scar thickening when compared to the control groups of an MI mouse (Kofidis et al., 2005). Similarly, when injected in a MI rat model there was improved fractional shortening and regional contractility when compared to control groups (Kofidis et al., 2004). In the recent years the natural scaffolds such as decellularized porcine small-intestinal submucosa, pericardium and other human heart tissues are used to generate 3D cardiac patches for myocardial regeneration in CTE (Godier-Furnémont et al., 2011; Haraguchi et al., 2006; Hata et al., 2010). Small Intestinal Submucosa (SIS) is derived from porcine in the form of lyophilized powder or decellularized tissue to generate hydrogels for CTE. It is composed of ECM components like collagens and a plethora of growth factors (Badylak et al., 2009; McDevitt et al., 2003). The patches generated from SIS ranges from 0.05 mm to 0.22 mm thickness with pore sizes varying from 20 to 30  $\mu$ m (Shi and Ronfard, 2013). In CTE, they have been applied to repair right ventricular free wall defects (Badylak et al., 2003). SIS ECM patch derived from porcine was used as an alter-

native patch to the other available synthetic and biological patch for CTE in a study conducted by Witt et al. (2013). They were sutured into the different cardiac locations of the infarcted region of 37 pediatric patients. SIS-ECM group had increased outflow tract gradients with ventricular outflow tract reconstructions. From this small group of study, SIS-ECM is a suitable scaffold for the closure of septal defect patching for CTE.

Similarly, pericardium is also a natural ECM that is extensively investigated as a scaffold for CTE applications. It is a fibrous sac composed of collagen and fibrin that surrounds the heart (Seif-Naraghi et al., 2010). One of the advantages of using pericardium is that autologous donor tissues can be obtained which can be processed as patches for artificial valves and durable grafts (Seif-Naraghi et al., 2010). Gálvez-Montón et al. (2017) prepared decellularized human pericardium which was seeded with porcine adipose tissue-derived progenitor cells to be used as a patch for the pig model of MI. The group with the pericardium patch has significant increase in the ejection fraction, stroke volume and reduced infarct size when compared to the control groups. ECM based patches with adequate structure and composition mimicking the native myocardium will be the key to generate 3D cardiac tissues for myocardial regeneration.

### 3.1.7 Decellularized tissues

Decellularized tissues (dECM) are natural polymers, which are derived from the native myocardium. They preserve the microstructure and composition of the native ECM (Moroni and Mirabella, 2014). dECM were first isolated from rat hearts by the removal of viable cells using detergents and leaving behind ECM (Ott et al., 2008). Decellularized tissues offer a natural matrix for individual cells to grow, which can be used to create cardiac tissues for heart failure patients (Iop et al., 2017). As an example, Wainwright et al. (2010) prepared a decellularized ECM of adult porcine hearts to produce suitable scaffold microenvironments for cardiomyocytes attachment and growth. This ECM maintains the complex structure and composition of the native ECM. In addition, it also supports the attachment and growth of chicken cardiomyocytes seeded in the porcine ECM.

While the adult human heart has very limited regenerative potential, certain evolutionarily primitive species have higher myocardial regenerative capacity. A species that is commonly known for its regenerative potential is the zebrafish. Chen et al. (2016) hypothesized that zebrafish cardiac ECM (zECM) may facilitate greater cardiac regeneration than mammalian cardiac ECM. They demonstrated that zECM promoted the proliferation of murine and human cardiac precursor cells and murine cardiomyocytes *in vitro*. When administrated intramyocardially to post-MI rats, zECM promoted the endogenous proliferation of murine cardiac stem cells. Even though dECM shows immense promise in CTE, better protocols are needed for complete cell removal from the dECM without the change in its structural integrity and composition (Bejleri and Davis, 2019; Gilpin and Yang, 2017). The current research focuses on addressing these limitations to employ dECM as a clinically relevant biomaterial for myocardial regeneration.

Table 1. Hybrid hydrogels currently studied in cardiac tissue engineering.

Hybrid Hydrogel	Cell type	<i>In Vivo</i> model	Outcomes	Reference
gelatin methacrylate (GelMA)/gold nanorod (GNR)	Neonatal rat ventricular cardiomyocytes (NRVM)	-	Excellent cell viability and retention; Improved electrical and mechanical property; Rhythmic contraction of the cells seeded in the GelMA/GNR hydrogel when compared to pure GelMA	(Navaei et al., 2016)
tetraaniline-polyethylene glycol diacrylate (TA-PEG)/thiolated hyaluronic acid (HA-SH)/DNA encoding eNOs (endothelial nitric oxide synthase)	adipose derived stem cells (ADSCs)	Sprague-Dawley rat MI model	Increased ejection fraction and vessel density; Reduction in infarct size and fibrosis area; Increase in electrical property of the gel	(Wang et al., 2018)
oxidized alginate (ALG-CHO)/2-aminopyridine-5-thiocarboxamide/tetraaniline	ADSCs	Sprague-Dawley rat MI model	Hydrogel exhibited excellent adhesive property; Upregulation of cardiac-related mRNA (Cx43, $\alpha$ -SMA, and cTnT) and angiogenic factors (VEGFA and Ang-1); Downregulation of inflammatory factors (tumor necrosis factor- $\alpha$ )	(Liang et al., 2019)
chitosan chloride/RoY peptide (CSCI/RoY)	human umbilical vein endothelial cells (HUVECs)	Sprague-Dawley rat MI model	Improved the cell survival, proliferation and migration seeded on CSCI/RoY when compared to CSCI hydrogel	(Shu et al., 2015)
Collagen/Chitosan/poly (3,4-ethylenedioxythiophene) : polystyrene sulfonate (PEDOT: PSS)	hiPSCs-CM	-	Improved electrical conductivity; Improved cell alignment and sarcomere organization; Enhanced connexin 43 expression	(Roshanbinfar et al., 2018)
Cross-linked hyaluronic acid and cross-linked polycaprolactone (CLMA) with Puramatrix peptide hydrogel	autologous adipose tissue-derived progenitor cells (ATDPCs)	Île-de-France sheep MI model	Reduced of infarct size when compared to control groups; Anchorage and integration of the cardiac patch with minimal fibrosis interface	(Chachques et al., 2019)
Methacrylated hyaluronic acid	-	Dorset sheep	Increased wall thickness in the apex and basilar infarct regions; Better cardiac output and ejection fraction when compared to control groups	(Ifkovits et al., 2010)
$\alpha$ (cyclodextrin) CD-MPEG-PCL-MPEG hydrogel	-	Rabbits	Prevented scar expansion and wall thinning; Increased ejection fraction when compared to the control groups	(Jiang et al., 2009)
Collagen I loaded 7-amino-acid-peptide hydrogel	H9C2 cardiac myoblast	C57/B6 mice	Increased stem cell recruitment and infarct wall thickness; Improved angiogenesis	(Zhang et al., 2019b)
gelatin methacrylate (GelMA)-(Au/SiO <sub>2</sub> ) NPs	H9C2 rat cardiomyoblasts	-	Improved cell adhesion and proliferation in the GelMA-Au/SiO <sub>2</sub> hydrogel compared to control; Uniform cell alignment in the GelMA-Au/SiO <sub>2</sub> hydrogel	(Maharjan et al., 2019)
Dextran-poly (ε-caprolactone)-2-hydroxyethyl methacrylate/poly (N-isopropylacrylamide) [Dex-PCL-HEMA/PNIPAM]	-	MI rabbits	Decreased scar expansion and thinning of wall compared with controls; Attenuated left ventricular systolic and diastolic dilatation; Increased left ventricular ejection fraction	(Wang et al., 2009)
Gelatin/Laponite® hydrogel loaded with secretome (nSi Gel+)	Human umbilical vein endothelial cells (HUVECs)	Fischer 344 rats	Increased capillary density; Reduced scar area; Improved cardiac function	(Waters et al., 2018)



### 3.2 Synthetically derived polymer-based hydrogels/Scaffolds

Disadvantages including batch-to-batch variability, risk of infection and weak mechanical strength limit the application of natural polymers in CTE (Han et al., 2019). The advantages of employing synthetic hydrogels in place of natural polymer-based hydrogels are that they can be easily fabricated with consistency and tuned material properties. Also, they can be produced in large quantities while scaling up without compromising quality (BaoLin and MA, 2014). Poly-N-isopropyl-acrylamide (PNIPAAm) and polycaprolactone (PCL) are some of the common synthetic polymer currently being discussed in this review for cardiac tissue regeneration.

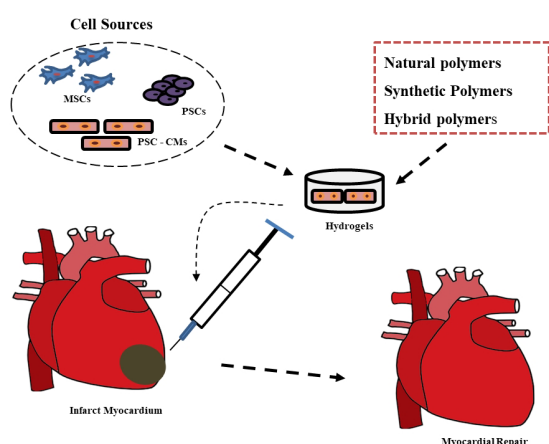


Figure 2. A schematic overview of injectable hydrogel mediated stem cell therapy for cardiac tissue engineering. Mesenchymal stem cells (MSCs), Pluripotent stem cells (PSCs), Pluripotent stem cells derived cardiomyocytes (PSCs-CM).

#### 3.2.1 poly(N-isopropylacrylamide)

Poly (N-isopropylacrylamide), abbreviated as PNIPAAm, is a temperature responsive polymer first synthesized in the 1950s. PNIPAAm hydrogels are formed by free-radical polymerization and can be readily functionalized for use in tissue engineering applications (Place et al., 2009; Schild, 1992). The arrangement of hydrophilic amide and hydrophobic isopropyl groups gives it a unique temperature sensitive property (Robb et al., 2007). PNIPAAm crosslinked with N, N'-methylene-bis-acrylamide remain as liquid at room temperature and changes to a hydrogel as the temperature rises above 32 °C (Alexander et al., 2014). Though this property is useful for injectable hydrogels, the therapeutic efficacy of PNIPAAm hydrogel alone is severely reduced due to its difficulty in degradation and reduced bioactivity of the encapsulated cells *in vivo* (Cui et al., 2011). Hence, it is used in combination with other polymers for CTE. Wang et al. (2009) prepared a PNIPAAm based biodegradable injectable hydrogel as a bulking agent for myocardial regeneration. It is composed of 2-hydroxyethyl methacrylate (HEMA)-PCL-grafted dextran chains embedded into the PNIPAAm network (Dex-PCL-HEMA/PNIPAAm). The hydrophilicity of the dextran chains improved the degradation of the PNIPAAm based hydrogel. The intramyocardial injection of this hydrogel in a post-MI rabbit helped

prevent left ventricle (LV) dilatation, improved LV contractility and LV wall thickness. To improve the bioactivity of the PNIPAAm hydrogels Li et al. (2014) employed single wall carbon nanotubes (SWCNTs). From the *in vitro* study, brown adipose-derived stem cells (BASCs) had better attachment and proliferation when grown in the PNIPAAm/SWCNT hydrogel compared to PNIPAAm hydrogel alone. In addition, from the *in vivo* experiment significant cell engraftment was observed when BASCs were encapsulated in PNIPAAm/SWCNTs hydrogel and injected into an MI rat model. PNIPAAm based thermosensitive injectable hydrogels offers new prospective to engineer better hydrogels for effective stem cell delivery for myocardial regeneration.

#### 3.2.2 Polycaprolactone

Polycaprolactone (PCL) is an important synthetic polymer whose properties can be tailored to match the mechanical and elastic strength of the native cardiac ECM. It is a FDA approved compound, which has excellent thermal stability and load bearing capacity (Siddiqui et al., 2018). The hydrophobicity and the poor conductivity of PCL prevent cells from attaching and cell to cell communication, which limits its use alone in CTE. It must be combined with other hydrophilic or electroactive polymers/material to make it more water soluble or electrically conductive respectively (Ciardelli et al., 2005). Spearman et al. (2015) prepared a conductive hydrogel by addition of polypyrrole (PPy) to the PCL hydrogels for the evaluation of electrophysiological property of HL-1 atrial myocytes. The addition of PPy and sodium hydroxide treatment improved the conductivity and the hydrophilicity of the PCL hydrogel. The cardiomyocyte attachment and viability were better for cells grown in PCL/PPy hydrogel compared to PCL hydrogels alone. Furthermore, there was also an improvement in the conduction velocity and calcium transient wave propagation, which is mediated by better cell to cell coupling *in vitro*.

Poly (glycolide-co-caprolactone) (PLGA) is a derivative of PCL, which has improved elasticity and may be better suited for the mechanically dynamic environment of the heart. In a study conducted by Piao et al. (2007), bone marrow-derived mononuclear cells (BMMNCs) were seeded onto PLGA and implanted on the epicardial surface of a post-MI rat. This PLGA scaffold influenced the migration, engraftment and differentiation of BMMNCs to cardiomyocytes, resulting in reduction of adverse LV remodeling. The properties associated with PCL hydrogels like high mechanical strength, non-immunogenicity and controlled degradation rate makes it a promising injectable hydrogel for CTE.

#### 3.3 Self assembling peptide nanofibers

Self-assembling peptide nanofibers are another important class of injectable hydrogels in myocardial repair. They are fabricated by employing macro-molecules like natural amino acids which self-assemble to form a molecular architecture at the physiological conditions (Hosseinkhani et al., 2013). These scaffold architectures are bioactive, biocompatible and nontoxic in nature. Also, when functionalized with peptide epitopes they mimic the native microenvironment which improves cell survival and also provides better cellular attachment (Yuan et al., 2014). For example, in a recent study, Li et al. (2018) synthesized a folic acid (FA) modified peptide hydrogel to differentiate iPSCs into functional cardiomyocytes in MI hearts of mice for myocardial repair. Post injection

the FA peptide hydrogel improved the survival and differentiation of the iPSC thereby augmenting the infarct size and fibrosis occurrence. Self-assembling peptides to date have been incorporated with many signaling molecules and growth factors to improve the tissue growth and its maintenance (French et al., 2016). Kim et al. (2011) incorporated two growth factors PDGF (Platelet derived growth factor) and FGF-1 (fibroblast growth factor 1) into RADA 16-II self-assembling peptides to promote revascularization in a MI heart of a rat. The peptide hydrogel along with growth factors significantly reduced the infarct size, apoptosis of cardiomyocytes and improved the blood vessel density in week 4 and 8 when compared to the control groups. These features make self-assembling peptide hydrogels a promising injectable hydrogels for CTE.

### 3.4 Hybrid hydrogels/Scaffolds

Blends of both natural and synthetic polymer based hydrogels are the most suitable options in cardiac tissue engineering (Shapira et al., 2016). Both natural and synthetic hydrogels have only a few desirable properties. Synthetic hydrogels provide better tunable physiochemical properties with strong mechanical strength but lack natural cell binding domains. Natural polymer-based hydrogels are bioactive and biocompatible but have undesirable features including uncontrolled degradation, poor mechanical strength, weak elastic property and long gelation times. Hybrid hydrogels comes into existence by conjugating both natural and synthetic polymers, which compensate for their individual weaknesses for successful tissue engineering practices (Li and Guan, 2011; Sheffield et al., 2018). Table 1 displays some of the hybrid hydrogels employed in the recent times in cardiac regeneration. Hybrid hydrogel offers innumerable opportunities for the materials to mimic the native tissue and to overcome the inherent challenges that will be needed to address in cardiac tissue engineering.

## 4. Future directions and concluding remarks

Over the years, several techniques and therapeutic approaches have been proposed to improve the regeneration of an impaired myocardium after myocardial infarction. Cell therapy is one such approach, which is of high interest and it is extensively investigated in CTE. The cells are injected into the infarcted myocardium with the hope that the cells electromechanically integrate into host tissue and replace the cardiomyocytes lost in an acute infarct. One of the most limiting factors in such therapy is that most of the injected cells die failing to adapt to the host tissue environment. Biomaterials have been employed to facilitate stem cell delivery to the infarct site and improve their survival in the post-infarct environment. Acellular biomaterials alone have also been proven to improve the stroke volume and positively influence the ventricular remodeling of the impaired heart. However, our understanding of the material-cell interaction *in vivo* is limited. Further research directed at development of novel composite and understanding the material-cell interactions will facilitate creation of translational paradigms.

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## Conflict of Interest

The authors declare no competing interests.

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