Published in final edited form as:

J Electrocardiol. 2007 ; 40(6 Suppl): S199–S201. doi:10.1016/j.jelectrocard.2007.05.025.

Replacing Damaged Myocardium

Irina A. Potapova, Sergey V. Doronin, Damon J. Kelly, Amy B. Rosen, Adam J.T. Schuldt, Zhongju Lu, Yuanjian Guo, Paul V. Kochupura, Richard B. Robinson, Michael R. Rosen, Peter R. Brink, Glenn R. Gaudette, and Ira S. Cohen

Introduction

Although there has been enormous investment in the research and development of pharmacologic treatments for heart failure, the survival statistics have barely changed for over half a century¹. The cold reality is that the only successful treatment for end-stage heart failure is cardiac transplantation and the need for hearts each year far outstrips the availability.

Traditional dogma suggests that the heart is a post-mitotic organ. However, recent evidence from a number of laboratories has challenged this position. Myocytes derived from recipients have been found in the donor hearts of cardiac transplant patients.² Also, a low rate of apoptosis has been demonstrated in the healthy heart indicating a limited ability for the heart to replace lost contractile elements if cardiac mass is to be maintained.³

These basic findings that have led to a number of clinical trials investigating various cell types as candidates to induce cardiac regeneration. On the positive front, whether it be skeletal myoblasts or bone marrow derived stem cells, there has been a measurable improvement in mechanical function following cell delivery.⁴ Disappointingly, this improvement is independent of cell type. Further, when the time period of the clinical trial is extended improvement is more evident in the short term (4–6 months)^{5,6} than for longer time periods (18 months).⁷

The lack of cell source specificity raised a serious question. What was the cells' mechanism of action? Improved wall movement or ejection fraction could be a result of increased active contractile function or alternatively, a change in the passive properties of the heart (increased compliance). Indeed a recent simulation published in *Circulation*⁸ suggested that injection of a passive material would have an effect on ejection fraction equal to that reported in any of the published clinical studies. Given the propensity of some of the cell replacement substrates to induce arrhythmias in both animals⁹ and humans¹⁰, it is natural to ask whether cellular therapy is worth the risk.

Properties of an Ideal Cellular Substrate

First and foremost, the heart serves as a rhythmic pump. In order for replacement “myocytes” to subserve this function they must be aligned appropriately with the native myocardium and be capable of synchronous longitudinal shortening. This means that they must form gap junctions with the native myocardium and have mature sarcomeres. The trigger for this mechanical event is an electrical event, the “action potential”. The electrical properties of the

native myocytes are region specific with the nodal regions and the ventricular conducting system having pacemaker function, while working atrial and ventricular muscle do not. In order for cellular transplantation to be successful, the delivered myocytes must take on the electrical phenotype of the host region. That is, delivery of cells to the left ventricular epicardium must result in myocytes whose electrical phenotype is identical to that of their neighbors. Even if mechanical function is enhanced, electrical discordance can result in life threatening arrhythmias.

Current Cellular Substrates

Embryonic stem cells, mesenchymal stem cells, and cardiac stem cells have all been shown to be capable of differentiating into cardiac phenotypes *in vitro*.^{3,11,12} Unfortunately, the usual criterion for cardiac differentiation is spontaneous contractions indicative of pacemaker function, an undesirable characteristic in working ventricular muscle. With the exception of embryonic stem cells, none of the other cell types convincingly develop sarcomeres capable of longitudinal shortening. In each case, when examined more closely, the resultant cardiomyocytes have heterogeneous electrical properties¹³. To be successfully employed for cell therapy, approaches must be developed that permit selection of myocytes according to electrical phenotype (and in the case of embryonic stem cells must also eliminate cells that remain undifferentiated). Since fully differentiated cardiac myocytes divide only rarely, successful cell therapy must also deal with delivery of as many as one billion myocytes.⁴

An Alternative Approach - Partially Differentiated Cells

If complete differentiation of stem cells *in vitro* results in a troublesome heterogeneity of phenotype, possibly partial differentiation may be the answer. Our laboratory has already demonstrated that a gradient in angiotensin II (A2) exists within the canine left ventricular wall. It is this gradient in A2 that determines electrical phenotype between endocardium (high A2) and epicardium (low A2).^{14,15} We decided to test the utility of partial differentiation with human mesenchymal stem cells (hMSCs) as our cellular substrate. Embryonic stem cells can be differentiated to a cardiac lineage *in vitro* by the formation of 3-D aggregates called embryoid bodies (EBs).¹¹ We wondered whether a similar approach might succeed with hMSCs. We formed hanging droplets of up to 250,000 hMSCs. Three days after hanging, the aggregates were plated in a culture dish and cells were observed to spread from the plated EBs. We examined the spreading cells for signs of cardiac differentiation and demonstrated that they expressed a number of cardiac proteins including sarcomeric alpha actinin, troponin T, and the alpha subunit of the L-type calcium channel Cav1.2. Although the cells were not spontaneously active, a fraction (5/31) expressed a large L-type calcium current similar in size to that found in human ventricular myocytes. Control hMSCs did not express the cardiac markers, nor did they express an L-type calcium current. In some cells, the pattern of sarcomeric alpha actinin was consistent with sarcomere spacing. However up to 10 days after plating, no cells exhibited clear striations *in vitro*. Thus, while these cells express cardiac specific proteins, they have not fully differentiated to the point of forming well organized sarcomeres. Since these cells were not fully differentiated, we wondered whether they might be capable of cell division. Given the large number of terminally differentiated cells that must be delivered to treat heart failure patients, cells that maintain the ability to proliferate have a distinct advantage in reducing the number of cells that must be delivered and the attendant delivery problems. Many of the spreading cells expressing the cardiac protein sarcomeric alpha actinin also expressed cyclin D-1 in the nucleus (a marker of entry into the cell cycle). We have previously used a full thickness defect model in the canine right ventricle to demonstrate the ability of an extracellular matrix to induce myocyte proliferation and restore mechanical function after 8 weeks *in vivo*.¹⁶ We employed the same model and used our high resolution method for determining regional deformation^{17,18} to examine whether our partially differentiated cardiogenic cells might have

more regenerative capacity than native hMSCs. Indeed, the cardiogenic cells increased regional mechanical function¹⁹. To track the delivered cells, we loaded them with highly fluorescent quantum dot nanoparticles. After residing 8 weeks *in vivo* some of these cardiogenic cells had become striated myocytes with mature sarcomere spacing.²⁰

Conclusions

Cell therapy to replace damaged myocardium is in its infancy. Current approaches to treat myocardial infarction have yielded only short term gains. Here we propose the use of hMSCs partially differentiated towards a cardiac lineage as a potential repair substrate. Differentiation is induced by a novel method employing hanging droplets adapted from work on embryonic stem cells. The approach results in a partially differentiated cardiogenic cell positive for a number of cardiac specific proteins and expressing an L-type calcium current. In addition, these cells express cell cycle markers, suggesting they maintain the ability to proliferate. This may greatly reduce the number of cells that need to be delivered for the treatment of heart failure. When delivered on a matrix, these cells can improve regional mechanical function and appear to become fully differentiated with well formed sarcomeres. Much remains to be done. For these cells to be an ideal substrate they must also take on the electrical phenotype of the region to which they are delivered. Experiments investigating this question are presently underway.

References

1. Levy D, Kenchaiah S, Larson MG, Benjamin EJ, Kupka MJ, Ho KK, Murabito JM, Vasan RS. Long-term trends in the incidence of and survival with heart failure. *N Engl J Med* 2002;347:1397–402. [PubMed: 12409541]
2. Muller P, Pfeiffer P, Koglin J, Schafers HJ, Seeland U, Janzen I, Urbchat S, Bohm M. Cardiomyocytes of noncardiac origin in myocardial biopsies of human transplanted hearts. *Circulation* 2002;106:31–5. [PubMed: 12093766]
3. 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;114:763–76. [PubMed: 14505575]
4. Murry CE, Reinecke H, Pabon LM. Regeneration gaps: observations on stem cells and cardiac repair. *J Am Coll Cardiol* 2006;47:1777–85. [PubMed: 16682301]
5. Wollert KC, Meyer GP, Lotz J, Ringes-Lichtenberg S, Lippolt P, Breidenbach C, Fichtner S, Korte T, Hornig B, Messinger D, Arseniev L, Hertenstein B, Ganser A, Drexler H. Intracoronary autologous bone-marrow cell transfer after myocardial infarction: the BOOST randomised controlled clinical trial. *Lancet* 2004;364:141–8. [PubMed: 15246726]
6. Schachinger V, Erbs S, Elsasser A, Haberbosch W, Hambrecht R, Holschermann H, Yu J, Corti R, Mathey DG, Hamm CW, Suselbeck T, Assmus B, Tonn T, Dimmeler S, Zeiher AM. Intracoronary bone marrow-derived progenitor cells in acute myocardial infarction. *N Engl J Med* 2006;355:1210–21. [PubMed: 16990384]
7. Meyer GP, Wollert KC, Lotz J, Steffens J, Lippolt P, Fichtner S, Hecker H, Schaefer A, Arseniev L, Hertenstein B, Ganser A, Drexler H. 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;113:1287–94. [PubMed: 16520413]
8. 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;114:2627–35. [PubMed: 17130342]
9. Mills WR, Mal N, Kiedrowski MJ, Unger R, Forudi F, Popovic ZB, Penn MS, Laurita KR. Stem cell therapy enhances electrical viability in myocardial infarction. *J Mol Cell Cardiol* 2007;42:304–14. [PubMed: 17070540]
10. Hagege AA, Marolleau JP, Vilquin JT, Alheritiere A, Peyrard S, Duboc D, Abergel E, Messas E, Mousseaux E, Schwartz K, Desnos M, Menasche P. Skeletal myoblast transplantation in ischemic

- heart failure: long-term follow-up of the first phase I cohort of patients. *Circulation* 2006;114:1108–13. [PubMed: 16820558]
11. Kehat I, Kenyagin-Karsenti D, Snir M, Segev H, Amit M, Gepstein A, Livne E, Binah O, Itskovitz-Eldor J, Gepstein L. Human embryonic stem cells can differentiate into myocytes with structural and functional properties of cardiomyocytes. *J Clin Invest* 2001;108:407–14. [PubMed: 11489934]
 12. Makino S, Fukuda K, Miyoshi S, Konishi F, Kodama H, Pan J, Sano M, Takahashi T, Hori S, Abe H, Hata J, Umezawa A, Ogawa S. Cardiomyocytes can be generated from marrow stromal cells in vitro. *J Clin Invest* 1999;103:697–705. [PubMed: 10074487]
 13. Cohen IS, Rosen AB, Gaudette GR. A Caveat Emptor for myocardial regeneration: mechanical without electrical recovery will not suffice. *J Mol Cell Cardiol* 2007;42:285–8. [PubMed: 17196613]
 14. Gao J, Kim J, Sun X, Cohen IS, Mathias RT. Gradients in Na/K pump current and angiotensin II across the canine ventricular wall. *Biophys J* 2007;92:1344.(abstract)
 15. Yu H, Gao J, Wang H, Wymore R, Steinberg S, McKinnon D, Rosen MR, Cohen IS. Effects of the renin-angiotensin system on the current $I_{(to)}$ in epicardial and endocardial ventricular myocytes from the canine heart. *Circ Res* 2000;86:1062–8. [PubMed: 10827136]
 16. Kochupura PV, Azeloglu EU, Kelly DJ, Doronin SV, Badylak SF, Krukenkamp IB, Cohen IS, Gaudette GR. Tissue-engineered myocardial patch derived from extracellular matrix provides regional mechanical function. *Circulation* 2005;112:1144–9. [PubMed: 16159807]
 17. Gaudette GR, Todaro J, Krukenkamp IB, Chiang FP. Computer aided speckle interferometry: a technique for measuring deformation of the surface of the heart. *Ann Biomed Eng* 2001;29:775–80. [PubMed: 11599585]
 18. Kelly DJ, Azeloglu EU, Kochupura PV, Sharma GS, Gaudette GR. Accuracy and reproducibility of a subpixel extended phase correlation method to determine micron level displacements in the heart. *Med Eng Phys* 2007;29:154–62. [PubMed: 16531092]
 19. Potapova I, Doronin S, Kelly D, Rosen A, Schuldt A, Lu J, Guo Y, Raptis N, Towner A, Robinson R, Rosen M, Brink P, Gaudette G, Cohen I. Functional Regeneration of the Canine Ventricle Using Adult Human Mesenchymal Stem Cells Committed In Vitro to a Cardiac Lineage. *Circ Res* 2006;99:E19.
 20. Rosen A, Kelly D, Brink P, Schuldt A, Lu J, Potapova I, Doronin S, Robinson R, Rosen M, Gaudette G, Cohen I. Finding Fluorescent Needles in the Cardiac Haystack: Reconstructing the 3-Dimensional Distribution of Quantum Dot-Loaded Human Mesenchymal Stem Cells Injected into the Rat Ventricle in Vivo. (abstract). *Circ Res* 2006;99:E34.