Published in final edited form as:

IEEE Eng Med Biol Mag. 2007 ; 26(4): 76–79.

Stem Cell Therapies for Heart Disease: Why Do We Need Bioengineers?

Nenad Bursac

The limited regenerative potential of the heart via cell proliferation or stem cell recruitment is insufficient to compensate for tissue loss after an acute myocardial infarction [1], [2].

Consequently, the increased workload placed on the surviving myocardium often leads to heart failure. Approximately 865,000 Americans per year suffer a myocardial infarction and nearly 4.9 million are afflicted with heart failure, demonstrating the vital need for new and more efficient therapies [3]. More than a decade ago, the transplantation of exogenous cells into the heart had been proposed as a method to augment compromised heart function in these diseases [2], [4]. Nowadays, the use of stem cells to rebuild a damaged heart has become a mainstream experimental concept in cardiac research. Moreover, the severity of heart disease and the increasing numbers of heart failure patients have prompted attempts to implement the stem cell therapies in clinical practice. While the number of enrolled patients in clinical studies is growing, our understanding of the potential role of transplanted cells in cardiac repair is limited. The main questions to be answered are: Which heart disease and when should it be treated? Which cell type or combination of cell types will be the most beneficial, safe, and yield long-lasting improvement of heart function? How should these cells be delivered? What are the mechanisms by which transplanted cells affect heart function, and how can this knowledge be used to promote the development of new methods for tissue repair and regeneration?

Despite a number of unoptimized variables, initial clinical attempts using autologous skeletal myoblasts [5] and bone marrow-derived stem cells [6] have demonstrated slight but significant improvements in heart function, most likely due to the paracrine action of implanted cells and increased vascularization of the infarct area. However, the latest results of double-blind randomized placebo-controlled trials have been less encouraging [7]–[10]. Some of the main hurdles have been recognized, including low retention and survival of implanted cells, as well as limited cardiogenic differentiation and functional integration of delivered cells within the host heart tissue. In particular, suboptimal methods for cell delivery into the heart have been shown to result in uncontrolled cell loss (even >90%) from the injection site and large variability of the resulting graft size [2]. The mixing of bioactive hydrogels with injected cells and subsequent cell/hydrogel polymerization *in situ* can be used to minimize cell loss [11], [12]. However, even if a high number of cells are efficiently retained at the injection site, their survival within the harsh infarct environment is not guaranteed and higher cell densities may lead to even higher cell mortality. Currently ~90% of successfully delivered cells are shown to die within the first week after injection [2]. Heat shock treatment prior to cell implantation [13] as well as exogenous delivery of anti-apoptotic and angiogenic factors [14]–[16] or their overexpression in implanted cells [17], [18] are being explored in an attempt to overcome this problem.

Nonetheless, the source of optimal donor cells for heart repair remains the most important and most studied area in this rapidly growing field. Interestingly, a large number of donor cell types in different animal studies have been shown to exert some functional benefit upon implantation in the heart (at least over a relatively short period after implantation). Ideally, the implanted cells should be nontumorigenic and nonimmunogenic, proliferate rapidly and abundantly *in vitro*, and structurally and functionally integrate in sufficient numbers with host

cardiomyocytes. Considering that the most desirable therapeutic outcome is a significant and long-term increase of heart contractile function, current emphasis is placed on identifying cells that can be manipulated to efficiently acquire a muscle phenotype prior to or shortly after implantation [1], [2]. The resulting functional improvement in this case would be mainly derived from remuscularization rather than revascularization of the infarcted area in the heart. Candidate cells include genetically modified autologous skeletal myoblasts [17], embryonic stem cell-derived cardiomyocytes (either allogeneic [19] or generated by somatic cell nuclear transfer [20]), and autologous resident cardiac progenitor cells [21]. Specifically, recently identified resident cardiac progenitors that can give rise to cardiomyocytes and endothelial and smooth muscle cells [22], [23] hold promise as an ideal stem cell type able to independently generate new cardiac muscle tissue together with its vascular support. Despite constant progress in this field, a lot of work still remains to be done in order to identify the cell source or mixture of cell sources [24] that would be most effective in replacing the dead or poorly contracting myocytes in the diseased heart. Furthermore, this process is further complicated by the fact that different types or stages of heart disease may require different cell types for optimal functional improvement.

In general, cell-based therapies for heart disease represent a field with extremely high expectations and potential impact. Naturally, this field is moving rapidly with a growing number of mostly trial-and-error animal experiments that usually involve the implantation of a specific cell type into the heart and a relatively short-term follow-up study. The bulk of this research is performed by clinical scientists. Simultaneously, basic studies by cell and developmental biologists, geneticists, and immunologists are focused on understanding the subcellular and molecular processes that govern the differentiation, proliferation, lineage specification, and immunogenicity of different stem cell types. These endeavors are crucial for the development of safe and efficient cell-based therapies for a number of diseases including heart disease. Unfortunately, well-defined basic science studies that would predict or explain a particular functional outcome of cell implantation in the heart are significantly less prevalent. Specifically, a number of factors that act at cellular and tissue levels to direct or modulate the fate of stem cells before and after implantation are largely unexplored. These factors include stem cell shape, structural and functional interactions of stem cells with surrounding extracellular matrix, other stem cells and host cardiac cells, as well as the presence of physical forces and flows in the heart (e.g., passive stretch and active contraction of heart cells, interstitial flow, and electric field). It is precisely within these research areas that significant contributions by bioengineers and biomaterial scientists are needed in order to further understand the processes and promote the technologies pertinent to future stem cell therapies for heart disease.

In particular, systematic studies of cell–cell and cell–surface interactions have been significantly advanced by a number of enabling technologies at the nanoscale and microscale. High throughput assays to explore the effects of different extracellular matrix proteins, synthetic biomaterials, and their combinations upon the stem cell proliferation and differentiation in two dimensions have recently become available [25], [26]. Similar methods have been also developed to culture multiple stem cell clusters of different size [27]. Furthermore, a variety of surface patterning and microfluidic techniques have been extensively used to reproducibly manipulate cellular microenvironment including the geometry of single cells [28], cell pairs [29], and larger groups of cells in two dimensions [30] and three dimensions [31], [32], as well as to create controlled co-cultures of stem cells and other cell types [33]. Patterned co-cultures of cardiac and stem cells, when combined with the optical measurements of electrical activity [34], could be used as well-defined in vitro assays for predicting the factors needed for efficient and safe functional integration of transplanted cells into the heart. Similar in vitro systems can allow controlled studies of cellular behavior in response to various external stimuli (e.g., drugs [35], gene vectors [36], stretch [37], or programmed electrical stimulation

[38]) with an advantage of a relatively straightforward interpretation of obtained measurements. One example would be studying the effect of chronic electrical, mechanical, or electromechanical stimulation on the cardiogenic differentiation of stem cells embedded within a three-dimensional cardiac cell culture.

Constant advancements in the areas of biomaterials and controlled drug and gene delivery offer further opportunities for improving existing cellular therapies for heart disease. The ability to independently tailor the physical, chemical, and degradation properties of biomaterials and control the temporal (and even spatial) release profiles of single or multiple genes and proteins is of paramount interest to the entire field of regenerative medicine. New generations of smart biomaterials [39], [40] that respond to local environmental conditions including protease activity, temperature, pH, or mechanical forces offer further control over encapsulated cell delivery in the heart and other organs [41]. Recent research emphasis is focused in the area of nanobiomaterials as they have been shown to distinctly regulate processes of stem cell differentiation, proliferation, and migration [42], [43]. For example, the use of injectable self-assembling nanofibers to simultaneously deliver cells and locally release insulin growth factor-1 in a sustained manner has been recently shown to improve cell therapy for myocardial infarction in rats [11]. Furthermore, the design of materials with precisely tunable viscoelastic properties offers a potentially independent route to direct stem cell lineage specification [44] and/or to control the compliance of the infarcted area in the heart [45]. These methods of “injectable tissue engineering” for heart repair are expected to reach the preclinical arena in the near future, primarily due to their potential for relatively noninvasive, percutaneous delivery of combined cells and biomaterials into the heart.

On the other hand, significant tissue engineering efforts are aimed toward the use of cells, bioengineered scaffolds, bioreactors, and different chemical and physical stimuli to engineer functional cardiac patches in vitro. The localized epicardial implantation of a large number of cells in the form of a bioengineered cardiac patch [46]–[49], although surgically more complex, is expected to provide additional benefits compared to cell injection, including: 1) better cell retention, survival, and localization at the injury site; 2) pre-engineered structural tissue repair; and 3) concentrated angiogenic and anti-apoptotic paracrine action of implanted cells. In fact, recent preliminary studies in rats and hamsters have shown that the implantation of rat H9c2 cardiomyoblasts seeded on collagen matrices or the use of scaffold-free skeletal myoblast tissue sheets [50], [51] outperformed the injection of a cell suspension for the treatment of heart damage. This benefit was mostly attributed to enhanced survival and paracrine action of donor cells when implanted as a tissue patch rather than as a cell injection [50]. Despite the ongoing progress, the field is faced with challenges to construct relatively thick (>1 mm) avascular cardiac tissue patches that would overcome diffusional limits in the delivery of oxygen and nutrients. The implantation of a thin (submillimeter) patch that would initially survive on diffusion and subsequently support controlled cell proliferation and vascular ingrowth is one alternative solution. Moreover, methods for controlling three-dimensional cell alignment over a relatively large patch area (>1 cm²) in order to mimic native tissue architecture and function are still lacking, even for tissue patches as thin as 50 μ m.

Importantly, the stem cell therapies for heart disease will heavily benefit from developments in noninvasive imaging techniques such as magnetic resonance imaging (MRI), positron emission tomography (PET), single-photon emission computed tomography (SPECT), and bioluminescent imaging [52]–[54]. These techniques are expected to allow short- and long-term tracking of stem cell survival, migration, and differentiation in the heart of the same subject. Ongoing research includes the development of gene reporter-based cell visualization for use in preclinical studies, as well as direct labeling of cells with magnetic nanoparticles or radioisotopes for both preclinical and clinical imaging [55]. Due to the specific limitations of

each methodology, a multimodality approach using combined PET or SPECT and MRI agents may ultimately prove most useful in the clinical setting.

Finally, the use of electrical [56] and mechanical [57], [58] computer models of the whole heart to predict or explain the functional outcomes of stem cell implantation in the heart represents yet another significant area of contribution by bioengineers and computational scientists. Recently, a finite element mechanical model of an infarcted ovine left ventricle was used to examine the short-term effect of injecting noncontractile material into the ventricular wall [59]. This important study has questioned the validity of using ejection fraction as a measure of improved heart function in both clinical and animal studies regarding that implantation of cells and/or biomaterials alters ventricular geometry. Similarly, electrophysiological models of different types of stem cells, developed from whole cell ion current recordings [60]–[62], can be incorporated into the available computer models of whole ventricles with realistic myofiber directions [56]. These large-scale multicellular models could be utilized to investigate the vulnerability of the heart to arrhythmias as a function of the type, number, and spatial distribution of implanted stem cells, as well as their ability to electrically couple with cardiac cells [63]. Along this path, electrotonic interactions between human ventricular myocytes and fibroblasts have already been explored in recent computational studies [64].

Currently, it is clear that the development of safe and efficient stem cell therapies for heart disease will be a long-term process that will perpetually build on synergistic advancements in a number of disciplines including basic sciences, engineering, and clinical research. With regard to the recent decline in federal funding, available resources should be carefully distributed to enable the most efficient and scientifically based progress of the field without the potential of compromising patient safety. Only this strategy will ensure the advancement of cell-based cardiac therapies to a level that will provide the most meaningful benefits to millions of people throughout the world.

Acknowledgments

This work is in part supported by National Institutes of Health Grant HL083342 and American Heart Association Grant 0530256N.

References

1. Anversa P, Kajstura J, Leri A, Bolli R. Life and death of cardiac stem cells: A paradigm shift in cardiac biology. *Circulation* 2006;113(no 11):1451–1463. [PubMed: 16549650]
2. Laflamme MA, Murry CE. Regenerating the heart. *Nat Biotechnol* 2005;23(no 7):845–856. [PubMed: 16003373]
3. Heart disease and stroke statistics-2006: A report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. *Circulation* 2005;113:85–151.
4. Murry CE, Field LJ, Menasche P. Cell-based cardiac repair: Reflections at the 10-year point. *Circulation* 2005;112(no 20):3174–3183. [PubMed: 16286608]
5. Menasche P. Myoblast-based cell transplantation. *Heart Fail Rev* 2003;8(no 3):221–227. [PubMed: 12878830]
6. Assmus B, Schachinger V, Teupe C, Britten M, Lehmann R, Dobert N, Grunwald F, Aicher A, Urbich C, Martin H, Hoelzer D, Dimmeler S, Zeiher AM. Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction (TOPCARE-AMI). *Circulation* 2002;106(no 24):3009–3017. [PubMed: 12473544]
7. Assmus B, Honold J, Schachinger V, Britten MB, Fischer-Rasokat U, Lehmann R, Teupe C, Pistorius K, Martin H, Abolmaali ND, Tonn T, Dimmeler S, Zeiher AM. Transcatheter transplantation of progenitor cells after myocardial infarction. *N Engl J Med* 2006;355(no 12):1222–1232. [PubMed: 16990385]

8. Lunde K, Solheim S, Aakhus S, Arnesen H, Abdelnoor M, Egeland T, Endresen K, Ilebekk A, Mangschau A, Fjeld JG, Smith HJ, Taraldsrud E, Grogaard HK, Bjornerheim R, Brekke M, Muller C, Hopp E, Ragnarsson A, Brinchmann JE, Forfang K. Intracoronary injection of mononuclear bone marrow cells in acute myocardial infarction. *N Engl J Med* 2006;355(no 12):1199–1209. [PubMed: 16990383]
9. Rosenzweig A. Cardiac cell therapy-mixed results from mixed cells. *N Engl J Med* 2006;355(no 12):1274–1277. [PubMed: 16990391]
10. Welt FG, Losordo DW. Cell therapy for acute myocardial infarction: Curb your enthusiasm? *Circulation* 2006;113(no 10):1272–1274. [PubMed: 16534025]
11. Davism ME, Hsieh PC, Takahashi T, Song Q, Zhang S, Kamm RD, Grodzinsky AJ, Anversa P, Lee RT. Local myocardial insulin-like growth factor 1 (IGF-1) delivery with biotinylated peptide nanofibers improves cell therapy for myocardial infarction. *Proc Natl Acad Sci USA* 2006;103(no 21):8155–8160. [PubMed: 16698918]
12. Huang NF, Yu J, Sievers R, Li S, Lee RJ. Injectable biopolymers enhance angiogenesis after myocardial infarction. *Tissue Eng* 2005;11(no 11–12):1860–1866. [PubMed: 16411832]
13. Zhang M, Methot D, Poppa V, Fujio Y, Walsh K, Murry CE. Cardiomyocyte grafting for cardiac repair: Graft cell death and anti-death strategies. *J Mol Cell Cardiol* 2001;33(no 5):907–921. [PubMed: 11343414]
14. Mirotsoy M, Zhang Z, Deb A, Zhang L, Gnicchi M, Noiseux N, Mu H, Pachori A, Dzau V. Secreted frizzled related protein 2 (Sfrp2) is the key Akt-mesenchymal stem cell-released paracrine factor mediating myocardial survival and repair. *Proc Natl Acad Sci USA* 2007;104(no 5):1643–1648. [PubMed: 17251350]
15. Kofidis T, de Bruin JL, Yamane T, Tanaka M, Lebl DR, Swijnenburg RJ, Weissman IL, Robbins RC. Stimulation of paracrine pathways with growth factors enhances embryonic stem cell engraftment and host-specific differentiation in the heart after ischemic myocardial injury. *Circulation* 2005;111(no 19):2486–2493. [PubMed: 15883216]
16. Cho SW, Gwak SJ, Kim UK, Cho MC, Hwang KK, Kwon JS, Choi CY, Yoo KJ, Kim BS. Granulocyte colony-stimulating factor treatment enhances the efficacy of cellular cardiomyoplasty with transplantation of embryonic stem cell-derived cardiomyocytes in infarcted myocardium. *Biochem Biophys Res Commun* 2006;340(no 2):573–582. [PubMed: 16376856]
17. Suzuki K, Brand NJ, Allen S, Khan MA, Farrell AO, Murtuza B, Oakley RE, Yacoub MH. Overexpression of connexin 43 in skeletal myoblasts: Relevance to cell transplantation to the heart. *J Thorac Cardiovasc Surg* 2001;122(no 4):759–766. [PubMed: 11581610]
18. Mangi AA. Mesenchymal stem cells modified with Akt prevent remodeling and restore performance of infarcted hearts. *Nat Med* 2003;9(no 9):1195–1201. [PubMed: 12910262]
19. Kolossov E, Bostani T, Roell W, Breitach M, Pillekamp F, Nygren JM, Sasse P, Rubenchik O, Fries JW, Wenzel D, Geisen C, Xia Y, Lu Z, Duan Y, Kettenhofen R, Jovinge S, Bloch W, Bohlen H, Welz A, Hescheler J, Jacobsen SE, Fleischmann BK. Engraftment of engineered ES cell-derived cardiomyocytes but not BM cells restores contractile function to the infarcted myocardium. *J Exp Med* 2006;203(no 10):2315–2327. [PubMed: 16954371]
20. Lanza R, Moore MA, Wakayama T, Perry AC, Shieh JH, Hendriks J, Leri A, Chimenti S, Monsen A, Nurzynska D, West MD, Kajstura J, Anversa P. Regeneration of the infarcted heart with stem cells derived by nuclear transplantation. *Circ Res* 2004;94(no 6):820–827. [PubMed: 14764454]
21. Torella D, Ellison GM, Mendez-Ferrer S, Ibanez B, Nadal-Ginard B. Resident human cardiac stem cells: Role in cardiac cellular homeostasis and potential for myocardial regeneration. *Nat Clin Pract Cardiovasc Med* 2006;3:S8–S13. [PubMed: 16501638]
22. Beltrami AP, Barlucchi L, Torella D, Baker M, Limana F, Chimenti S, Kasahara H, Rota M, Musso E, Urbanek K, Leri A, Kajstura J, Nadal-Ginard B, Anversa P. Adult cardiac stem cells are multipotent and support myocardial regeneration. *Cell* 2003;114(no 6):763–776. [PubMed: 14505575]
23. Garry DJ, Olson EN. A common progenitor at the heart of development. *Cell* 2006;127(no 6):1101–1104. [PubMed: 17174889]
24. Carvalho KA, Guarita-Souza LC, Rebelatto CL, Senegaglia AC, Hansen P, Mendonca JG, Cury CC, Francisco JC, Brofman PR. Could the coculture of skeletal myoblasts and mesenchymal stem cells

- be a solution for postinfarction myocardial scar? *Transplant Proc* 2004;36(no 4):991–992. [PubMed: 15194344]
25. Anderson DG, Levenberg S, Langer R. Nanoliter-scale synthesis of arrayed biomaterials and application to human embryonic stem cells. *Nat Biotechnol* 2004;22(no 7):863–866. [PubMed: 15195101]
 26. Flaim CJ, Chien S, Bhatia SN. An extracellular matrix microarray for probing cellular differentiation. *Nat Methods* 2005;2(no 2):119–125. [PubMed: 15782209]
 27. Khademhosseini A, Langer R, Borenstein J, Vacanti JP. Microscale technologies for tissue engineering and biology. *Proc Natl Acad Sci USA* 2006;103(no 8):2480–2487. [PubMed: 16477028]
 28. McBeath R, Pirone DM, Nelson CM, Bhadriraju K, Chen CS. Cell shape, cytoskeletal tension, and RhoA regulate stem cell lineage commitment. *Dev Cell* 2004;6(no 4):483–495. [PubMed: 15068789]
 29. Nelson CM, Chen CS. Cell-cell signaling by direct contact increases cell proliferation via a PI3K-dependent signal. *FEBS Lett* 2002;514(no 2–3):238–242. [PubMed: 11943158]
 30. Nelson CM, Jean RP, Tan JL, Liu WF, Sniadecki NJ, Spector AA, Chen CS. Emergent patterns of growth controlled by multicellular form and mechanics. *Proc Natl Acad Sci USA* 2005;102(no 33):11594–11599. [PubMed: 16049098]
 31. Nelson CM, Vanduijn MM, Inman JL, Fletcher DA, Bissell MJ. Tissue geometry determines sites of mammary branching morphogenesis in organotypic cultures. *Science* 2006;314(no 5797):298–300. [PubMed: 17038622]
 32. Tsang VL, Bhatia SN. Fabrication of three-dimensional tissues. *Adv Biochem Eng Biotechnol* 2007;103:189–205. [PubMed: 17195464]
 33. Khademhosseini A, Ferreira L, Bluming J III, Yeh J, Karp JM, Fukada J, Langer R. Co-culture of human embryonic stem cells with murine embryonic fibroblasts on microwell-patterned substrates. *Biomaterials* 2006;27(no 36):5968–5977. [PubMed: 16901537]
 34. Bursac N, Aguel F, Tung L. Multiarm spirals in a two-dimensional cardiac substrate. in *Proc Natl Acad Sci USA* 2004;101(no 43):15530–15534.
 35. Rohr S, Kucera J, Fast V, Kleber A. Paradoxical improvement of impulse conduction in cardiac tissue by partial cellular uncoupling. *Science* 1997;275(no 5301):841–844. [PubMed: 9012353]
 36. Abraham MR, Henrikson CA, Tung L, Chang MG, Aon M, Xue T, Li RA, O'Rourke B, Marban E. Antiarrhythmic engineering of skeletal myoblasts for cardiac transplantation. *Circ Res* 2005;97(no 2):159–167. [PubMed: 15976318]
 37. Yamada K, Green KG, Samarel AM, Saffitz JE. Distinct pathways regulate expression of cardiac electrical and mechanical junction proteins in response to stretch. *Circ Res* 2005;97(no 4):346–353. [PubMed: 16037569]
 38. Bursac N, Tung L. Acceleration of functional reentry by rapid pacing in anisotropic cardiac monolayers: Formation of multi-wave functional reentries. *Cardiovasc Res* 2006;69(no 2):381–390. [PubMed: 16274682]
 39. Anderson DG, Burdick JA, Langer R. Materials science. Smart biomaterials. *Science* 2004;305(no 5692):1923–1924. [PubMed: 15448260]
 40. Lutolf MP, Hubbell JA. Synthetic biomaterials as instructive extracellular microenvironments for morphogenesis in tissue engineering. *Nat Biotechnol* 2005;23(no 1):47–55. [PubMed: 15637621]
 41. Davis ME, Hsieh PC, Grozinsky AJ, Lee RT. Custom design of the cardiac microenvironment with biomaterials. *Circ Res* 2005;97(no 1):8–15. [PubMed: 16002755]
 42. Nur EKA, Ahmed I, Kamal J, Schindler M, Meiners S. Three-dimensional nanofibrillar surfaces promote self-renewal in mouse embryonic stem cells. *Stem Cells* 2006;24(no 2):426–433. [PubMed: 16150921]
 43. Shih YR, Chen CN, Tsai SW, Wang YJ, Lee OK. Growth of mesenchymal stem cells on electrospun type I collagen nanofibers. *Stem Cells* 2006;24(no 11):2391–2397. [PubMed: 17071856]
 44. Engler AJ, Sen S, Sweeney HL, Discher DE. Matrix elasticity directs stem cell lineage specification. *Cell* 2006;126(no 4):677–689. [PubMed: 16923388]
 45. Berry MF, Engler AJ, Woo YJ, Pirolli TJ, Bush LT, Jayasankar V, Morine KJ, Gardner TJ, Discher DE, Sweeney HL. Mesenchymal stem cell injection after myocardial infarction improves myocardial compliance. *Am J Physiol Heart Circ Physiol* 2006;290(no 6):H2196–2203. [PubMed: 16473959]

46. Zimmermann WH, Melnychenko I, Wasmeier G, Didie M, Naito H, Nixdorff U, Hess A, Budinsky L, Brune K, Michaelis B, Dhein S, Schwoerer A, Ehmke H, Eschenhagen T. Engineered heart tissue grafts improve systolic and diastolic function in infarcted rat hearts. *Nat Med* 2006;12(no 4):452–458. [PubMed: 16582915]
47. Miyahara Y, Nagaya N, Kataoka M, Yanagawa B, Tanaka K, Hao H, Ishino K, Ishida H, Shimizu T, Kangawa K, Sano S, Okano T, Kitamura S, Mori H. Monolayered mesenchymal stem cells repair scarred myocardium after myocardial infarction. *Nat Med* 2006;12(no 4):459–465. [PubMed: 16582917]
48. Furuta A, Miyoshi S, Itabishi Y, Shimizu T, Kira S, Hayakawa K, Nishiyama N, Tanimoto K, Hagiwara Y, Satoh T, Fukuda K, Okana T, Ogawa S. Pulsatile cardiac tissue grafts using a novel three-dimensional cell sheet manipulation technique functionally integrates with the host heart, in vivo. *Circ Res* 2006;98(no 5):705–712. [PubMed: 16469955]
49. Kutschka I, Chen IY, Kofidis T, Arai T, von Degenfeld G, Sheikh AY, Hendry SL, Pearl J, Hoyt G, Sista R, Yang PC, Blau HM, Gambhir SS, Robbins RC. Collagen matrices enhance survival of transplanted cardiomyoblasts and contribute to functional improvement of ischemic rat hearts. *Circulation* 2006;114(no 1):1167–173. [PubMed: 16820568]
50. Kondoh H, Sawa Y, Miyagawa S, Sakakida-Kitagawa S, Memon IA, Kawaguchi N, Matsuura N, Shimizu T, Okano T, Matsuda H. Longer preservation of cardiac performance by sheet-shaped myoblast implantation in dilated cardiomyopathic hamsters. *Cardiovasc Res* 2006;69(no 2):466–475. [PubMed: 16423569]
51. Memon IA, Sawa Y, Fukushima N, Matsumiya G, Miyagawa S, Taketani S, Sakakida SK, Kondoh H, Aleshin AN, Shimizu T, Okano T, Matsuda H. Repair of impaired myocardium by means of implantation of engineered autologous myoblast sheets. *J Thorac Cardiovasc Surg* 2005;130(no 5):1333–1341. [PubMed: 16256786]
52. Kraitchman DL, Heldman AW, Atalar E, Amado LC, Martin BJ, Pittenger MF, Hare JM, Bulte J. In vivo magnetic resonance imaging of mesenchymal stem cells in myocardial infarction. *Circulation* 2003;107(no 18):2290–2293. [PubMed: 12732608]
53. Cao F, Lin S, Xie X, Ray P, Patel M, Zhang X, Drukker M, Dylla SJ, Connolly AJ, Chen X, Weissman IL, Ghambhir SS, Wu JC. In vivo visualization of embryonic stem cell survival, proliferation, and migration after cardiac delivery. *Circulation* 2006;113(no 7):1005–1014. [PubMed: 16476845]
54. Shen D, Liu D, Cao Z, Acton PD, Zhou R. Coregistration of magnetic resonance and single photon emission computed tomography images for noninvasive localization of stem cells grafted in the infarcted rat myocardium. *Mol Imaging Biol* 2007;9(no 1):24–31. [PubMed: 17053860]
55. Zhou R, Acton PD, Ferrari VA. Imaging stem cells implanted in infarcted myocardium. *J Am Coll Cardiol* 2006;48(no 10):2094–2106. [PubMed: 17112999]
56. Henriquez, CS.; Tranquillo, JV.; Weinstein, D.; Hsu, EW.; Johnson, CJ. Three-dimensional propagation in mathematical models: Integrative model of the mouse heart. In: Zipes, DP.; Jalife, J., editors. *Cardiac Electrophysiology from Cell to Bedside*. Vol. 4th. Philadelphia, PA: Saunders; 2004.
57. Nickerson D, Smith N, Hunter P. New developments in a strongly coupled cardiac electromechanical model. *Europace* 2005;7:118–127. [PubMed: 16102509]
58. Usyk TP, McCulloch AD. Relationship between regional shortening and asynchronous electrical activation in a three-dimensional model of ventricular electromechanics. *J Cardiovasc Electrophysiol* 2003;14:S196–202. [PubMed: 14760924]
59. Wall ST, Walker JC, Healy KE, Ratcliffe MB, Guccione JM. Theoretical impact of the injection of material into the myocardium: A finite element model simulation. *Circulation* 2006;114(no 24):2627–2635. [PubMed: 17130342]
60. Sartiani L, Bettiol E, Stillitano F, Mugelli A, Cerbai E, Jaconi M. Developmental changes in cardiomyocytes differentiated from human embryonic stem cells: A molecular and electrophysiological approach. *Stem Cells* 2007;25:1136–1144. [PubMed: 17255522]
61. Li GR, Sun H, Deng X, Lau CP. Characterization of ionic currents in human mesenchymal stem cells from bone marrow. *Stem Cells* 2005;23(no 3):371–382. [PubMed: 15749932]
62. Heubach JF, Graf EM, Leutheuser J, Bock M, Balana B, Zahanich I, Christ T, Boxberger S, Wettwer E, Ravens U. Electrophysiological properties of human mesenchymal stem cells. *J Physiol* 2004;554(pt 3):659–672. [PubMed: 14578475]

63. Valiunas V, Doronin S, Valiuniene L, Potapova I, Zuckerman J, Walcott B, Robinson RB, Rosen MR, Brink PR, Cohen IS. Human mesenchymal stem cells make cardiac connexins and form functional gap junctions. *J Physiol* 2004;555(pt 3):617–626. [PubMed: 14766937]
64. Maccannell KA, Bazzazi H, Chilton L, Shibukawa Y, Clark RB, Giles WR. A mathematical model of electrotonic interactions between ventricular myocytes and fibroblasts. *Biophys J* 2007;92:4121–4132. [PubMed: 17307821]

Nenad Bursac