Cell Transplantation, Vol. 16, pp. 963–970, 2007

0963-6897/07 $90.00 + .00
E-ISSN 1555-3892
www.cognizantcommunication.com

REVIEW

Adipose-Derived Cells

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Heart failure is by far the most common cause of hospitalization in Western countries, with onerous economic consequences. Cell therapy holds great promise for use in tissue regeneration and is increasingly used in an effort to improve outcomes in cardiac disease. Recently it has been shown that adipose tissue, in addition to committed adipogenic, endothelial progenitor cells and pluripotent vascular progenitor cells, also contains multipotent cell types (adipose-derived stem cells, ADSCs) that, in cell culture conditions, have shown to have an impressive developmental plasticity including the ability to undergo multilineage differentiation and self-renewal. ADSCs express multiple CD marker antigens similar to those observed on MSCs and are also capable of secreting a large number of angiogenesis-related cytokines, including vascular endothelial growth factor, granulocyte/macrophage colony stimulating factor, stromal-derived factor-1α, and hepatocyte growth factor. Adipose tissue can be harvested in large quantities with minimal morbidity in several regions of the body and, on average, 100 ml of human adipose tissue yields about 1×10^6 stem cells. Studies conducted in porcine AMI models have shown a significant LV functional improvement, with no report of any potentially fatal arrhythmias. The APOLLO trial, a prospective, double blind, randomized, placebo-controlled trial currently in the recruiting phase, is a "first-in-man" study that explores the safety and feasibility of ADSC transplantation in patients with acute MI.

Key words: Cardiac regeneration; Adipose-derived stem cells; Heart failure; Acute myocardial infarction

INTRODUCTION

Primary PCI, thanks to enormous improvements in materials and devices, is nowadays considered the gold standard for the treatment of AMI and leads to excellent safety and efficacy results. Nevertheless, revascularization therapies, though able to restore perfusion and contractile function of postinfarct stunned or hibernated myocardium, have no effect on necrotic tissue.

Postinfarction myocardial necrosis and subsequent formation of fibrotic scar that replaces viable myocardium leads to depressed systolic function, reduced diastolic compliance, left ventricular remodeling, and ultimately to congestive heart failure progression, by far the most common cause of hospitalization in Western countries, with onerous economic consequences.

The potential of cell transplantation to repair damaged myocardium and to grow new viable tissue is attractive, and has been widely studied in both experimental and clinical conditions using various cell types (8–10,24,35,42).

The characteristics of the ideal cell still remain to be defined, but it appears clear that, among the cells efficient in the treatment of heart disease, cells that are autologous, nonembryonic, do not require culturing to obtain a therapeutic dose, and can be administered during the same procedure may be logistically easier to use.

Mesenchymal stem cells, referred also to as marrow stromal cells (MSCs), have shown to have some of the above-mentioned ideal properties; MSCs in fact are multipotent adult stem cells that can expand in culture and are able to differentiate into multiple mesenchymal cell phenotypes, including bone, cartilage, and fat. In addition, MSCs are capable of nonmesenchymal phenotypic differentiation into neurons, skeletal muscle progenitor cells, vascular endothelial cells, and cardiomyocytes (14,20,22,31,40,53,55).

So far, these cells have been harvested from bone

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marrow, a tissue source that presents multiple limitations, including: a) donor site morbidity limits the amount of marrow that can be obtained (generally 40–50 ml) (3,18); b) MSCs represent <0.01% of all nucleated bone marrow cells in healthy volunteers (approximately 1 in 25,000 to 1 in 100,000) (4,5); and c) requires extended time in culture to obtain therapeutic cell doses by ex vivo cell expansion, rendering treatment in the acute phase of myocardial infarction impractical.

Recently it has been shown that adipose tissue, in addition to committed adipogenic, endothelial progenitor cells and pluripotent vascular progenitor cells, contains multipotent cells, similar to MSCs (58,59). This finding has generated major interest because, in contrast to bone marrow, large quantities of adipose tissue can be easily and safely harvested with minimal morbidity, making it an appealing source for cell therapy.

CELL CHARACTERISTICS

Adipose tissue has a notable plasticity during life. Although cellular hypertrophy can partially enable increases in volume, large variations are usually associated with an increase in adipocyte count (cellular hyperplasia) accompanied by a concomitant expansion and remodeling of the microvasculature supplying these cells (1).

The hyperplastic process of the adipose tissue was formerly thought to be mediated by a population of unipotent progenitor cells called preadipocytes. These cells have been demonstrated to have potential beyond that of the adipocytic lineage, with an impressive developmental plasticity (23,58), including the ability to undergo multilineage differentiation and self-renewal (49, 54,57,59) (Fig. 1). These cells, present within the stromal vascular fraction of adipose tissue, are generally referred to as adipose-derived stem cells (ADSCs).ADSCs are a cell population with properties that are very similar, though not identical, to those of marrow-derived MSCs (7,38,39,52).

These cells have extensive proliferative capacity and are able to undergo differentiation along both mesenchymal lineages (adipogenesis, chondrogenesis, osteogenesis) (11,14,20,22,31,40,53,55) and nonmesenchymal lineages (endothelial, smooth muscle and neurogenic), confirming the transdifferentiation ability of ADSCs (40,41).

In culture, these cells have a surface phenotype rather similar to the MSC phenotype (Table 1): both cell types express CD117 (stem cell factor receptor), CD105, STRO1, and CD166 (multilineage differentiation markers) (6,19,26,43), CD90, CD54 (ICAM-1), CD44 and CD29 (β1-integrin) (13,14), whereas both cell types do not express the endothelial markers CD31, CD34, and the hematopoietic marker CD45 (7,19).

However, MSCs and ADSCs have a number of relatively slight distinctions, the most interesting of which is the reciprocal expression pattern of the very-late-activation antigen 4 or VLA-4 (CD49d/CD29) and its cognate receptor vascular cell-adhesion molecule 1 or VCAM-1 (CD106). ADSCs express VLA-4 but not VCAM-1 in the majority of donors, while MSCs usually express VCAM-1 and not VLA-4 (7).

The consequences of these properties are not clear yet, but this observation is fascinating because both these molecules play an important role in the homing and mobilization process of hematopoietic stem cell from bone marrow (44,48).

MSCs and ADSCs are also known to secrete a large number of angiogenesis-related cytokines (37). ADSCs in standard cultures secrete high levels of hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF), placental growth factor (PlGF), transforming growth factor-β (TGF-β), fibroblast growth factor (FGF), granulocyte/macrophage colony stimulating factor (GM-CSF), monocyte chemotactic protein-1 (MCP-1), and stromal-derived factor-1α (SDF-1α), suggesting an important role of ADSCs in neovascularization (36,37).

Clonally derived, multipotent cells from adipose tissue display an immunoprivileged behavior (39). Less than 1% of ADSCs express the cell surface MHC class-I, while class-II is almost absent (25), being therefore poorly recognized by T lymphocytes. Moreover, ADSCs have the ability to suppress lymphocyte reaction in a dose- and time-dependent fashion; such immunosuppression probably occurs via the production of IL-4, IL-10, and TGF-β, responsible for the inhibition of lymphocyte proliferation (34).

These characteristics, together with the easy availability and the small amount of tissue needed for their isolation, suggest that ADSCs might even have potential as immunoprivileged universal donor cells with the prospective possibility to be used also for allogenic transplantation.

Large quantities of subcutaneous adipose tissue can be safely harvested using liposuction in several regions of the body. A typical harvest out of 100–200 ml of human adipose tissue contains about 5×10^7 nucleated cells. After enzymatic digestion, filtration, and centrifugation processes, the nonbuoyant stromal cells part from the buoyant adipocytes (29,32,33). The nonbuoyant fraction can yield in excess of 1×10^6 stem cells (from 100 cc of fat tissue), about 45-fold more than a typical harvest of bone marrow aspiration (of 40 ml), which normally contains 2.5×10^4 MSCs in a mature adult (3,30). The ADSC-containing fraction can be cultured with different media (e.g., DMEM-F12; EGM-2-MV, α-MEM, etc.) and supplemented with different growth factors (e.g., VEGF, insulin-like growth factor, basic FGF, etc.)

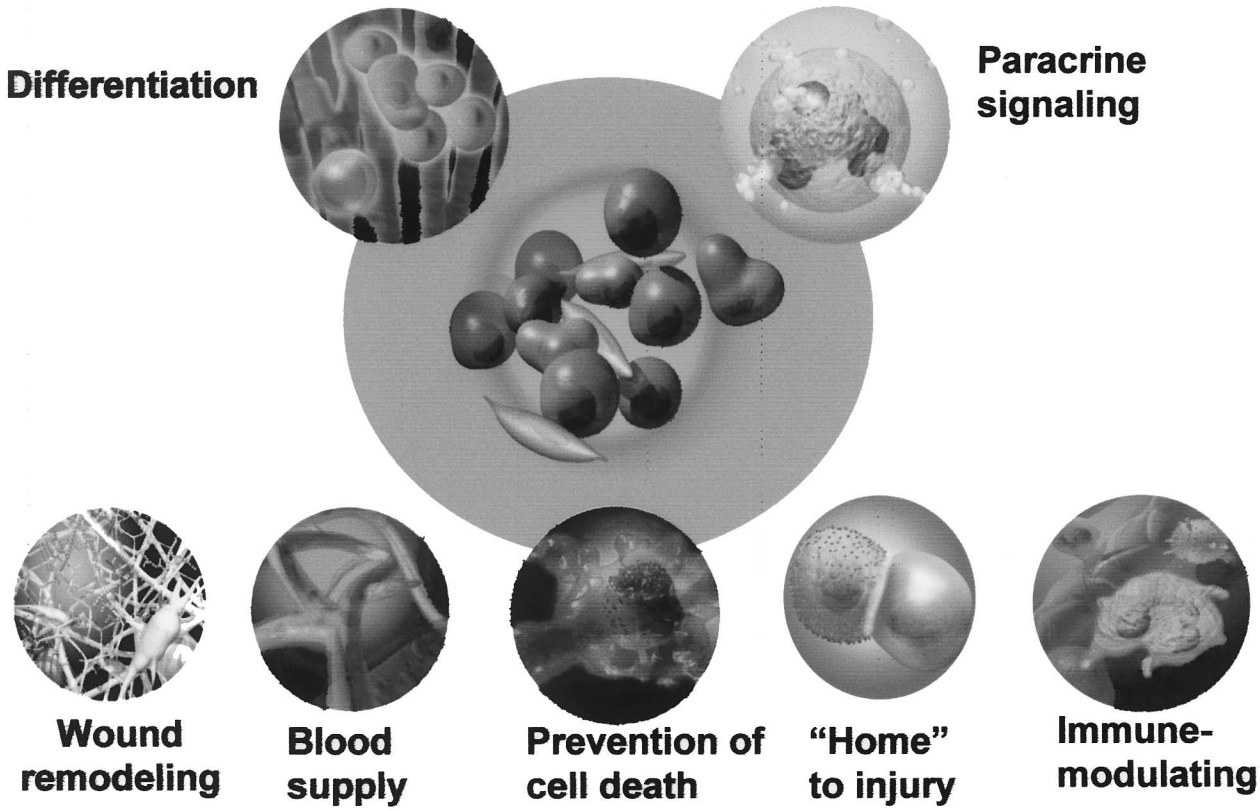

Figure 1. Multiple mechanisms of cell regeneration enacted by ADSCs.

to stimulate expansion and differentiation into dedicated lineages. Due to the great concentration of ADSCs normally present in an adipose tissue harvest, it is also possible to obtain therapeutic cell doses in as little as 1 after liposuction, without further expansion in culture (32,33), therefore avoiding cell alterations often associated with culturing.

Table 1. Cell Surface Characteristics of Adipose-Derived Stem Cells and Mesenchymal Stem Cells

Cell Surface Marker	ADSCs	MSCs
CD29	+	+
CD44	+	+
CD54	+	+
CD90	+	+
CD105	+	+
CD166	+	+
STRO-1	+	+
CD31	−	−
CD34	−	−
CD45	−	−
CD49D	+	−
CD106	−	+

DIFFERENTIATION CAPABILITIES OF ADSCs

ADSCs, similarly to MSCs, have the ability to undergo differentiation along both mesenchymal (fat, bone, cartilage) and nonmesenchymal lineages (endothelial cells, smooth muscle) (13,15,20,22,44,45), demonstrating that adipose tissue contains pluripotent cells. Furthermore, concerning muscle differentiation, evidence shows that ADSCs have the capability to differentiate into contractile cells with either striated muscle cells or cardiomyocyte features (12,21,27,50,56).

In Vitro Studies of ADSC Differentiation

After isolation and expansion of processed lipoaspirate (PLA) clones, PLA cells expressed multiple CD marker antigens similar to those observed on MSCs, as observed by Zuk et al. in 2002 (58). During in vitro culture with various supplementation, PLA clones differentiated into different mesodermal lineages (examined lineages were: osteogenic, chondrogenic, adipogenic), while some clones exhibited differentiation potential into all of these lineages. These trilineage clones were designated as ADSCs, confirming therefore the presence of a stem cell population within adipose tissue. In addition to mesodermal lineages, PLA cells

and clones showed the capability to differentiate into putative neurogenic cells, exhibiting morphology and a protein pattern consistent with the neuronal phenotype.

In 2005, Planat-Benard et al. (32) obtained extraordinary results by plating fresh ADSCs in a semisolid culture. After 3 weeks of culture, ADSCs morphologically developed into ventricle-like, atrial-like, and pacemaker-like cells, displaying tight intercellular connections (evidenced by Cx-43) and spontaneous as well as triggered action potentials with beating. Moreover, these differentiated cells expressed the cardiac-specific transcription factors Nkx2.5, GATA-4, and MEF-2C, the structural cardiac proteins β-myosin heavy chain (MHC), myosin light chain-2 ventricular (MLC-2v), myosin light chain-2 atrial (MLC-2a) as well as the atrial natriuretic peptide (ANP), whereas the skeletal marker MyoD and smooth muscle actin were not expressed. Consistent with these results, after 3 weeks of exposure of ADSCs with a permeable cell membrane to rat cardiomyocyte extracts, the ADSCs expressed cardiomyocyte markers including sarcomeric alpha-actin, desmin, and cardiac troponin I, as well as the gap junction protein connexin-43 (Cx-43) and started to beat spontaneously, as observed by Gaustad et al. in 2004 (17).

In Vivo Studies of ADSC Differentiation

Several groups have so far reported data about the safety and efficacy of ADSCs to regenerate damaged myocardium in both small (rats) and large animals (pigs). Strem and colleagues (46) in 2005 used intraventricular injections to deliver freshly isolated ADSCs from Rosa 26 mice (expressing the β-galactosidase transgene) into syngeneic recipient mice, following myocardial cryoinjury. β-Galactosidase-positive cells were found in treated mice together with the expression of MHC, Nkx2.5, and troponin I at 14 days posttransplantation, suggesting that ADSCs have the ability to engraft injured myocardium and express specific cardiomyocyte markers in vivo.

In another study conducted by the same group, syngeneic freshly isolated ADSCs were injected into the LV chamber of 10 female Lewis rats after 60-min occlusion of the LAD, resulting in significant increases of 5.1% and 7.7% delta in LVEF at 4 and 12 weeks (Fig. 2), respectively, compared with control rats (saline treated, $n = 10$). This study also demonstrated significant improvements in both dP/dt_{max} and dP/dt_{min} in the ADSC-treated rats compared to controls (Table 2) (47). Moreover, in accordance with the previous study, cell engraftment was demonstrated 7 days after transplantation (Fig. 3).

Delivery of ADSCs by intracoronary infusion into pigs 2 days after an acute MI through a LAD balloon occlusion resulted in a 3% absolute increase in LVEF while the untreated pigs showed a 9% decline at 6-month follow-up ($p < 0.01$) (51). Likewise, intracoronary infusion of either ADSCs or MSCs in an acute MI pig model resulted in an 11.4% improvement of absolute LVEF after 4 weeks, compared to only 2% improvement in the nontreated group ($p < 0.003$) (50).

Freshly isolated ADSCs given immediately after reperfusion in a porcine AMI model resulted in a 8% LVEF increase compared to controls at 8-week follow-up ($p = 0.023$) (2).

Interestingly, concordant with the functional improvement, wall thickness and capillary density were both significantly increased in the border infarct areas of the treated group, compared to the control group (28).

In a study recently published by Miyahara and colleagues (29), ADSCs were transplanted directly onto infarcted murine hearts as a monolayered cell sheet. After transplantation, the engrafted sheet gradually grew to form a thick stratum that included newly formed vessels, undifferentiated cells, and cardiomyocytes. Autologous transplantation of ADSC sheets resulted in a decreased LVEDP, improved maximum and minimum dP/dt, increased diastolic wall thickness, increased left ventricular fractional shortening, and decreased diastolic wall stress, compared to dermal fibroblast sheets taken as a control.

Human Studies of ADSC Differentiation

ADSCs have been used successfully in a variety of indications in humans, including bone reconstruction, the treatment of Crohn's disease fistulas (15,16), osteogenesis imperfecta (21), and for breast augmentation and reconstruction after partial mastectomy (56). The APOLLO trial, currently recruiting patients, is the "first-in-man" study that explores the safety and feasibility of ADSC transplantation in patients with acute MI. The APOLLO trial is a prospective, double blind, randomized, placebo-controlled, sequential dose escalation study that will enroll up to 48 patients (4 cohorts of 12 patients each) with acute MI at the Thoraxcenter, Erasmus MC, Rotterdam.

Eligible patients are enrolled following primary PCI and undergo liposuction and isolation of the adipose-derived regenerative cells fraction (ADRCs) using the Celution™ system (Fig. 4). ADRCs are then injected within 24 h from the primary PCI into the culprit coronary artery.

Main safety end points include: major adverse cardiac or cerebral event (MACCE) rates, serious adverse events (SAEs)/adverse events (AEs) rates and a composite clinical end point of death, MI, stroke, and rehospitalization for heart failure. Main feasibility end points are: absolute LVEF and changes in LVEF from baseline to 6 months, MI size, regional wall thickness and thickening in all segments, LV end systolic volume (LV-

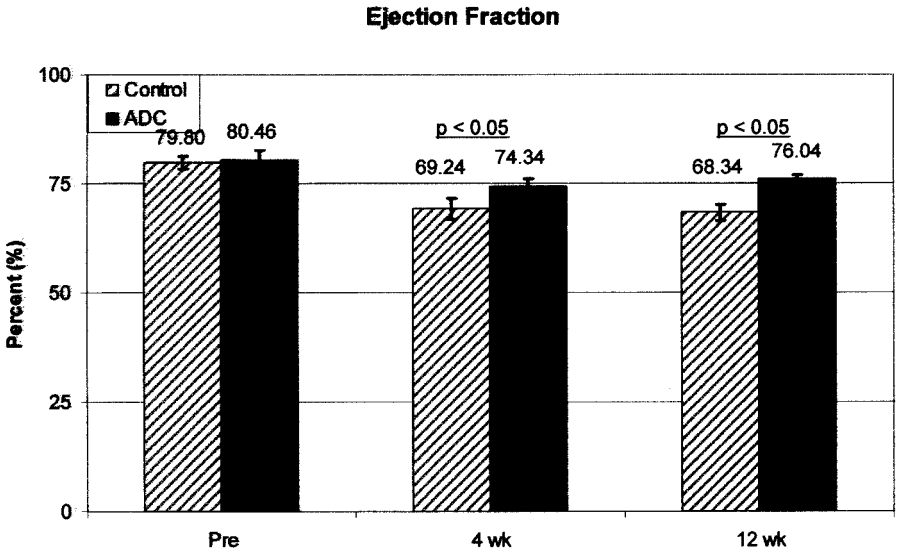

Figure 2. LVEF evaluated by echocardiography at 4 and 12 weeks showed a significant improvement in the group treated with ACSCs (ADC) compared to the control group (Control).

ESV), LV end diastolic volume (LV-EDV), and change in perfusion defect after revascularization to 6 months as measured by contrast enhanced MRI, echocardiography, and scintigraphy.

Patients will be followed for 36 months and will undergo imaging studies (2D/3D echocardiography, LV angiography, MRI, SPECT), functional evaluation (left ventricular pressure–volume relationship), clinical evaluations, and laboratory testing at 6, 12, and 18 months.

SIDE EFFECTS, ADVERSE EVENTS AND POTENTIAL HURDLES

The main issue related to transplanted cells is their potential arrhythmogenicity. Previous reports conducted using skeletal myoblasts showed an increased rate of arrhythmic events in the treated groups that raised important interrogatives on the safety of this treatment and that led often to prophylactic AICD implantation (27).

Although limited safety data are available to date, in vivo preliminary animal studies conducted using ADSCs have not reported an increase in any potentially fatal arrhythmias. For instance, loop recorder monitoring in pigs treated with ADRCs (12) did not show an increase in fatal arrhythmic events or sustained arrhythmias (e.g., bradycardia lower than 45/min or tachycardia above 165/min) in the treated animals. Programmed right ventricular stimulation did not show an increase in susceptibility for arrhythmias by ADRC treatment. On the contrary, cycle length was significantly longer in the ADRC group at the beginning of an induced arrhythmia, suggesting rather a reduction in the overall inducibility of malignant arrhythmias than an increase in susceptibility. In addition, there have been no reports on arrhythmias in patients who underwent intramyocardial injections of ADRCs using the NOGA delivery system in patients with chronic myocardial ishemia in the PRECISE trial.

Issues associated with tissue harvest in patients with a recent AMI under high doses of anticoagulants, GP IIb-IIIa inhibitors, and aspirin are a potential concern, owing to the highly developed vascularization of adipose tissue and the consequent bleeding risk. However, only a relatively small amount of tissue is required to obtain an effective cell dose, and hemostasis at the site of liposuction can easily be achieved by local pressure.

Table 2. dP/dt Evaluated at 12 Weeks by Millar Pressure Tip Catheter Is Significantly Higher in the Group Treated With ADSCs Compared to the Control Group (Saline Treated)

	Saline Treated	ADC Treated	*p*-Value
+dP/dt	2837.61 (301.19)	5494.46 (550.76)	0.015
–dP/dt	–2716.49 (331.83)	–6323.28 (544.61)	0.003

Values are mmHg/s with SE in parentheses.

CONCLUSIONS

Human adipose tissue is an interesting source of multipotent stem cells that have the ability to differentiate into several mesenchymal and nonmesenchymal lineages (including vascular and cardiomyocytes lineages), demonstrate stem cell-like extensive self-renewal, and secrete several factors with angiogenic and antiapoptotic effects. Moreover, ADSCs/ADRCs, unlike bone mar-

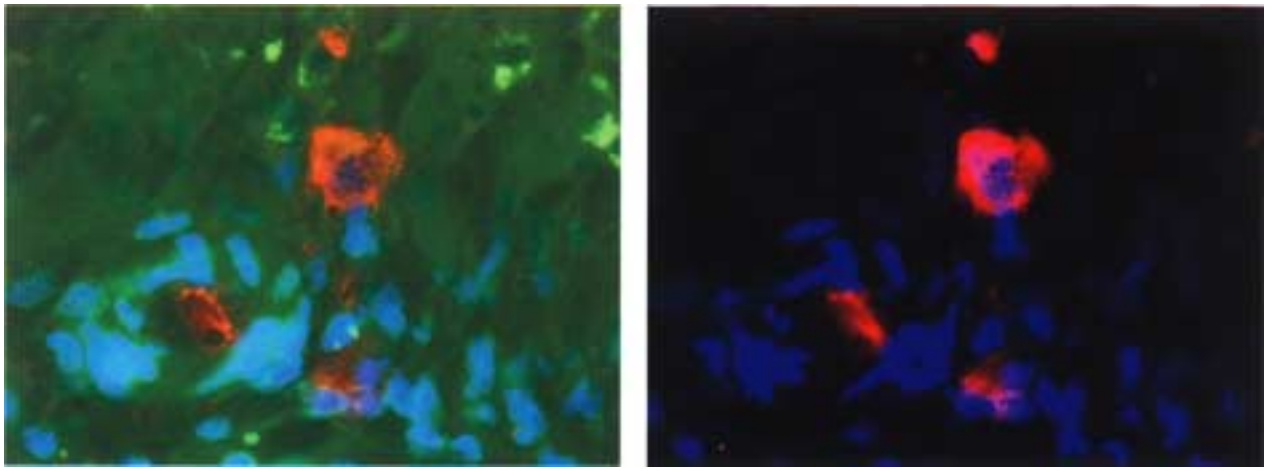

Figure 3. Histologic samples taken at day 7 posttransplantation in the AMI broder region of ADC-treated rats showing ADSC engraftment. Left: green channel on. Right: green channel off. Red = anti-GFP immunostaining, blue = DAPI.

row-derived MSCs, can be easily and safely obtained in large numbers, without the necessity to culture and expand them to obtain a therapeutic dose.

ADRCs have already been used in humans in different clinical settings with excellent results and few side effects. Preclinical studies conducted in animal AMI models showed that ADSCs have the capability to differentiate into cardiac muscle cells as well as to excrete different growth factors with important proangiogenic effects that led to a significant improvement of ejection fraction and wall thickness.

The APOLLO trial, a "first-in-men" study conducted with ADSCs in AMI patients, will provide fundamental data about the safety and efficacy of these cells in humans, providing an essential insight on what could be their role in the treatment of cardiovascular disease.

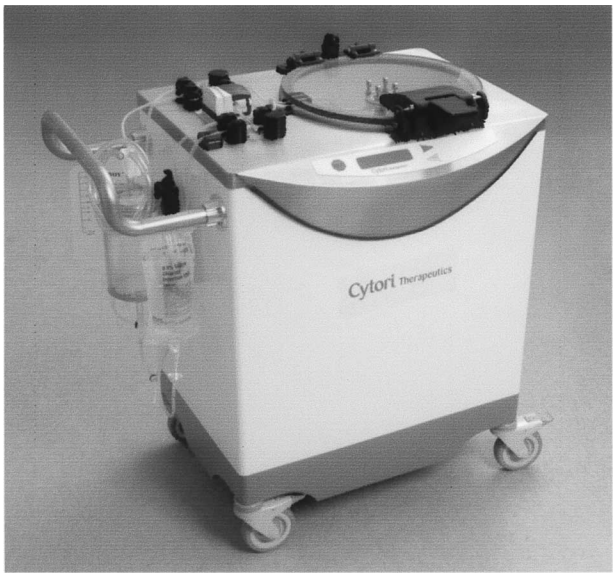

Figure 4. The Celution™ system. Reproduced with permission from EuroIntervention and Cytori Therapeutics Inc.

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