Cytotherapy, 2015; 17: 1220–1229

International Society for Cellular Therapy
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ADIPOSE-DERIVED CELLS

Storage effect on viability and biofunctionality of human adipose tissue-derived stromal cells

MIZIED FALAH[1], ANWAR RAYAN[1] & SAMER SROUJI[2,3]

[1]*Drug Discovery Informatics Lab, QRC-Qasemi Research Center, Al-Qasemi Academic College, Baka El-Garbiah, Israel,* [2]*Oral and Maxillofacial Surgery Department, Bone Regeneration Lab, Galilee Medical Center, Nahariya, Israel, and* [3]*Faculty of Medicine in the Galilee, Bar-ilan University, Ramat Gan, Israel*

Abstract

Background aims. In our recent studies, the transplantation of human adipose tissue–derived stromal cells (ASCs) has shown promise for treatment of diseases related to bone and joint disorders. *Methods.* For the current clinical applications, ASCs were formulated and suspended in PlasmaLyte A supplemented with heparin, glucose and human serum albumin, balanced to pH 7.4 with sodium bicarbonate. This cell solution constitutes 20% of the overall transplanted mixture and is supplemented with hyaluronic acid (60%) and OraGraft particles (20%). We intended to investigate the effect of this transplantation mixture on the viability and biofunctionality of ASCs in bone formation. Freshly harvested cells were resuspended and incubated in the indicated mixture for up to 48 h at 4°C. Cell viability was assessed using trypan blue and AlamarBlue, and cell functionality was determined by quantifying their adhesion rate *in vitro* and bone formation in an ectopic mouse model. *Results.* More than 80% of the ASCs stored in the transplantation mixture were viable for up to 24 h. Cell viability beyond 24 h in storage decreased to approximately 50%. In addition, an equal degree of bone formation was observed between the cells transplanted following incubation in transplantation mixture for up to 24 h and zero-time non-incubated cells (control). *Conclusions.* The viability and functionality of ASCs stored in the presented formulation will make such cell therapy accessible to larger and more remote populations.

Key Words: *adipose tissue–derived cells, cell functionality, cell viability, human serum albumin, hyaluronic acid, OraGraft, transplantation mixture*

Introduction

Human adipose tissue–derived stromal cells (ASCs) share both structural markers and self-renewal, proliferation and differentiation capacities with mesenchymal stromal cells (MSCs), yielding multiple lineages *in vitro*, including adipocytes, osteoblasts, chondrocytes, myocytes and neuronal cells [1,2]. These cells bear therapeutic potential in regenerative medicine and tissue engineering applications [3–5]. Currently, ASCs are widely used in treatment of a variety of tissue injuries [6,7] and degenerative diseases [8,9] and have been assessed in a number of clinical trials [5,10,11]. ASCs can be isolated from various sources of adipose tissues, which are distributed across multiple locations in the body. The superficial adipose tissue and the deep subcutaneous fat constitute the largest ASCs stores, the latter accounting for approximately 80% of the total body fat. This unique reservoir of ASCs is advantageous because of its accessibility, large quantities, minimally invasive cell isolation procedures [1,5,12] and relatively lenient ethics and safety regulations. Therefore, the isolated ASCs present an attractive tool for curing a broad spectrum of disorders, injuries and diseases [4,5,13,14], including bone reconstruction [15,16]. The existing surgical techniques to reconstruct bone defects (including transplantation of autografts, allografts, xenografts and alloplasts) are at present limited and somewhat disadvantageous [15,17]. In view of this and because of the increasing incidence of osteodegenerative diseases and the large number of people suffering bone fractures, skeletal disabilities and craniofacial skeletal pathology

Correspondence: **Samer Srouji**, PhD, DMD, Department of Anatomy and Cell Biology, Bone Regeneration Lab, Technion-Israel Institute of Technology, Haifa 32000, Israel. E-mail: dr.samersrouji@gmail.com

(Received 21 January 2015; accepted 26 May 2015)

ISSN 1465-3249
http://dx.doi.org/10.1016/j.jcyt.2015.05.014

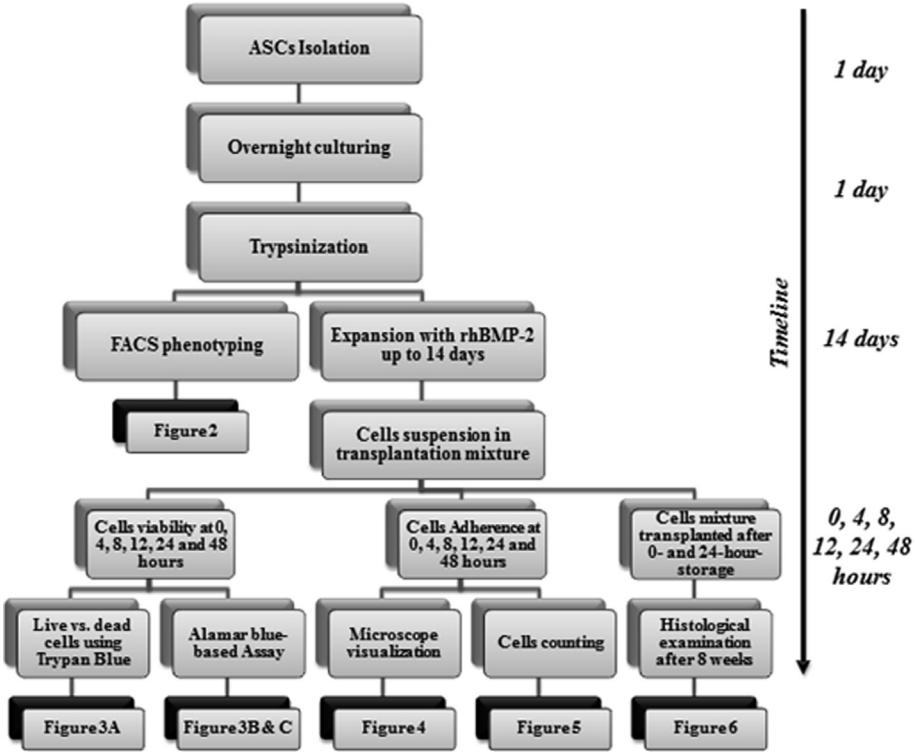

Figure 1. Schematic illustration of overall study strategy, indicating steps and assays, referring to corresponding experiment and figure.

[18], tissue engineering is enormously required. ASCs, which are available in large quantities, constitute a major player in bone regeneration [15].

The reported clinical applications using therapeutic cells have demonstrated varying degrees of effectiveness [19]. These variances have been suggested to arise from the many factors that affect cell viability and function, including cell source, inter-donor variability, cell isolation procedures, culture conditions and implantation methods [20–22]. Most important, the quality of cells awaiting transplantation and the storage conditions, including solution formulations, temperatures and duration, have a critical impact on cell quality [23].

For clinical applications, cells must be stored in a solution that is clinically approved for transplantation, safe for both the cells and the recipient. Moreover, the transplantation mixture must provide optimal conditions to preserve cells in their viable and functional state. Supportive conditions of prolonged preservation of cells enable service of larger and more remote populations that lack the facilities for cells isolation and culturing. Human serum albumin (HSA), the most abundant blood protein component (3–5 g/100 mL blood, i.e., 3–5%), provides antioxidant protection to cells in suspension and is clinically approved for use in transplantation solutions [24–26]. Likewise, hyaluronic acid (HA), a polysaccharide approved for human use, can serve as a biological scaffold and molecular "shock absorber" and stabilizer for suspended cells. In addition, it possesses anti-oxidative properties and as such reduces the toxic effects of other materials and preservatives [27–29]. Its viscoelastic properties are valuable in separating between tissues and maintaining their shape, and as such, it constitutes a component suitable for cellular therapeutics [27–29]. Moreover, the naturally occurring HA, present in connective, epithelial and neural tissues, facilitates cell proliferation and migration, two key processes required for bone formation. HA also promotes tissue hydration because it can absorb water volumes up to 3000 times its own weight [30].

We have recently formulated a mixture that can ensure safe and effective storage of ASCs until their transplantation, especially for bone regeneration. The formulation contains HSA and HA, two components shown to effectively maintain cell function and viability. The transplantation mixture is further supplemented with high concentrations of glucose, which contributes to ATP synthesis. Moreover, the added PlasmaLyte contains heparin, to prevent cell aggregation, and sodium bicarbonate, which aids in stabilizing the pH of the transplantation mixture. Similar to our pre-clinical studies with MSCs isolated from various sources [31–34], OraGraft particles are included in the transplantation mixture along with the ASCs to cure bone defects. OraGraft is a popular bone substitute used mostly for bone regeneration.