Adipose tissue-derived stem cells: can impure cell preparations give pure results?

Marlies E.J. Reinders¹ and Ton J. Rabelink¹,²

¹Department of Nephrology, and ²Einthoven Laboratory for Experimental Vascular Medicine, Leiden University Medical Center, Leiden, The Netherlands

Correspondence and offprint requests to: Marlies E.J. Reinders; E-mail: m.e.j.reinders@lumc.nl

Stem cells are undifferentiated cells defined by their ability for self-renewal and the capacity for multi-lineage differentiation. Over the last decade, there has been a growing interest in the use of stem cells to restore organ function, including renal failure. Biotechnology companies have also picked up on this scientific development, which is an essential step in development towards clinically applicable cell therapy products. Unfortunately, unregulated commercial exploitation of the scientific potential of stem cell therapy occurs as well, and careful scientific evaluation of its benefits and potential risks is warranted [1]. Currently, there is no universal source for stem cells available. Embryonic stem cells (ESc) exhibit unlimited differentiation potential both in vitro and in vivo, and thus would constitute the ultimate source for generation of various tissues. However, they are subject to significant legal, ethical and political concerns [2]. In addition, teratoma formation is a critical obstacle to safe clinical translation of human ESc-based therapies in the future. Another interesting cell type for possible future therapeutic applications is the induced pluripotent stem (iPS) cell. These are differentiated cells that are reprogrammed to stem cells by introducing a set of embryological transcription factors that results in (epi)genetic reprogramming of the cell [3]. However, similar to the use of ESc, the risk of malignant transformation has been shown to be high in iPS cells, while results in rodents may be difficult to translate to human cell reprogramming [3]. Therefore, the development of current therapies is mostly limited to progenitor cells isolated from adult tissue, which in comparison with totipotent stem cells have more specialization and less differentiation potential [4,5]. These cells include haematopoietic stem cells (HSCs), bone marrow-derived mesenchymal stromal cells (BM-MSCs), endothelial progenitor cells (EPCs) and adipose tissue-derived mesenchymal stromal cells (AT-MSC). Encouraging results have in particular been obtained with BM-MSCs in both pre-clinical and early-phase clinical studies, and >100 trials have been registered so far for a wide range of indications including graft-versus-host disease, osteogenesis imperfecta, myocardial infarction, systemic lupus erythematosus and diabetes [6,7]. A big challenge with the development of MSCs as a clinical therapy is the requirement to culture and expand these cells for 3–5 weeks, which necessitates technological advanced facilities and procedures, while the culture procedure may also change the phenotype and function of MSCs. Recently, adipose tissue has been identified as a novel and rich source of MSCs (AT-MSCs). AT-MSCs appear to be different from BM-MSCs in that they exhibit a different immune phenotype and a higher proliferative potential. In addition, the numbers of MSCs that can be generated from adipose tissue are higher than those that can be isolated from bone marrow. AT-MSCs can therefore be generated in a shorter time period, facilitating their clinical application. It is still largely unknown, however, to what extent the functional properties of these cells are different from BM-MSCs.

In this journal, Feng et al. take this concept one step further by investigating the potential for renoprotection of freshly isolated, uncultured adipose tissue cell extract that can be isolated in real time in sufficient quantity to apply for therapeutic applications without ex vivo expansion using the elegant CellultionTM system (Cytori Therapeutics) [8]. Depending on the method of cell isolation and harvesting, ~10⁵–10⁶ final cell therapy products per gram tissue can be obtained within 1–2 h. Time-consuming invasive protocols, which carry a risk of culture contamination and/or genetic modification with possible malignant transformation, are not necessary with this method [9–11]. In addition, there is no need of using culture serum (including fetal calf serum), which may be associated with the transmission of disease and antibody formation. Strikingly, both the fresh and cryopreserved isolates can alleviate ischaemia–reperfusion injury in rats.

Despite these fascinating observations, there still remain fundamental uncertainties regarding the use of adipose tissue-derived cell product harvested by this method. One of the biggest concerns may in fact be the heterogeneous nature of the cell product. The cell isolate is coined adipose tissue-derived stem and regenerative cells (ADRCs) by the authors [8]. Although MSCs and other progenitors may be in the cell product, the final cell product will contain a mixture of cells including fibroblasts, endothelial cells,
Obtain SVF: ADRC
1. Direct injection of ‘ADRC’
   (Cell isolation system)
2. Cryopreserve uncultured ADSCs

![Diagram](https://example.com/diagram.png)

**Fig. 1.** The stromal vascular fraction (SVF) (ADRCs named by Feng et al.) is a heterogeneous cell population derived from the adipose tissue after manipulation, including collagenase digestion. It can be used for direct injection of fresh cells and cryopreserved without culturing, or the cells can be plated and cultured. The plastic-adherent cell population consists of ADRCs that include vascular cells, adipocyte progenitor cells and adult multipotent mesenchymal stromal cells (AT-MSC), besides circulating blood cells, fibroblasts, endothelial cells (EC), smooth muscle cells (SMC) and immune cells. The AT-MSC can be expanded in culture for several passages, and the adherently derived cell population maintains its mesenchymal phenotype and multi-potency.

CD45⁺ leucocytes and smooth muscle cells (SMCs) (Figure 1) [12]. Only a small amount of the heterogeneous mixture of the cells will adhere to plastic and expand, which has been put forward as a characteristic of adipose tissue-derived stem cells that include MSCs, but also vascular (pericytes and endothelial progenitor) cells/adipocyte progenitor cells. In addition, it is uncertain to what extent freshly isolated adipose tissue-derived progenitor populations can be compared with the existing literature on culture-expanded MSCs [13]. The heterogeneity probably has implications for the mechanism of action and the safety of its use. A particular concern is the possibility of immunogenicity since CD45⁺ cells [8] and HLA-DR positivity are found in the cell product [13,14]. This not only is of relevance with respect to safety but also could offset the immunosuppressive potential of MSCs which has in fact been forwarded as an important mechanism of therapeutic action. In that respective, it would have been interesting to test whether the ADRCs by Feng provoke a mixed lymphocyte reaction when co-cultured with allogeneic peripheral blood monocytes and can suppress cytotoxic T-cell responses in vivo. To this end, some research groups have tried to remove the CD45⁺ cells and CD31⁺ cells from the isolated cells prior to their experiments [15,16].

While the use of freshly isolated adipose tissue-derived progenitors may curtail some of the discussions on potential malignant transformation in prolonged culture, the impurity of the preparation may carry risks as well. Recently, Thirabanjasak et al. reported a case of lupus nephritis treated by direct renal injection of autologous stem cells recovered from peripheral blood in both kidneys [17]. The patient developed haematuria and masses, which were seen on ultrasound and magnetic resonance imaging studies. The clinicians suspected translational cell carcinoma, but nephrectomy revealed that the masses were angiomyloloproliferative lesions. The time course and pathology entity strongly suggested that the lesion was stem cell (HSC)-derived or stem cell (HSC)-induced [17]. Another concern is that, given the lack of standardization of the fresh cell product, the influence of background diseases (e.g. renal failure and diabetes) on this heterogeneous cell population is difficult to estimate and is certainly something which needs to be taken into consideration for future experiments. It is one thing to improve ischaemia–reperfusion injury in a healthy rat; it is quite another thing to translate these observations to acute renal failure in sick people. Finally, to obtain solid clinical evidence on efficacy and safety, one has to be able to compare results between patients and between centres. This would require standardization of the final therapeutic product.

In conclusion, the observations of Feng et al. are elegant and very appealing, due to its relatively easy clinical applicability. However, as also acknowledged by the authors, a thorough scientific exploration into mechanisms, safety aspects, reproducibility and thus characterization of the cell isolate is imperative not to create unrealistic hopes and impose dangers on our patients. It is of importance that the European Union to this purpose has issued the Tissue and Cells Directives (EUTCD), which set out to ensure a safe and harmonized approach to the regulation of therapy, based on tissues and cells across Europe.

**Acknowledgements.** The authors have received funding from the European Community’s Seventh Framework Programme (FP7/2007–13) under grant agreement number HEALTH-F5-2008-223007 STAR-T REK.
Circulating cells and dialysis: improving cell number or increasing session number?

Matthieu Monge, Anton Jan van Zonneveld and Ton J. Rabelink

Department of Nephrology and the Einthoven Laboratory for Experimental Vascular Medicine, Leiden University Medical Center, Leiden, The Netherlands

Correspondence and offprint requests to: Matthieu Monge; E-mail: m.monge@lumc.nl

What—if anything—is an endothelial progenitor cell?

Chronic renal failure (CRF) and dialysis therapy are marked by a dramatic increase in cardiovascular (CV) morbidity and mortality [1]. This multifactorial outcome of CRF and dialysis is due to a combination of fluid overload, hypertension, peripheral arterial disease, coronary heart disease, and heart failure [2]. Apart from the traditional CV risk factors, CRF is associated with increased oxidative stress and endothelial progenitor cell (EPC) dysfunction. The concept of endothelial progenitor cells as vasculogenic bone marrow-derived circulating cells was first put forward in 1997 [3]. Ever since that seminal paper, numerous research laboratories have engaged in measuring EPCs in CRF and dialysis, trying to correlate the findings with the CV outcome of patients.

However, the definition of EPC has been diverse explaining some contradictory results. Indeed, two approaches are currently performed to define EPC, leading to some confusion as they assess different cellular phenotypes.

In the first approach, cells or colonies are counted that are selected by these assays do have the associated and stem cell-associated markers. Stem Cells 2006; 24: 376–385

In the second approach, cells or colonies are counted that are CFU-Hill colonies after 4 days [4]. It has now become clear that the ‘EPCs’ selected by these assays do have the capacity to augment neovascularization but lack the capacity to generate endothelial cells. In fact, the early outgrowth

References

1. Gornall J. Stem cell renegades or pioneers? BMJ 2010; 340: c2041

Received for publication: 28.9.10; Accepted in revised form: 29.9.10

© The Author 2010. Published by Oxford University Press on behalf of ERA-EDTA. All rights reserved.
For Permissions, please e-mail: journals.permissions@oxfordjournals.org

doi: 10.1093/ndt/gfq654
Advance Access publication 18 October 2010