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Human adipose-derived stromal cells in a clinically applicable injectable alginate hydrogel: Phenotypic and immunomodulatory evaluation

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Abstract

Background aims. Clinical trials have documented beneficial effects of mesenchymal stromal cells from bone marrow and adipose tissue (ASCs) as treatment in patients with ischemic heart disease. However, retention of transplanted cells is poor. One potential way to increase cell retention is to inject the cells in an *in situ* cross-linked alginate hydrogel. *Methods.* ASCs from abdominal human tissue were embedded in alginate hydrogel and alginate hydrogel modified with Arg-Gly-Asp motifs (RGD-alginate) and cultured for 1 week. Cell viability, phenotype, immunogenicity and paracrine activity were determined by confocal microscopy, dendritic cell co-culture, flow cytometry, reverse transcriptase quantitative polymerase chain reaction, Luminex multiplex, and lymphocyte proliferation experiments. *Results.* ASCs performed equally well in alginate and RGD-alginate. After 1 week of alginate culture, cell viability was >93%. Mesenchymal markers CD90 and CD29 were reduced compared with International Society for Cellular Therapy criteria. Cells sedimented from the alginates during cultivation regained the typical level of these markers, and trilineage differentiation was performed by standard protocols. Hepatocyte growth factor mRNA was increased in ASCs cultivated in alginates compared with monolayer controls. Alginates and alginates containing ASCs did not induce dendritic cell maturation. ASCs in alginate responded like controls to interferon-gamma stimulation (licensing), and alginate culture increased the ability of ASCs to inhibit lymphocyte proliferation. *Discussion.* ASCs remain viable in alginates; they transiently change phenotype in alginate hydrogel but regain the phenotype of monolayer controls upon release. Cells maintain their paracrine potential while in alginates; the combination of ASCs and alginate is non-immunogenic and, in fact, immunosuppressive.

Key Words: *adipose tissue–derived stromal cells (ASCs), alginate, immunogenicity, immunosuppression, injectable hydrogel, licensing*

Introduction

Mesenchymal stromal cells (MSCs) have been used as cell-based therapies in several clinical trials in patients suffering from heart disease [1–3]. As such, MSCs have significant proven clinical regenerative abilities, although the first phase 3 study remains to be completed [4]. Originally, the mechanism of action behind this clinically proven efficiency was believed to be exerted by differentiation of MSCs to endothelial cells or cardiomyocytes and incorporation as replacement of damaged tissue. Indeed, this has been shown to occur but only for a small fraction of the injected cells, and not nearly enough to explain the functional benefits of the treatment [5,6]. Therefore, the paradigm has shifted to MSCs exerting their effects mainly by paracrine functions, stimulating angiogenesis, immunosuppression, cytoprotection, synthesis of extracellular matrix and recruitment of resident stem cells and progenitor cells [7].

Clinical studies that test treatment of heart failure with MSCs from bone marrow (BMSCs) have shown a correlation between the number of injected cells and clinical efficacy [1,8]. As such, it is reasonable to suggest that the regenerative potential of MSC paracrine activity can be enhanced by increasing

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either the number of administered cells or the time of cell residence.

Cellular tracking studies performed on humans and animals have led to the conclusion that the majority of applied MSCs are not retained around the injection site but are dispersed throughout the body of the recipient [8–11]. Therefore, it is of utmost importance to enhance the retention of injected MSCs for the cells to remain and secrete beneficial cytokines, growth factors, and extracellular matrix constituents for as long as possible [6]. This has been shown to enhance the efficacy of cellular treatment in several animal studies [12–14].

The chosen carrier must preserve viability and characteristics of co-injected cells and must be biocompatible and non-immunogenic. One method that will potentially increase retention of injected cells is injection in a hydrogel capable of solidifying at the site of injection. Alginate is an anionic polysaccharide extracted from brown seaweed, capable of forming an *in situ* hydrogel in the presence of calcium ions, such as those released in tissues at a site of injury and/or injection [15,16]. An alginate solution is already in clinical development for treatment of patients after acute myocardial infarction and seems a good candidate for a clinically approvable hydrogel in the near future [16,17]. Multipotent mesenchymal stromal cells from bone marrow and adipose tissue (ASCs) have been integrated into alginate hydrogels in previous studies [18,19]. These studies used medium- or low-viscosity solutions of alginate with high glucuronic acid content. When using biomaterials for clinical use, the clearance of the product is important. Because no human enzymes capable of cleaving the algal-derived alginate polymers have been identified, it has been suggested that only polymers smaller than 50 kDa should be used in clinical studies [20]. The suggestion of size is based on a study performed with Wistar rats, but the vascular permeability of rat kidneys resembles that of humans [21,22]. These are some of the considerations that are often overlooked in early academic research, with many carrier systems from small animal models not being translational [17]. We therefore use very low-viscosity solutions of alginate with high glucuronic acid content (VLVG) to allow for easier translation in the future.

The aim of this study was to investigate the behaviour of ASCs in a clinically applicable alginate hydrogel in terms of cell viability, phenotype, immunogenicity, and paracrine function. This was done by live/dead staining, flow cytometry and differentiation; dendritic cell (DC) co-culture; and licensing assay and lymphocyte proliferation assays, respectively. We cultured ASCs in alginate hydrogels made from VLVG. VLVG alginate with and without attached tripeptide Arg-Gly-Asp (RGD) were tested to clarify the clinical potential of VLVG alginate as an injectable carrier of regenerative ASCs and to assess whether the addition of adhesion motifs was necessary for preservation of viability and phenotype.

Methods

Donors

Lipoaspirate was obtained from eight healthy donors (six women and two men; mean age: 46 years, range 21-84 years). All participants signed an informed consent. This complied with the Declaration of Helsinki, and the study was approved by the Ethical Committee of the Capital Region of Denmark (protocol no. H-3-2009-119).

Isolation and culture of ASCs

Isolation and culture expansion of ASCs isolated form abdominal adipose tissue was performed as described earlier [23] with minor modifications. Briefly, approximately 100 mL lipoaspirate from subcutaneous abdominal fat was washed twice with phosphate-buffered saline (PBS) pH 7.4 (Gibco, Life Technologies) before digestion by incubation with Collagenase NB 4 (SERVA Electrophoresis) dissolved in Hank's Balanced Salt Solution (2 mmol/L Ca^{2+}, Gibco) at 37°C for 45 min under continuous rotation. The collagenase was neutralised with complete medium (modified Eagle's medium alpha modulation [α-MEM] low glucose 1 g/L supplemented with 25 mmol/L HEPES buffer [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid] and L-glutamin, [Gibco, Life Technologies], 10% pharmaceutical grade fetal bovine serum [FBS, Gibco, Life Technologies], 1% penicillin/streptomycin [P/S; Gibco, Life Technologies]) and filtered through a 100-μL mesh (Cell Strainer, BD Bioscience). The remaining cells were centrifuged at 1200*g* for 10 min at room temperature, re-suspended and counted using NucleoCounter NC-100 (Chemometec) according to the manufacturer's instructions.

The cells were seeded in T75 flasks (Thermo Fischer Scientific) in complete medium in a density of 4.5×10^6 cells/flask, and incubated at standard conditions (37°C, 5% CO_2 humidified air). The cells were washed after 4 days of culture to remove non-adherent cells. When reaching a confluence of 90%, the cells were passaged using TrypLE Select for detachment (Gibco, Life Technologies) and reseeded in a density of 3.5×10^5 cells/flask. When reaching confluence after the first passage, the cells were detached and frozen 1×10^6 cells/1 mL in FBS with 5% dimethyl sulfoxide (WAK-Chemie Medical)