

Systemic and Coronary Delivery of Marrow Stromal Cells for Cellular Cardiomyoplasty: Advantages and Precautions

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Abstract

Implanting multipotent adult stem cells and progenitor cells is undergoing extensive laboratory studies as well as early clinical trials. In most of these studies, localized myocardial infarcts had been the target lesion, and donor cells had been implanted by local injections. However in heart failure due to diffuse cardiomyopathies, coronary or systemic delivery of donor cells may be preferable. The advantages and feasibility of such cell delivery techniques will be discussed, while possible risks associated with embolization by aggregated donor cells will be reported. With appropriate precautions, we believe such alternate delivery approaches would be clinically desirable in selected patients.

Key words: administration, adult stem cells, aggregation, cellular cardiomyoplasty, embolism, marrow stromal cells.

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Cell therapy to regenerate damaged myocardium, or “cellular cardiomyoplasty” [5], is undergoing active experimental studies [10, 14, 16, 19] and early clinical trials [6, 7] at this time. The donor cells most commonly used for implantation are skeletal myoblasts and bone marrow derived adult stem cells and progenitor cells. Most of the experimental models of cardiac lesion, in various animal species ranging from mouse to pigs, are localized myocardial injuries produced by myocardial cryo-injury [16] or infarction following coronary artery ligation [14]. In clinical trials to date, most of the patients had myocardial infarction with scar formation [11, 15]. Thus, in order to deliver the donor cells to the damaged segment of the myocardium, direct local injections of donor cells have been the predominant technique used both in experimental and clinical cellular cardiomyoplasty. The donor cells have been injected with a needle directly into the myocardial scar, or into the peri-infarct zone, the latter allowing the donor cells to come into direct contact with the native cardiomyocytes, which have been thought to promote *milieu dependent differentiation* of adult stem cells [7, 17]. During cardiac surgical operations, such as coronary artery bypass grafting, a segment of non-bypassable and irreversibly damaged myocardium may be identified and receive cell therapy by multiple punctures of the epicardium to implant the donor cells. In patients who are not undergoing concomitant surgery, epicardial injection of donor cells can also be achieved by minimally invasive surgical techniques. Alternately, the cells may be introduced into the target zone of the myocardium by transluminal cardiac catheterization, using specially designed

catheters. They may be guided by various mapping and monitoring techniques, such as echocardiography and fluoroscopy, such that the needle at the tip of the catheter can be positioned and fixed with the ventricular wall so that the cells can be injected from the endocardial side of the myocardium. In all these injection techniques, avoidance of damage to major coronary arteries, and the prevention of leakage of the injected cells are important considerations. The latter is particularly significant when the cells are injected into a contracting peri-infarct myocardium rather than into an akinetic scar, since a contracting myocardium may compress upon the bolus of injectate, squeezing implanted cells to leak back out of the puncture hole. A number of techniques are being suggested to minimize these shortcomings.

Coronary and Venous Administration of Donor Cells: Indications, Feasibility and Advantages

In contrast to myocardial infarction patients, those who suffer heart failure as the result of cardiomyopathies of various etiologies would require donor cells delivered diffusely throughout the myocardium, rather than to a specific segment. Although this could be achieved by multiple punctures all over the ventricular wall for cell injections, this would appear less than optimal. Ideally donor cells could be delivered diffusely by a single injection of cells via the coronary artery, either through coronary artery catheterization, or injected into aortic root during cardiac surgery under cardiopulmonary bypass, since such cells would enter the coronary circulation as the pump will deliver retrograde blood flow in the aortic root.

We [18] studied the feasibility of this approach, and had experimentally shown that these cells would distribute widely throughout the coronary arterial system, and reach the microvasculature of the myocardium. We observed that these cells were then able to migrate out of the vascular space, and underwent *in situ* differentiation to express various phenotypes. When the bone marrow stroma derived cells were used as donors, they expressed phenotypes ranging from cardiomyocytes, to vascular endothelium and smooth muscles as well as myofibroblasts. In a recent non-randomized clinical study, Assmus B et al. [1] infused either bone marrow derived or circulating blood derived progenitor cells into the involved coronary artery in reperfused acute myocardial infarction, and reported improved ventricular function and myocardial perfusion. They reported that in those 20 patients, no malignant arrhythmias were observed.

Perhaps the least invasive technique to administer cells is through systemic venous injection, such as that used for bone marrow cell transplantation [3]. Recent studies indicate that after myocardial infarction, autologous bone marrow stromal cells are mobilized from the bone marrow, traffic through the blood circulation, and home-in to the myocardial injury site, where they can undergo *in situ* milieu dependent differentiation [2, 13]. Following acute myocardial infarction, cells injected therapeutically into the vein can likewise traffic through the blood stream, and reach the myocardium via the coronary circulation [4]. They are able to home-in and localize within and around the infarcted segment of the myocardium where they undergo *in situ* differentiation and improve ventricular function. This highly convenient route of cell administration is clinically useful primarily for patients who suffer acute myocardial infarction, since the signaling molecules to recruit these cells to the infarct site appear to be inflammatory cytokines, such as GC-CSF (granulocyte-colony stimulating factor) and MC-CSF (macrophage-colony stimulating factor) [8, 12] etc. In a chronic stable infarction, however, the inflammatory response to acute myocardial infarction would have subsided, and we have preliminary data (MacDonald, D et al, unpublished data) indicating that the homing mechanism of marrow derived stem cells and progenitor cells are lost, thus no longer able to localize at the scar tissue. The clinical advantage of intravenous infusion of donor cells in acute myocardial infarction is the simplicity and the minimally invasive nature of this technique, which could be literally carried out in emergency rooms and primary care hospitals without the need for surgical intervention or cardiac catheterization [6].

Precautions for the Systemic and Coronary Delivery Approaches

Although coronary or intravenous administration of donor cells for cellular cardiomyoplasty is clinically advantageous in selected patients as described above, one important potential complication which may be associ-

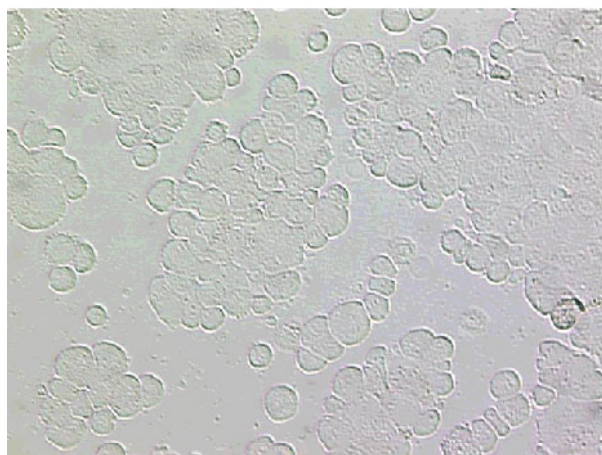


Figure 1. Aggregated MSCs (Marrow Stromal Cells) in a smear of infusate, untreated with high cellular concentration. 40×10^6 MSCs suspended in 0.25cc of culture medium viewed at 100X magnification.

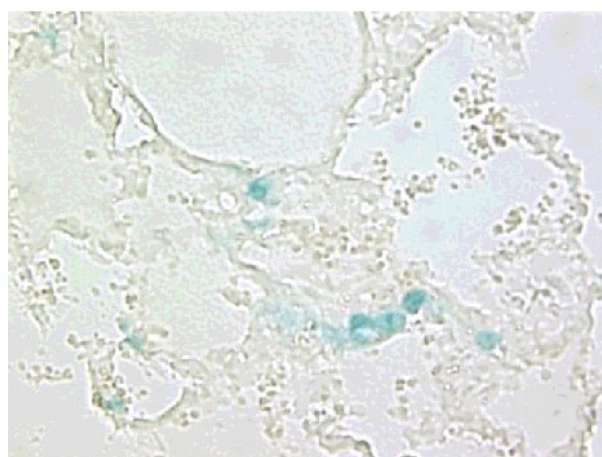


Figure 2. Histology (200X) showing embolized aggregates of LacZ reporter gene labeled MSCs (blue stained) in the pulmonary vasculature.

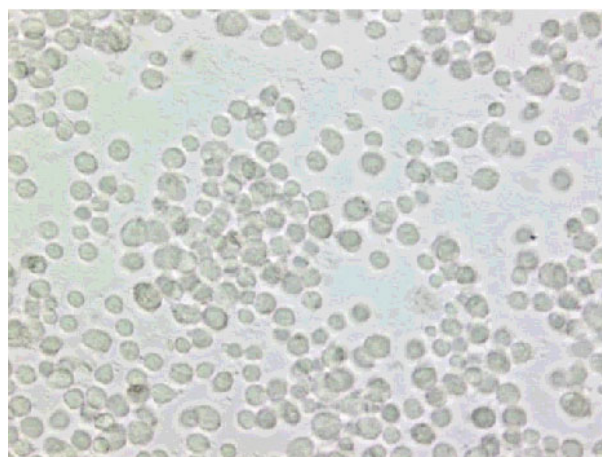


Figure 3. Dispersed MSCs in a smear of infusate, diluted to reduce cellular concentration. 20×10^6 MSCs suspended in 0.50cc of culture medium viewed at 100X magnification.

ated with such approaches needs to be recognized and prevented. During our laboratory studies, we found that a rapid injection of a high concentration of marrow stroma derived cells (MSCs) could cause acute death of the recipient animals (unpublished observation). Histological examinations indicate that such death might be associated with vascular embolization by these cells. We have found that in the suspension of these cells prior to injection, they have a tendency to aggregate into multi-cellular globules (Figure 1), which are estimated to be able to reach 100 microns in diameter so that they are large enough to embolize and occlude arterioles. Histologically, findings of embolization in the coronary vessels when MSCs are injected via the coronary artery, or embolization in the pulmonary vasculature (Figure 2) following intravenous injection, confirm our suspicion that unless measures are taken to reduce the risk of cellular aggregation, such complications may pose a safety risk for the recipients. We have experimented with a number of ways to reduce MSC aggregation in the cell suspension to be used for intravascular administration, and found that reduction of cell density by dilution is the most reliable technique to avoid this complication (Figure 3). Various other measures undertaken, such as the addition of heparin and mechanical agitation, were not effective. We would, therefore, recommend that sufficient dilution of these cell suspensions and slow intravascular infusion rather than a bolus injection, should be the technique of choice for administering these cells. Recently, Gao et al [9] reported on using gamma camera real time imaging to study the dynamic distribution of systematically delivered, isogenic, in-oxine labeled MSCs. After both intra-arterial and intravenous infusions, radioactivity associated with marrow stroma cells was detected primarily in the lung and then secondarily in the liver and other excised organs, including kidney, spleen and long bones. When these authors administered a vasodilator, sodium nitroprusside, they found more labeled marrow stroma cells cleared the lungs, resulting in a larger proportion being detected in the liver and marrow of the long bones. It is, however, not clear by what mechanism this vasodilator allowed these aggregates to clear the pulmonary vasculature.

We have used cells labeled with β -gal to observe the fate of these cells originally embolized in the pulmonary vasculature. If the pulmonary embolism was not sufficiently severe to be lethal, they were able to clear the pulmonary vasculature over the next several days, indicating that the greatest risks, both in the coronary and in the pulmonary vasculatures, will be immediately after injection. Thus in conclusion, we advocate slow infusion of diluted cells in clinical application when the donor cells are administered through the coronary artery or systemic veins for cellular cardiomyoplasty.

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References

- [1]Assumus B, Schachinger V, Teupe C, et al: Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction (TOP-CARE-AMI). *Circulation* 2002; 106: 3009-3017.
- [2]Bittira B, Kuang JQ, Piquer S, Shum-Tim D, Al-Khalidi A, Chiu RCJ: The pathophysiological roles of bone marrow stromal cells (MSCs) in myocardial infarction. *Circulation* 2001; 104 (Suppl) II-523.
- [3]Bradstock K: Wanderings of the bone-marrow stem cell. *The Lancet* 2001; 358: 5-6.
- [4]Chen J, Li Y, Wang L, Zhang Z, et al: Therapeutic benefit of intravenous administration of bone marrow stromal cells after cerebral ischemia in rats. *Stroke* 2001; 32: 1005-1011.
- [5]Chiu RCJ, Zibaitis A, Kao RL: Cellular cardiomyoplasty: myocardial regeneration with satellite cell implantation. *Ann Thorac Surg* 1995; 60: 12-18.
- [6]Chiu RCJ: Adult stem cell therapy for heart failure. *Expert Opinion on Biological Therapy*. In press.
- [7]Chiu RCJ: Cellular cardiomyoplasty: The biology and clinical importance of "milieu dependent differentiation", in Kipshidzen N, Serruys PW (eds): *Handbook of Cardiac Cell Transplantation*. London, UK, Martin Dunitz Ltd, 2002, in press.
- [8]Fukuhara S, Tomita S, Ohtsu Y, et al: G-CSF promoted bone marrow cells to migrate into infarcted heart and differentiate into cardiomyocytes. *Circulation* 2002; 106: II-376.
- [9]Gao J, Dennis JE, Muzic RF, Lundberg M, Caplan AL: The dynamic in vivo distribution of bone-marrow derived mesenchymal stem cells after infusion. *Cells, Tissues Organs* 2001; 169: 12-20.
- [10]Marelli D, Desrosiers C, el Alfy M, Kao RL, Chiu RC: Cell transplantation for myocardial repair: an experimental approach. *Cell Transplantation* 1992; 1 (6): 383-390.
- [11]Menasche P, Hagege AA, Scorsin M, et al: Myoblast transplantation for myocardial infarction. *Lancet* 2001; 357: 279-80.
- [12]Mike T, Sakamoto J, Nakano A, et al: Mobilization of bone marrow cells by G-CSF/M-CSF improves ventricular function in the heart undergoing post-infarct remodeling. *Circulation* 2002; 106: II-132.
- [13]Orlic D, Kajstura J, Chimenti S, Limana F, Jakoniuk I, Quaini F, et al: Mobilized bone marrow cells repair the infarcted heart, improving function and survival. *Pro-*

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- ceedings of the National Academy of Sciences of the United States of America* 2001; 98 (18): 10344-10349.
- [14] Orlic D, Kajstura J, Chimentl S, et al: Bone marrow cells regenerate infarcted myocardium. *Nature* 2001; 410: 701-5.
- [15] Stamm C, Westphal B, Kleine HD, et al: Bone marrow stem cell transplantation for myocardial regeneration in post infarction CABG patients. *Circulation* 2002; 106: II-375.
- [16] Taylor DA, Atkins BZ, Hungspreugs P, et al: Regenerating functional myocardium: Improved performance after skeletal myoblast transplantation. *Nature Medicine* 1998; 4: 929-933.
- [17] Tomita S, Nakatani T, Fukuhara S, et al: Bone marrow stromal cells can differentiate into cardiac lineage and contract synchronously with cardiomyocytes by direct cell-to-cell interaction in vitro. Presented at the 82nd Annual Meeting of the American Association for Thoracic Surgery, Washington DC, May 5, 2002.
- [18] Wang JS, Shum-Tim D, Chedrawy E, Chiu RCJ: The coronary delivery of marrow stromal cells for myocardial regeneration: Pathophysiologic and therapeutic implications. *J Thor Cardiovasc Surg* 2001; 122: 699-705.
- [19] Wang JS, Shum-Tim D, Galipeau J, et al: Marrow Stromal Cells for Cellular Cardiomyoplasty: Feasibility and Clinical Advantages. *J Thorac Cardiovasc Surg*. 2000; 120: 999-1006.