Published in final edited form as:

Methods Mol Biol. 2017 ; 1553: 293–304. doi:10.1007/978-1-4939-6756-8_23.

Optimal Environmental Stiffness for Stem Cell Mediated Ischemic Myocardium Repair

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Abstract

Cardiovascular diseases related to myocardial infarction (MI) contribute significantly to morbidity and mortality worldwide. The loss of cardiomyocytes during MI is a key factor in the impairment of cardiac-pump functions. Employing cell transplantation has shown great potential as a therapeutic approach in regenerating ischemic myocardium. Several studies have suggested that the therapeutic effects of stem cells vary based on the timing of cell administration. It has been clearly established that the myocardium post-infarction experiences a time-dependent stiffness change, and many studies have highlighted the importance of stiffness (elasticity) of microenvironment on modulating the fate and function of stem cells. Therefore, this chapter outlines our studies and other experiments designed to establish the optimal stiffness of microenvironment that maximizes benefits for maintaining cell survival, promoting phenotypic plasticity, and improving functional specification of the engrafted stem cells.

Keywords

Myocardial infarction; Stem cell therapy; Stiffness of microenvironment; Hydrogel

1 Introduction

Cardiovascular diseases related to myocardial infarction (MI) contribute significantly to morbidity and mortality worldwide despite advancements in medicine. The loss of cardiomyocytes during MI is a key factor in the impairment of cardiac-pump function due to the limited regenerative capacity of heart tissues. Using cell transplantation to regenerate ischemic myocardium has been considered as a new potential therapeutic approach to replace the lost or damaged cardiac cells. The results from experimental studies have suggested that transplanted stem cells can promote cardiac functional recovery after acute myocardial infarction (AMI) [1–4]. Mesenchymal stem cells (MSCs) are a particularly attractive option because they are both multipotent and immune privileged. There is some evidence that stem cells can improve cardiac function in patients suffering from AMI [5–8]. It has also been demonstrated that the ability of stem cells to repair the myocardium is not only dependent on the transdifferentiation of stem cells into cardiac phenotypes [9–11], but also on the protection of the native myocardium which is mediated primarily by paracrine factors released from stem cells [1, 3]. However, several studies have suggested that the therapeutic effects of stem cells are varied based on the timing of cell administration [12–14]. An analysis of seven trials with 660 patients indicated that bone marrow stem cells (BMSCs) transferred at 4–7 days post-AMI is superior to that within 24 h in improving cardiac function [14]. It has been observed that stem cell therapy at 1 week after AMI

facilitates integration of transplanted cells and functional recovery [13]. The optimal efficacy of bone marrow stem cell therapy at 7–14 days after MI may result from non-VEGF dependent angiogenesis [15]. Cell transfer within 24 h post-AMI does not augment recovery of global left ventricular contractile function [8]. Therefore, the optimal window of opportunity for stem cell therapy for MI might range in the period from day 5 to week 2 after the infarction [13–15].

The microenvironment around transplanted cells after MI potentially plays an essential role in deciding the optimal timing of cell therapy [15]. Many studies have highlighted the importance of stiffness (elasticity) of myocardium and composition of the extracellular matrix (ECM) on modulating the fate and function of stem cells including renewal, proliferation, differentiation, and regenerative potential [16–21]. Thus, the optimal stiffness of myocardium within a certain time frame post-AMI might offer some benefits for maintaining cell survival, promoting phenotypic plasticity, and improving functional specification of the engrafted stem cells [15].

2 The Myocardium Post-infarction Experiences a Time-Dependent Stiffness Change

In most soft tissues, cells added to an ECM establish a relatively elastic microenvironment. Myocardial stiffness is an index of muscle properties and is important in understanding normal and abnormal physiology [22]. Titin is a large elastic protein that extends across each half-sarcomere and is stretched in diastole when the sarcomere relaxes. However, at long sarcomere lengths or in damaged cardiomyocytes, collagen in the ECM increasingly contributes to stiffness and changes in a time-dependent manner from flexible to rigid following MI [23]. This translates into myocardial loss, subsequent remodeling, progressive ventricular dilatation, and fibrosis. Cardiomyocytes first undergo irreversible cell death, inducing an acute inflammatory reaction in the ischemic myocardium. Neutrophils and macrophages then quickly infiltrate the infarct region and release inflammatory mediators and matrix metalloproteinases (MMPs) to degrade ECM between 24 and 72 h [24]. Given that cardiomyocytes possess limited regenerative capacity, the spared myocardium becomes composed of the surviving hypertrophic cardiomyocytes as well as remodeling and degradation of the surrounding ECM resulting in scar formation. Finally, a matured collagen-rich reparative scar is formed to replace the extensive loss of cardiomyocytes in the infarct zone [25]. During this period, the proper balance between ECM synthesis and degradation is critical for optimal infarct healing. Excessive ECM accumulation increases wall stiffness and impairs compliance, leading to diastolic dysfunction [25, 26]. Atomic force microscopy has been used to map myocardial elasticity and establish the baseline elastic modulus for normal heart muscle at 18 ± 2 kPa [4]. The stiffness of infarcted myocardium between 1 to 24 h after AMI is relatively soft (4–17 kPa) [27]. Two weeks post-ischemia, infarcted myocardium formed significant fibrosis, with a similar threefold increase in the elastic modulus (55 ± 15 kPa). Injection of MSCs exhibited a significantly softer tissue modulus (40 ± 10 kPa) compared to the infarcted area in animals without MSC treatment [4]. This potentially indicates that the stiffness of infarcted myocardium may play

a significant role in the post-infarction remodeling process and any intervention that softens the infarct region may reduce deleterious remodeling.

3 The Role of Environmental Stiffness on Function and Fate of Stem Cells

Successful engraftment and survival of stem cells are the most important factors when assessing the effectiveness of stem cell therapies [28]. Implantation of stem cells via intramyocardial or coronary injection is plagued by limited cell retention and survival due to the lack of ECM in the infarcted myocardium [29, 30]. Injected or transplanted cells remain at the site of treatment only for a very short duration, leading to reduced therapeutic efficacy of the transplanted cells [30–32]. Cells delivered into the heart are rapidly redistributed to the lungs, liver, spleen, other organs throughout the body, or/and cleared by the lymphatic system [33]. The infarct region is poorly oxygenated, contains dead and apoptotic cells, and has a progressively increasing stiffness, which does not support the viability and well-being of implanted cells [33]. Transplanted cells do not survive in this hostile environment in the early days after MI and most cells die within 4 days after transplantation [34, 35]. Acute retention of stem cells varies depending upon route of delivery. In an elegant study utilizing radiolabeled bone marrow mononuclear cells [36], only $11 \pm 3\%$ of cells remained in the heart just 90 min after intramyocardial injection. The low intrinsic capacity to differentiate into cardiomyocytes may also influence their direct participation in myocardial regeneration. However, stem cells are able to feel and respond to the mechanical rigidity of a matrix changes in their mechanical environment [37, 38]. Cameron et al. [39] have recently reported that loss of rigidity has an influence on differentiation and proliferation of stem cells. Generally, soft matrices promoted significantly more proliferation and chondrogenic differentiation, whereas hard matrices promoted osteogenic differentiation [40]. The differentiation of MSCs into a specific lineage is enhanced on a matrix designed to create stiffness that is similar to the targeted tissue type [41]. It has also been reported that soft matrices (0.1–1.0 kPa) (which mimic brain tissue) have neurogenic and stiffer matrices (11 kPa) which mimic striated myogenic muscle. Comparatively rigid matrices (34 kPa) which are heavily cross-linked with collagen more commonly initiate bone growth [42]. Stem cells cultured in a medium with a matrix stiffness (31 kPa) similar to the elasticity of infarcted myocardium at day 7 had a greater ability to differentiate into endothelial lineage cells. However, those cells grown in the medium with a relatively soft matrix (4–17 kPa) showed minimal differentiation [27]. The differentiation of ES cells into cardiomyocyte was very limited when cells were cultured on the hydrogel with stiffness similar to that of brain or fat tissue (0.2 kPa) [38].

Microenvironment stiffness also influences the proliferation of stem cells. The percentage of actively proliferating cardiomyoblasts falls from 23 % to 1 % between embryonic day 14.5 and neonatal day 7 [43], which coincides with the rise in myocardial stiffness during embryological development [44]. In addition, the marked reduction in proliferative capacity of cardiomyoblasts in vivo between embryonic day 14.5 and neonatal day 7 is surprisingly similar with that of a cell cycle altered by the gel stiffness in vitro [45].

The environmental stiffness not only influences the survival, proliferation, and differentiation of stem cells, but also influences the function of cells. A series of flexible

substrates of hydrogels with stiffness similar to that of embryonic, healthy, ischemic, or fibrotic myocardium have been used to test the effects of stiffness on the contractile output of cardiomyocytes [46]. The matrices with stiffness in the range of 11–17 kPa of the developing myocardial microenvironment were optimal for promoting actomyosin striation and cardiomyocyte beating. The scar-like stiff substrate (35–70 kPa) region that mimicked a post-infarct fibrotic scar lacked striated myofibrils and stopped beating. However, on very soft matrices, cells preserved contractile beating for several days but did very little work [46]. The mechanical output has also been demonstrated using cardiomyocytes differentiated from human pluripotent stem cells that it was the highest on hydrogels with stiffness similar to that of healthy myocardium [47].

4 Hydrogel with Optimized Stiffness Improves Stem Cell Efficacy for Heart Repair

4.1 Natural and Synthetic Hydrogel

A variety of natural and synthetically formulated materials have been utilized to determine the effects of stiffness on stem cell mediated heart repair. The natural biomaterial is consisted of a three-dimensional decellularized cardiac ECM which is composed of a network of interstitial collagens. Cardiac ECM demonstrated excellent biological properties for cellular recognition, biocompatibility, and had the potential to degrade through known metabolic processes [48–50]. These experiments were initially designed as a protective platform for cellular therapy because they can provide many benefits in maintaining the appropriate phenotypic and functional characteristics of cells [51, 52]. Cardiac ECM may improve the survival of transplanted cells via providing a suitable scaffold that structurally and biochemically mimics the native cardiac environment [52]. These ECM also provide many heparin-binding sites that enhance the loading and release of soluble growth factors [53]. A range of stiffness of natural ECM-protein-derived hydrogels are achieved by changing the density of these proteins and altering the surface ligand concentration [54].

Synthetic materials have several advantages in manufacturability and are typically inexpensive and easily reproducible. Synthetic polymer gels can serve as ECM mimetic scaffolds for stem cell fate control [42, 55, 56]. The stiffness of polymer gels can be adjusted by altering the amount of a cross-linker. Polyacrylamide (PA) is critical for biomedical applications because it is hydrophilic and bioinert. Because of the toxicity of its monomer, acrylamide [48, 57], it is necessary to characterize their biocompatibility and mechanical properties before being used in vivo [58]. Fibrin glue is a hydrogel that is widely used in surgery, cell culture, and tissue engineering. Both autologous and recombinant off-the-shelf fibrin glues have been extensively used as pro-coagulants during cardiac and other surgeries. Additionally, its angiogenic role following myocardial ischemia makes this an attractive system for possible catheter-based cell therapy of the heart [59]. A semisynthetic hydrogel has also previously been used for cardiac and skeletal muscle regeneration [60]. This type of hydrogel has a distinct advantage over other types of scaffolds because its mechanical properties are highly malleable while leaving the functionality of the encapsulated cells well preserved by the backbone of the polymeric network.

4.2 Hydrogel Improves the Function of Stem Cells

Biocompatible hydrogels have been considered in situ as a cell delivery vehicle to improve cell retention, survival, and function following delivery into the ischemic myocardium. The potential of injectable biomaterials for the delivery of stem cells and their roles in myocardium regeneration have been reported previously. Intra-myocardial polymerization of polymers in situ represents one possible solution to address both leakage and clearance-mediated cell loss [33].

A tissue-engineered, hydrogel-based endothelial progenitor cell-mediated therapy can enhance cell delivery, cell retention, vasculogenesis, and preservation of myocardial structure and function [61, 62]. Revascularization and hemodynamic parameters of infarcted heart are significantly improved by injection of cells into the infarct region of optimal stiffness of the PEG-fibrinogen (PF) scaffold [60]. The functional integration of transplanted cells and host myocardium in the ischemic heart is significantly improved [60].

The phenotype, cell proliferation, and viability of cardiomyocytes are well maintained and are higher in cells cultured with ECM than that of cells cultured without the ECM sheets. It has thus been suggested that natural ECM sheets could be used in the future to improve strategies for cardiomyocyte transplantation [50]. Intramyocardial injection of BMSCs with α -CD/MPEG-PCL-MPEG hydrogel can increase the survival and retention of transplanted cells and vessel density around the infarct zone when compared to BMSC implantation alone [63]. Injection of BMSCs with biomaterials significantly increased the LV ejection function and attenuated LV dilatation [63]. Hydrogel may also provide an ideal delivery system of growth factors for stem cell transplantation. Engineering vasculogenic endothelial cells and angiogenic effector cells with chitosan hydrogel containing VEGF-loaded microtubes significantly prolonged cell survival and effectively induced neovascularization and enhanced vascular repair [64]. Thus, a feasible strategy in cardiac muscle reconstruction would combine the bioengineering of therapeutic cells to facilitate vascularization while delivering them with a support system optimized for the damaged myocardium [60].

5 In Vitro Mimicry of Various Myocardial Stiffness and Preparation of Cell Culture Dishes

It has been well established that a standard culture system with rigid substrate does not effectively mimic the physical or mechanical microenvironment. Stem cells cultured on traditional plastic dishes concomitantly lose their self-renewal and regenerative potential rapidly, as shown by their limited contribution to muscle regeneration following transplantation [62, 65]. Excessive rigidification constrains cell-based cardiac repair by limiting favorable phenotype specifications [15]. Rowlands et al. [66] showed that PA gels containing singular ECM proteins of varying stiffnesses could affect MSC differentiation in a composition dependent manner. It is expected that the results obtained from flexible cell culture substrates will have significant implications for understanding physical effects of the in vivo microenvironment.

The following represents a method for preparing a series of hydrogel stiffness for testing the effects of microenvironment stiffness on survival, renewal, and differentiation of cultured stem cells in vitro. The variably compliant polyacrylamide hydrogels are prepared as previously described [67, 68].

5.1 Materials

5.1.1 Cleaning Cover Glass

1. NaOH, 1 N.
2. HCl, 1 N.
3. Distilled water.
4. 250 ml beaker.

5.1.2 Polyacrylamide Gel

1. No. 1.5 cover glass, 22 mm × 22 mm.
2. NaOH, 0.1 N.
3. PBS, 500 ml.
4. 3-aminopropyltrimethoxy saline.
5. Glutaraldehyde, 0.5 %, mix 357 µl of 70 % stock tightly sealed in zip bags in a closed container at 4 °C.
6. HEPES, 1 M, pH = 8.5.
7. HEPES, 50 mM, pH = 8.5.
8. Acrylamide (40 %).
9. Bis (2 %).
10. Ammonium persulfate (Bio-Rad) solution, 10 mg in 100 µl distilled water. Prepare immediately before use.
11. TEMED (Bio-Rad).
12. Sulfo-SANPAH (pierce), 0.5 mg/ml in 50 mM HEPES (pH = 8.5), need 200 µl per cover glass. Prepare immediately before use. Handle Sulfo-SANPAH in the dark. Weigh the appropriate amount, add 1 µl DMSO per mg of sulfo-SANPAH. While vortexing the DMSO and sulfo-SANPAH mixture, add 50 mM HEPES at room temperature to obtain the final concentration.

5.2 Gel Preparation

5.2.1 Cleaning Cover Glass

1. Add NaOH solution (1 N) to a beaker and seal the beaker with Parafilm.
2. Shake the beaker at 100 rpm for 4 h at RT.
3. Wash the cover glass with distilled water 3 times.

4. Add HCl solution (1 M) to the beaker and seal the beaker with Parafilm.
5. Shake the beaker at 100 rpm for 4 h.
6. Wash the cover glass with distilled water 3 times.
7. Add 100 % EtOH to the beaker, then shake for 2 min.
8. Put a piece of paper tissue in the fume hood; and put the cover glass separately on the paper tissue.
9. Wait until the cover glass dries.
10. Sterilize the cover glass through UV exposure for 15 min.
11. Store cover glass in a petri dish and seal the petri dish using Parafilm for future use.

5.2.2 Preparing Polyacrylamide Gel

1. Place the clean cover glass on plastic surface and add ~100 μ l of 0.1 N NaOH to the cover glass, then smear the NaOH solution with cell scraper until the entire surface of the cover glass is covered. Wait until the NaOH solution dry.
2. Smear surface with ~100 μ l 3-aminopropyltrimethoxy silane using a cell scraper, and then wait for 5 min at room temperature.
3. Remove the 3-aminopropyltrimethoxy silane by washing the cover glass with distilled water, and then wipe the cover glass with a Kimwipes to ensure that most of the silane is removed.
4. Collect the cover glass in a plastic container and rinse with distilled water. Place the plastic container on a shaker for 15 min. Remove the distilled water, replace with fresh distilled water, and shake for 15 min, repeat the washing four times.
5. Remove the distilled water and let the cover glass dry. Place the cover glass on aluminum foil or a metal surface, and then add 0.5 % glutaraldehyde to cover the cover glass. Wait for 30 min at room temperature.
6. Remove the glutaraldehyde and wash the cover glass with distilled water on a shaker. Make sure the treated surface of the cover glass is always facing up. The cover glass may be stored in desiccator for 2 weeks.
7. The stiffness of the PA gel depends upon the ratio of acryl-amide to bis-acrylamide mix 5 ml of acrylamide solution in 15-ml Corning tubes according to Table 1.
8. Put the 15-ml Corning tubes in a vacuum chamber, and degas the solution for 60 min to remove the dissolved oxygen in the solution.
9. Add 30 μ l freshly prepared ammonium persulfate solution, 20 μ l TEMED to the Corning tubes, then seal the Corning tubes with Parafilm and swirl the mixture gently.

10. Transfer 15 μ l the acrylamide solution to the activated surface of the cover glass, and then place an un-activated clean cover glass on top of the acrylamide solution droplet to make a cover glass–acrylamide solution–cover glass sandwich.
(Steps 9 and 10 should be finished within 2 min for all the cover glasses.)
11. Wait until the acrylamide polymerizes. Use remaining acrylamide solution in the Corning tubes as a measure, until the acrylamide solution polymerizes to become gel.
12. Flood cover glass sandwich with 50 mM HEPES, and then remove the un-activated cover glass with fine tweezers.
13. Rinse the remaining activated cover glass with the formed polyacrylamide gel with 50 mM HEPES. The gel maybe stored at 4 °C for 2 weeks.
14. Remove as much liquid from the polyacrylamide surface as possible and add 100 μ l sulfo-SANPAH solution.
15. Place under UV lamp for 5–8 min. The sulfo-SANPAH solution will become dark.
16. Repeat steps 14 and 15.
17. Rinse the polyacrylamide surface with 50 mM HEPES to remove excess sulfo-SANPAH solution. Do this quickly.
18. Add 100 μ l fibronectin (20 μ g/ml in PBS) to surface for 1 h at room temperature or overnight in 4 °C.
19. Before plating the cells, expose the surface in UV light for 15 min to sterilize the surface.
20. Rinse the surface with PBS.
21. The fibronectin-coated polyacrylamide surface is ready for cell culture.

6 Notes

1. Before being used for cell culture, the gels covered with PBS are exposed to ultraviolet light with wavelength of 275 nm for 15 min.
2. Thereafter PBS is replaced with complete culture medium and cell culture dishes are placed in incubator for 2 h to allow equilibrium.
3. Stem cells can be cultured in primary or passage culture.

Acknowledgments

This work was supported by the National Institutes of Health grants HL105176 and HL114654 (M. Xu).

References

1. Tang YL, et al. Paracrine action enhances the effects of autologous mesenchymal stem cell transplantation on vascular regeneration in rat model of myocardial infarction. *Ann Thorac Surg.* 2005; 80(1):229–236. discussion 236–7. [PubMed: 15975372]
2. Feygin J, et al. Functional and bioenergetic modulations in the infarct border zone following autologous mesenchymal stem cell transplantation. *Am J Physiol Heart Circ Physiol.* 2007; 293(3):H1772–H1780. [PubMed: 17573463]
3. Kinnaird T, et al. Local delivery of marrow-derived stromal cells augments collateral perfusion through paracrine mechanisms. *Circulation.* 2004; 109(12):1543–1549. [PubMed: 15023891]
4. Berry MF, et al. Mesenchymal stem cell injection after myocardial infarction improves myocardial compliance. *Am J Physiol Heart Circ Physiol.* 2006; 290(6):H2196–H2203. [PubMed: 16473959]
5. Schachinger V, et al. Intracoronary bone marrow-derived progenitor cells in acute myocardial infarction. *N Engl J Med.* 2006; 355(12):1210–1221. [PubMed: 16990384]
6. Meyer GP, et al. Intracoronary bone marrow cell transfer after myocardial infarction: eighteen months' follow-up data from the randomized, controlled BOOST (BOne marrOw transfer to enhance ST-elevation infarct regeneration) trial. *Circulation.* 2006; 113(10):1287–1294. [PubMed: 16520413]
7. Wollert KC, et al. Intracoronary autologous bone-marrow cell transfer after myocardial infarction: the BOOST randomised controlled clinical trial. *Lancet.* 2004; 364(9429):141–148. [PubMed: 15246726]
8. Janssens S, et al. Autologous bone marrow-derived stem-cell transfer in patients with ST-segment elevation myocardial infarction: double-blind, randomised controlled trial. *Lancet.* 2006; 367(9505):113–121. [PubMed: 16413875]
9. Duran JM, et al. Bone-derived stem cells repair the heart after myocardial infarction through transdifferentiation and paracrine signaling mechanisms. *Circ Res.* 2013; 113(5):539–552. [PubMed: 23801066]
10. Thal MA, et al. Enhanced angiogenic and cardiomyocyte differentiation capacity of epigenetically reprogrammed mouse and human endothelial progenitor cells augments their efficacy for ischemic myocardial repair. *Circ Res.* 2012; 111(2):180–190. [PubMed: 22589372]
11. Hatzistergos KE, et al. Bone marrow mesenchymal stem cells stimulate cardiac stem cell proliferation and differentiation. *Circ Res.* 2010; 107(7):913–922. [PubMed: 20671238]
12. Bartunek J, et al. Timing of intracoronary bone-marrow-derived stem cell transplantation after ST-elevation myocardial infarction. *Nat Clin Pract Cardiovasc Med.* 2006; 3(Suppl 1):S52–S56. [PubMed: 16501632]
13. Hu X, et al. Optimal temporal delivery of bone marrow mesenchymal stem cells in rats with myocardial infarction. *Eur J Cardiothorac Surg.* 2007; 31(3):438–443. [PubMed: 17239611]
14. Zhang S, et al. Impact of timing on efficacy and safety of intracoronary autologous bone marrow stem cells transplantation in acute myocardial infarction: a pooled subgroup analysis of randomized controlled trials. *Clin Cardiol.* 2009; 32(8):458–466. [PubMed: 19685520]
15. Zhang S, et al. Infarcted myocardium-like stiffness contributes to endothelial progenitor lineage commitment of bone marrow mononuclear cells. *J Cell Mol Med.* 2011; 15(10):2245–2261. [PubMed: 21091632]
16. Das RK, Zouani OF. A review of the effects of the cell environment physicochemical nanoarchitecture on stem cell commitment. *Biomaterials.* 2014; 35(20):5278–5293. [PubMed: 24720880]
17. Peyton SR, Putnam AJ. Extracellular matrix rigidity governs smooth muscle cell motility in a biphasic fashion. *J Cell Physiol.* 2005; 204(1):198–209. [PubMed: 15669099]
18. Gilbert PM, et al. Substrate elasticity regulates skeletal muscle stem cell self-renewal in culture. *Science.* 2010; 329(5995):1078–1081. [PubMed: 20647425]
19. Guilak F, et al. Control of stem cell fate by physical interactions with the extracellular matrix. *Cell Stem Cell.* 2009; 5(1):17–26. [PubMed: 19570510]
20. Gattazzo F, Urciuolo A, Bonaldo P. Extracellular matrix: a dynamic microenvironment for stem cell niche. *Biochim Biophys Acta.* 2014; 1840(8):2506–2519. [PubMed: 24418517]

21. Wen JH, et al. Interplay of matrix stiffness and protein tethering in stem cell differentiation. *Nat Mater*. 2014; 13(10):979–987. [PubMed: 25108614]
22. Raya TE, et al. Serial changes in left ventricular relaxation and chamber stiffness after large myocardial infarction in rats. *Circulation*. 1988; 77(6):1424–1431. [PubMed: 3370778]
23. Chaturvedi RR, et al. Passive stiffness of myocardium from congenital heart disease and implications for diastole. *Circulation*. 2010; 121(8):979–988. [PubMed: 20159832]
24. Ma Y, Yabluchanskiy A, Lindsey ML. Neutrophil roles in left ventricular remodeling following myocardial infarction. *Fibrogenesis Tissue Repair*. 2013; 6(1):11. [PubMed: 23731794]
25. Ma Y, Halade GV, Lindsey ML. Extracellular matrix and fibroblast communication following myocardial infarction. *J Cardiovasc Transl Res*. 2012; 5(6):848–857. [PubMed: 22926488]
26. Ma Y, et al. Matrix metalloproteinase-28 deletion exacerbates cardiac dysfunction and rupture after myocardial infarction in mice by inhibiting M2 macrophage activation. *Circ Res*. 2013; 112(4):675–688. [PubMed: 23261783]
27. Zhang S, et al. A role of myocardial stiffness in cell-based cardiac repair: a hypothesis. *J Cell Mol Med*. 2009; 13(4):660–663. [PubMed: 19243474]
28. Hoover-Plow J, Gong Y. Challenges for heart disease stem cell therapy. *Vasc Health Risk Manag*. 2012; 8:99–113. [PubMed: 22399855]
29. Barbash IM, et al. Systemic delivery of bone marrow-derived mesenchymal stem cells to the infarcted myocardium: feasibility, cell migration, and body distribution. *Circulation*. 2003; 108(7):863–868. [PubMed: 12900340]
30. Hofmann M, et al. Monitoring of bone marrow cell homing into the infarcted human myocardium. *Circulation*. 2005; 111(17):2198–2202. [PubMed: 15851598]
31. Wollert KC, Drexler H. Clinical applications of stem cells for the heart. *Circ Res*. 2005; 96(2):151–163. [PubMed: 15692093]
32. Qian H, et al. Intracoronary delivery of autologous bone marrow mononuclear cells radiolabeled by ¹⁸F-fluoro-deoxy-glucose: tissue distribution and impact on post-infarct swine hearts. *J Cell Biochem*. 2007; 102(1):64–74. [PubMed: 17407141]
33. Martens TP, et al. Percutaneous cell delivery into the heart using hydrogels polymerizing in situ. *Cell Transplant*. 2009; 18(3):297–304. [PubMed: 19558778]
34. Zhang M, et al. Cardiomyocyte grafting for cardiac repair: graft cell death and anti-death strategies. *J Mol Cell Cardiol*. 2001; 33(5):907–921. [PubMed: 11343414]
35. Toma C, et al. Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart. *Circulation*. 2002; 105(1):93–98. [PubMed: 11772882]
36. Hou D, et al. Radiolabeled cell distribution after intramyocardial, intracoronary, and interstitial retrograde coronary venous delivery: implications for current clinical trials. *Circulation*. 2005; 112(9 Suppl):I150–I156. [PubMed: 16159808]
37. Kong HJ, Boontheekul T, Mooney DJ. Quantifying the relation between adhesion ligand-receptor bond formation and cell phenotype. *Proc Natl Acad Sci U S A*. 2006; 103(49):18534–18539. [PubMed: 17124175]
38. Discher DE, Janmey P, Wang YL. Tissue cells feel and respond to the stiffness of their substrate. *Science*. 2005; 310(5751):1139–1143. [PubMed: 16293750]
39. Cameron AR, Frith JE, Cooper-White JJ. The influence of substrate creep on mesenchymal stem cell behaviour and phenotype. *Biomaterials*. 2011; 32(26):5979–5993. [PubMed: 21621838]
40. Navaro Y, et al. Matrix stiffness determines the fate of nucleus pulposus-derived stem cells. *Biomaterials*. 2015; 49:68–76. [PubMed: 25725556]
41. Discher DE, Mooney DJ, Zandstra PW. Growth factors, matrices, and forces combine and control stem cells. *Science*. 2009; 324(5935):1673–1677. [PubMed: 19556500]
42. Engler AJ, et al. Matrix elasticity directs stem cell lineage specification. *Cell*. 2006; 126(4):677–689. [PubMed: 16923388]
43. Walsh S, et al. Cardiomyocyte cell cycle control and growth estimation in vivo—an analysis based on cardiomyocyte nuclei. *Cardiovasc Res*. 2010; 86(3):365–373. [PubMed: 20071355]
44. Jacot JG, Martin JC, Hunt DL. Mechanobiology of cardiomyocyte development. *J Biomech*. 2010; 43(1):93–98. [PubMed: 19819458]

45. Shkumatov A, Baek K, Kong H. Matrix rigidity-modulated cardiovascular organoid formation from embryoid bodies. *PLoS One*. 2014; 9(4):e94764. [PubMed: 24732893]
46. Engler AJ, et al. Embryonic cardiomyocytes beat best on a matrix with heart-like elasticity: scar-like rigidity inhibits beating. *J Cell Sci*. 2008; 121(Pt 22):3794–3802. [PubMed: 18957515]
47. Ribeiro AJ, et al. Contractility of single cardiomyocytes differentiated from pluripotent stem cells depends on physiological shape and substrate stiffness. *Proc Natl Acad Sci U S A*. 2015; 112(41):12705–12710. [PubMed: 26417073]
48. Laflamme MA, Murry CE. Heart regeneration. *Nature*. 2011; 473(7347):326–335. [PubMed: 21593865]
49. Bouten CV, et al. Substrates for cardiovascular tissue engineering. *Adv Drug Deliv Rev*. 2011; 63(4–5):221–241. [PubMed: 21277921]
50. Lee KM, et al. Natural cardiac extracellular matrix sheet as a biomaterial for cardiomyocyte transplantation. *Transplant Proc*. 2015; 47(3):751–756. [PubMed: 25891725]
51. Robertson MJ, et al. Optimizing recellularization of whole decellularized heart extracellular matrix. *PLoS One*. 2014; 9(2):e90406. [PubMed: 24587354]
52. Singelyn JM, et al. Catheter-deliverable hydrogel derived from decellularized ventricular extracellular matrix increases endogenous cardiomyocytes and preserves cardiac function post-myocardial infarction. *J Am Coll Cardiol*. 2012; 59(8):751–763. [PubMed: 22340268]
53. Pati F, et al. Printing three-dimensional tissue analogues with decellularized extracellular matrix bioink. *Nat Commun*. 2014; 5:3935. [PubMed: 24887553]
54. Ingber DE. Mechanical signaling and the cellular response to extracellular matrix in angiogenesis and cardiovascular physiology. *Circ Res*. 2002; 91(10):877–887. [PubMed: 12433832]
55. Khetan S, et al. Degradation-mediated cellular traction directs stem cell fate in covalently crosslinked three-dimensional hydrogels. *Nat Mater*. 2013; 12(5):458–465. [PubMed: 23524375]
56. Huebsch N, et al. Harnessing traction-mediated manipulation of the cell/matrix interface to control stem-cell fate. *Nat Mater*. 2010; 9(6):518–526. [PubMed: 20418863]
57. Exon JH. A review of the toxicology of acrylamide. *J Toxicol Environ Health B Crit Rev*. 2006; 9(5):397–412. [PubMed: 17492525]
58. Darnell MC, et al. Performance and biocompatibility of extremely tough alginate/polyacrylamide hydrogels. *Biomaterials*. 2013; 34(33):8042–8048. [PubMed: 23896005]
59. Christman KL, et al. Injectable fibrin scaffold improves cell transplant survival, reduces infarct expansion, and induces neovasculature formation in ischemic myocardium. *J Am Coll Cardiol*. 2004; 44(3):654–660. [PubMed: 15358036]
60. Bearzi C, et al. PIGF-MMP9-engineered iPS cells supported on a PEG-fibrinogen hydrogel scaffold possess an enhanced capacity to repair damaged myocardium. *Cell Death Dis*. 2014; 5:e1053. [PubMed: 24525729]
61. Atluri P, et al. Tissue-engineered, hydrogel-based endothelial progenitor cell therapy robustly revascularizes ischemic myocardium and preserves ventricular function. *J Thorac Cardiovasc Surg*. 2014; 148(3):1090–1097. discussion 1097–8. [PubMed: 25129603]
62. Collins CA, et al. Stem cell function, self-renewal, and behavioral heterogeneity of cells from the adult muscle satellite cell niche. *Cell*. 2005; 122(2):289–301. [PubMed: 16051152]
63. Wang T, et al. Bone marrow stem cells implantation with alpha-cyclodextrin/MPEG-PCL-MPEG hydrogel improves cardiac function after myocardial infarction. *Acta Biomater*. 2009; 5(8):2939–2944. [PubMed: 19426843]
64. Lee S, et al. Enhanced therapeutic neovascularization by CD31-expressing cells and embryonic stem cell-derived endothelial cells engineered with chitosan hydrogel containing VEGF-releasing microtubes. *Biomaterials*. 2015; 63:158–167. [PubMed: 26102992]
65. Gussoni E, et al. Normal dystrophin transcripts detected in Duchenne muscular dystrophy patients after myoblast transplantation. *Nature*. 1992; 356(6368):435–438. [PubMed: 1557125]
66. Rowlands AS, George PA, Cooper-White JJ. Directing osteogenic and myogenic differentiation of MSCs: interplay of stiffness and adhesive ligand presentation. *Am J Physiol Cell Physiol*. 2008; 295(4):C1037–C1044. [PubMed: 18753317]

67. Pelham RJ Jr, Wang Y. Cell locomotion and focal adhesions are regulated by substrate flexibility. *Proc Natl Acad Sci U S A*. 1997; 94(25):13661–13665. [PubMed: 9391082]
68. Beningo KA, Lo CM, Wang YL. Flexible polyacrylamide substrata for the analysis of mechanical interactions at cell-substratum adhesions. *Methods Cell Biol*. 2002; 69:325–339. [PubMed: 12071003]

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Table 1

Acrylamide solution to make polyacrylamide gels of different stiffness

Young's Modulus (kPa)	40 % Acrylamide (μl)	2 % Bis (μl)	1 M HEPES (μl)	H ₂ O (μl)
75	1000	200	50	3750
30	1000	150	50	3800
10	1000	50	50	3900
24	625	200	50	4125
15	625	150	50	4175
7	625	63	50	4262