Cultured and freshly isolated adipose tissue-derived cells: fat years for cardiac stem cell therapy

Pedro L. Sánchez¹, Ricardo Sanz-Ruiz¹, María Eugenia Fernández-Santos², and Francisco Fernández-Avilés¹*

¹Cardiology Department, Hospital General Universitario Gregorio Marañón, 28007 Madrid, Spain; and ²Cell Processing Unit, Hospital General Universitario Gregorio Marañón, 28007 Madrid, Spain

This editorial refers to ‘Both cultured and freshly isolated adipose tissue-derived stem cells enhance cardiac function after acute myocardial infarction’, by X. Bai et al. on page 489

As understanding has grown about what happens when heart muscle cells die en masse after a heart attack, so has the development of treatments aimed at avoiding the loss or failure of cardiovascular function. These treatments include stem cell transplantation. The first pre-clinical and clinical experiences in stem cell transplantation were reported around a decade ago. ¹,² However, and although the field has moved quickly in these years, the most important questions—the ‘cell product’ to be used, delivery method, outcome measurement, organizational problems, and funding—remain unanswered.

The quest for the best cell type has been wheeled around from bedside to bench and back again. ³ The ideal cell type should be capable of differentiating into functional cardiomyocytes and forming new vessels. Cell types from several different sources have already been tested in animal models, and bone marrow-derived cells and skeletal myoblasts have already been used in clinical trials. Each subtype has its advantages and disadvantages, but one important issue is the time needed for their preparation: time-consuming protocols hinder easy implementation in the acute clinical setting and carry a risk of culture contamination and/or genetic modification. Furthermore, whether the beneficial effect is mediated by one specific cell type or many cell types in concert remains unknown. It is obviously of great scientific interest to test all possible combinations of progenitor types, numbers of cells, times of injection after damage, frequencies of injection, mechanisms of action, as well as homing/grafting and survival, all through different routes in animal models and then in man.

The report by Bai et al.⁴ is significant for different reasons. First, it demonstrates that freshly isolated adipose tissue-derived stem cells (ASCs) transplanted into ischaemic hearts after acute myocardial infarction promote cardiac function as well as do cultured ASCs. Secondly, it indicates that transplanted cells could act through differentiation and paracrine effects, and are able functionally to engraft and proliferate in the infarcted heart. Thirdly, it demonstrates that injected cultured ASCs can survive in injured hearts up to 4 months after myocardial infarction without migrating to other organs.

Adipose tissue-derived ‘cell products’

Bai et al.⁴ selected adipose tissue for their investigation. ASCs have emerged as a new and promising type of stem cells with two clear advantages over previously used types (bone marrow, blood, or skeletal muscle): first, the easy and repeatable access that makes it possible to harvest large amounts of adipose tissue by a minimally invasive method and, secondly, their increased proliferative potential in culture, either because of the properties of the cells themselves or because of the greater frequency of stem cells within the population used to initiate the culture. Multipotent cell types have also been found in adipose tissue stroma, in addition to committed adipogenic, endothelial, and pluripotent vascular progenitor cells. There is no consensus on the nomenclature used in describing progenitor cells isolated from adipose tissue-derived stroma, and this can sometimes lead to confusion. Thus, the terms adipose tissue-derived stromal cell, adipose stroma–vascular cell fraction (SVF), and adipose-derived regenerative cells correspond to cells obtained immediately after collagenase digestion of adipose tissue. Processed lipoaspirate cells and plastic-adherent ASCs are obtained after culture of the aforementioned cells. Other terms that have been used are multipotent...
adipose tissue-derived mesenchymal stem cells, which include SVF subpopulations or even ‘pre-adipocytes’ (adipocyte progenitors). Both cell types can be referred to as ASCs, according to the International Fat Applied Technology Society Consensus. 5

The study by Bai et al.,4 also confirms that cultured and freshly isolated ASCs have different cell surface characteristics, which are homogeneous in cultured ASCs showing a fibroblast-like morphology but heterogeneous in freshly isolated ASCs containing different populations, including functional endothelial cells (Table 1). In this study, the rigorous use of magnetic resonance imaging to examine the long-term effect of both cultured and freshly isolated ASCs revealed a functional improvement in left ventricular function compared with mice intramyocardially injected with phosphate-buffered saline alone (controls). Freshly isolated ASCs induced a similar effect in the injured hearts when compared with cultured ASCs. Thus, this study does not enable us to determine which ASC products are better or which specific freshly isolated adipose tissue-derived cell type mediates the beneficial effect. As in other studies, this area of uncertainty warrants further study. However, the advantages of using freshly isolated ASCs obviate the need for prior cell expansion in vitro, thus allowing for immediate autologous cell transplantation at the time of an acute infarction.

### ASCs: mechanism of action

Both freshly isolated and cultured ASCs appear to be a representative example of plasticity, because of their outstanding ability to generate a wide range of cell types from different organs. Some authors have suggested that the mechanism by which bone marrow-derived cells change their phenotype might be a process of in vivo fusion of stem cells with differentiated host cells.4 Bai et al.,4 confirming previous observations,7,8 were unable to detect any fused cells, suggesting that effective repair depends at least on the differentiation of ASCs into cardiomyocytes. Moreover, the authors were able to show direct evidence of the vascular differentiation potential (neovascularization) in vivo of cultured and freshly isolated ASCs, since both types of cells expressed von Willebrand factor (vWF) or smooth muscle actin (SMA), thus indicating that some injected cells differentiated into vascular cells. Therefore, Bai et al. suggest that functional recovery could be mediated by fusion-independent differentiation (cardiomyocytes, endothelial cells, and smooth muscle cells) and probably also by a paracrine effect on host tissue regeneration. Ideally, these results should be confirmed by other groups.

### ASCs: survival and migration

Bai et al. used a well-established murine model of acute myocardial infarction and an elegant luciferase-based bioluminescence imaging method for in vivo tracking of the survival and migration of injected ASCs, features that are particularly precious today, when almost all reported results show poor survival with any transplanted cell type (even cardiac resident stem cells) in injured hearts.9–11

It is remarkable that previous studies using murine cultured ASCs in a chronic infarction model failed to detect any injected cells 30 days after transplantation12 (the survival of freshly isolated ASCs in this setting has yet to be elucidicated). Therefore, we could reasonably propose that the experimental model of acute myocardial infarction is a suitable environment for ASCs to survive and reach a cardiac phenotype, as shown in the study of Bai and colleagues and in other recently published studies,8 whereas the harsh environment inherent to the chronic infarction model (e.g. fibrotic areas, mechanical forces) is probably responsible for the limited engraftment observed. It is well known that both ischaemia and reperfusion are accompanied by an inflammatory reaction in which neutrophils and monocytes are recruited from the bloodstream as part of the cell-mediated inflammatory response. The factors (cytokines and growth factors) that control the complex inflammatory process may also guide the behaviour of ASCs in tissue repair. However, to date, it is difficult to establish the role and influence of each cytokine, because their effects are time and location dependent.

The intensity of homing and survival, which also depend on the transfer method, may vary. Direct intramyocardial delivery significantly increases the number of cells introduced into the heart in comparison with other intravascular methods.11 Bai et al. showed that intramyocardially injected cultured ASCs were retained in the heart and did not migrate in significant numbers to other organs or tissues up to 4 months after transplantation. Post-mortem histological analysis confirmed this finding, and the presence of freshly isolated ASCs was not found in other organs. Until now, molecular imaging with radioactively labelled stem cells showed that the percentage of cells homing to the heart was low, with other organs (kidney, liver, and spleen) receiving the vast majority of progenitors, both in animal models11,15 and in humans.16 The contrast between heart-homing ASCs and bone marrow-derived stem cells suggests that ASCs transdifferentiate into cardiomyocytes and vascular cells, as shown by Bai et al., and that further paracrine factors secreted by these cells are also fundamental for cell retention.

### ASCs: therapeutic applications

Lastly, the observation that transplantation of freshly isolated ASCs into ischaemic hearts promotes cardiac function as well as cultured ASC transplants do has obvious therapeutic consequences for human cardiac repair (Figure 1). Although there is some experience

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**Table 1** Cell surface characteristics of cultured and freshly isolated adipose tissue-derived stem cells (ASCs)

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<th>Freshly isolated ASCs</th>
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in the fields of plastic and reconstructive surgery and digestive surgery,\textsuperscript{17–19} no cardiovascular clinical trials on ASCs have been published to date. Nevertheless, two ongoing trials—APOLLO and PRECISE—are exploring the safety, feasibility, and efficacy of freshly isolated ASCs with the Cellution\textsuperscript{TM} system (Cytori Therapeutic Inc.) in both acute myocardial infarction and chronic myocardial ischaemia. Both trials have just completed enrolment and are finishing follow-up. PRECISE is a prospective, double-blind,
randomized, placebo-controlled, sequential dose-escalation trial randomizing patients with end-stage coronary artery disease not amenable to revascularization and with moderate-severe left ventricular dysfunction to receive freshly isolated ASCs or placebo in a 3:1 ratio. The cells are delivered via transcendocardial injections after left ventricle electromechanical mapping with the NOGA XP™ delivery system (BDS). APOLLO aims to investigate the same source of freshly isolated ASCs, although in patients with acute myocardial infarction and left ventricular ejection fraction impairment. It is also a prospective, double-blind, randomized, placebo-controlled, sequential dose-escalation trial in which ASCs are delivered through intracoronary infusion after appropriate infarct-related artery repair with stent implantation.

Several scientific and medical questions related to ASCs have yet to be answered before this approach can be applied in routine clinical practice. First, further research is necessary to establish the ideal subpopulation for cardiovascular repair, as well as the isolation and culture processes to be adopted. Therefore, consensus must be reached on the cell terminology to be used when describing multipotent precursor cells from adipose tissue stroma. Secondly, the immunogenicity of ASCs is worthy of extensive research—class I and II major histocompatibility complexes are expressed in only 1% of ASCs, and, therefore, it has been hypothesized that these could behave as universal donor cells and could be used for autologous and allogenic transplantation. In this sense, the study by Bai et al. could have provided data on inflammatory reactions due to graft rejection or the immunosuppressive regimen, if any, used in their experiment. Finally, the possibility of adverse events must be ruled out. The two main concerns are haeostasis when harvesting adipose tissue in patients with acute myocardial infarction who are on antplatelet and anticoagulant treatment and the exceptional chance of long-term tumour development.20 No cases of arrhythmia have been reported in any of the studies performed to date.

To summarize, we think that human adipose tissue is a promising alternative source of stem cells for cardiovascular repair that, as shown by Bai et al., has revealed encouraging results in the pre-clinical field. Nevertheless, the applicability of these results to humans is an important issue. The ongoing PRECISE and APOLLO trials will shed light on the safety and efficacy issues of the cardiovascular application of these excelling cells. Fat years for cardiac stem cell therapy are on the way.

Conflict of interest: none declared.

References