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Stem Cells in Clinical Practice: Biological performance of human adipose -stem cells

Teodor N and Praphairaksit

Institute of Bioorganic Chemistry and Petrochemistry of the Ukrainian National Academy of Sciences., 1, Murmanska St., Kiev 94, 02660, Ukraine

***Corresponding Author:** Teodor N, Institute of Bioorganic Chemistry and Petrochemistry of the Ukrainian National Academy of Sciences., 1, Murmanska St., Kiev 94, 02660, Ukraine

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Abstract

Background: The reconstruction of adipose tissue defects is often challenged by the complications that may occur following plastic and reconstructive surgery, including donor-site morbidity, implant migration and foreign body reaction. To overcome these problems, adipose tissue engineering (ATE) using stem cell-based regeneration strategies has been widely explored in the last years. Mounting evidence has shown that adipose-derived stem cells (ADSCs) represent a promising cell source for ATE. In the context of a small number of reports concerning adipose tissue regeneration using three-dimensional (3-D) systems, the present study was designed to evaluate the biological performance of a novel alginate matrix that incorporates human ADSCs (hADSCs).

Results: Culture-expanded cells isolated from the stromal vascular fraction (SVF), corresponding to the third passage which showed the expression of mesenchymal stem cell (MSC) markers, were used in the 3-D culture systems. The latter represented a calcium alginate hydrogel, obtained by the diffusion of calcium gluconate (CGH matrix), and shaped as discoid-thin layer. For comparative purposes, a similar hADSC-laden alginate hydrogel cross-linked with calcium chloride was considered as reference hydrogel (RH matrix). Both hydrogels showed a porous structure under scanning electron microscopy (SEM) and the hADSCs embedded displayed normal spherical morphologies, some of them showing signs of mitosis. More than 85% of the entrapped cells survived throughout the incubation period of 7 days. The percentage of viable cells was significantly higher within CGH matrix at 2 days post-seeding, and approximately similar within both hydrogels after 7 days of culture. Moreover, both alginate-based hydrogels stimulated cell proliferation. The number of hADSC within hydrogels has increased during the incubation period of 7 days and was higher in the case of CGH matrix. Cells grown under adipogenic conditions for 21 days showed that both analyzed 3-D culture systems support adipogenic differentiation in terms of neutral lipid accumulation and perillipin expression. Furthermore, the cells encapsulated in CGH matrix displayed a more differentiated phenotype.

Conclusions: The results of this study suggest that both CGH and RH matrices successfully support the survival and adipogenesis of hADSC. An enhancement of biological performance was detected in the case of CGH matrix, suggesting its promising application in ATE.

Keywords: Adipose tissue engineering, hADSCs, Alginate hydrogel, 3-D culture, Adipogenesis, Viability

Background

In the last decade, advances in bioengineering and cell biology of the adipose tissue have been made and new strategies, which effectively reconstruct the soft tissue defects, have been developed [1,2]. Standard approaches on soft-tissue reconstruction are represented by autologous fat transplantation, alloplastic implants and autologous tissue flaps. However, these approaches have several disadvantages, including donor-site morbidity, implant migration and foreign body reaction. To overcome the limitations of the current restorative techniques, the engineering of the adipose tissue has been proposed as an alternative approach [3,4].

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Thus, new tissue-engineered systems for the generation of de novo adipose tissue [5,6] are increasingly developing, using the patient's own fat stem cells.

Adipose tissue-derived stem cells (ADSCs) share many similar characteristics to their counterparts in the bone marrow, including the extensive proliferative potential and the capacity to differentiate into a variety of cell types (adipocytes, osteocytes, chondrocytes, myocytes, and neurons), when cultured with the appropriate stimuli [7-11]. As a result of these features, ADSCs can be used for the regeneration and repair of acute and chronically damaged tissues [12]. In addition to the restorative medicine, ADSCs can be used for cosmetic treatments. Currently, there are two possible tissue engineering strategies to induce de novo adipogenesis [13]. One strategy consists in the in vivo induction of adipose tissue from precursor or stem cells originally existing in the body. These cells are able to proliferate and mature into adipocytes by creating a biomimetic environment through site-specific delivery of potent bioactive factors [14,15]. The second strategy is to grow in vitro the cells isolated from a patient's own tissue and seed them onto a biocompatible scaffold [12,16,17].

To create adipose tissue-engineered constructs, a diversity of biodegradable natural or synthetic polymer scaffolds has been tested in combination with animal or human adipocyte precursor cells. For instance, such synthetic scaffolds include polylactic-co-glycolic acid (PLGA) [18,19], polyglycolic acid (PGA) [20], and polyethylene terephthalate (PET) [21]. At the same time, a number of biomaterials of natural origin have been investigated for adipose tissue engineering applications, such as collagen sponges [12,22], hyaluronic acid-based scaffolds [23,24], matrigel [25], fibrin [26], and alginate gels [27,28].

The alginate gels cross-linked with calcium ions (Ca^{2+}) have been widely used for tissue engineering studies [29,30] due to their high biocompatibility, relatively low cost, reduced immunogenicity, and capacity of forming hydrogels under gentle conditions. Hydrogel-based materials have been frequently used in tissue engineering applications (especially for soft tissues) due to their particular viscoelastic properties, amiability of fabrication into specific shapes, and ability to form biocompatible solid constructs with homogeneous distribution of cells [31]. Their structures provide encapsulated cells with a 3-D environment similar to that of the extracellular matrix (ECM) of soft tissues, allowing a good transfer of gases and nutrients to maintain cell viability [32], adherence, proliferation and differentiation [33]. Furthermore, the alginate may be easily separated from the embedded cells. Thus, exposed to mild chelating agents, alginate can release the entrapped cells, and exposed to a number of ions, including sodium, the alginate may degrade itself [34].

In a previous report it was shown that attachment-dependent cells are unable to specifically interact with alginate, which promotes minimal protein adsorption, probably due to its high hydrophilic nature [32]. This shortcoming was overcome by modifying substrate surface with a peptide containing the Arg-Gly-Asp (RGD) recognition sequence, known for its ability to mimic extracellular matrix molecule binding sites and stimulate cell adhesion to material [35,36].

Numerous studies regarding cellular behavior in or on unmodified alginate hydrogels have been issued. A considerable number of these studies have been devoted to studying the effects of encapsulation of adult MSCs in alginate gels, especially on chondrogenic [37-39] and osteoblastic differentiation [40].

In the context of a small number of reports concerning adipose tissue regeneration using alginate three-dimensional (3-D) systems, the present study was designed to evaluate the biological performance of a novel alginate matrix that incorporates human adipose-derived stem cells (hADSCs). Therefore, the biological performances of two alginate hydrogel matrices, as temporary physical support for hADSCs, were compared in order to identify an appropriate environment for cell proliferation and adipogenic differentiation. These hydrogels were designed as thin layer disks and prepared by the diffusion of two different cross-linking agents (calcium chloride and calcium gluconate) in cell-loaded alginate solution. The behavior of hADSC cultured under 3-D conditions within alginate hydrogels was analyzed in terms of viability, proliferation, morphology, and adipogenic differentiation.

We found that both calcium gluconate and calcium chloride alginate hydrogels successfully support survival and adipogenic differentiation of hADSC. Moreover, an enhancement of biological performance was detected in the case of CGH matrix, suggesting its promising application in soft tissue engineering.

Methods

Primary cultures

The human subcutaneous abdominal white adipose tissue was obtained from moderately overweight women undergoing elective liposuction. All the medical procedures were performed in compliance with the Helsinki Declaration, with the approval of the Emergency Hospital for Plastic Surgery and Burns Ethical Committee (reference no. 3076/10.06.

2010). All subjects were in good health and provided their written consent before participating to in the study. None of them had diabetes, severe systemic illness, or was taking medications known as impairing the adipose tissue metabolism. Upon sampling, the lipoaspirates (LAs) were immediately processed for obtaining the stromal vascular fraction (SVF).

hADSCs were isolated as described by Gimble et al. [41]. Briefly, LAs were subjected to collagenase digestion and the obtained SVF was centrifuged at 420 g for 10 min. Then, the pellet was resuspended in growth culture medium (GCM) consisting in Dulbecco's modified Eagle's medium (DMEM, Sigma-Aldrich, Co): Ham-F12 supplemented with 1% ABAM, 1.2 g/L NaHCO3 (Sigma-Aldrich, Co) and 0.5 mM sodium pyruvate (Sigma-Aldrich, Co) and seeded at an initial density of 1.5 × 104 cells/cm2 in standard cell culture conditions. For the first 24 h, the cells have been maintained in GCM supplemented with 40% FBS to allow their attachment and then, this medium was changed to GCM containing 10% FBS.

Subcultivation

When the cells reached 80% confluence, they were harvested by trypsinization with 0.25% trypsin - 0.5 mM EDTA solution (Sigma-Aldrich, Co). After centrifugation, the cellular pellet was suspended in GCM and plated on T75 cell culture flasks (Nunc) at a cell density of 1.5 × 104 cells/cm2. Due to a decrease in the cell proliferation, the culture was propagated up to eight passages. Cell morphology was analyzed every day by phase contrast microscopy (Nikon Eclipse TS 100).

Characterization of hADSCs cells grown in 2-D culture system

Phenotypic characterization

Immunophenotypic characterization of hADSCs at the third passage was achieved by flow cytometry. We examined the expression of the following cell surface antigens: CD34 (mouse anti-human-PE conjugated monoclonal antibody, code A07776, Beckman Coulter) as a hematopoietic marker and CD44 (mouse anti-human monoclonal antibody, 1:50, code Sc-9960, Santa-Cruz Biotechnology), CD73 (rabbit anti-human polyclonal antibody, 1:50, code Sc-25603, Santa-Cruz Biotechnology), CD90 (mouse anti-human monoclonal antibody, 1:50, code Sc-59396, Santa-Cruz Biotechnology) and CD105 (mouse anti-human-PE conjugated monoclonal antibody, code A07414, Beckman Coulter) as typical protein markers in MSCs.

Flow cytometric analysis was issued on a FC 500 Cytometer (Beckman Coulter). Approximately 18000 events were acquired on flow cytometer and analyzed using CXP 2.2 software. The third passage cells were trypsinized, washed twice with 1% bovine serum albumin (BSA, Sigma-Aldrich, Co) solution, and aliquots of 1.4 × 105 cells were incubated at 4°C for 30 min with fluorescent primary antibodies. Upon removal of the excess antibodies by several washes with PBS (Gibco), the samples were subjected to cytometric analysis. Concomitantly, a negative control was prepared by incubating the cells with phycoerythrin (PE) and fluorescein (FITC) conjugated isotype control antibody solution, under the same conditions (IgG1 mouse – PE, code A07796, Beckman Coulter and IgG1 mouse – FITC, code A07795, Beckman Coulter).

3-D cell cultures within alginate hydrogel matrices

Third passage cells were detached from the monolayer by trypsin-EDTA treatment, centrifuged, counted and mixed with sterile 1.5% (w/v) low viscosity sodium alginate in 0.9% NaCl at a concentration of 7 x 105 cells/ml. The cell-alginate mixture was distributed into the wells of a 6-multiwell culture plate (Nunc) for further flow cytometric studies. For microscopy studies and spectrophotometric MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay, the cell suspension was distributed into the wells of a 12 multi-well culture plate (Nunc). To produce alginate gel, a sterile disc of Whatmann filter paper, was soaked with the cross-linking agent and placed above the alginate-cell solution. Equal volume of gelling agent was placed above the disk, and plates were incubated for 45–60 min at 37°C in a humidified atmosphere of 5% CO2. After gelling, the paper disks were removed and the remaining fluid was aspirated. The resulting thin layer alginate hydrogels were sequentially washed with 0.9% saline solution and GCM, covered with culture medium, and subjected to incubation in standard conditions.

Herein, we used two gelling agents: (1) calcium chloride solution (Sigma-Aldrich, Co) currently used for obtaining cell-laden alginate hydrogels and considered as the reference hydrogel material, and (2) calcium gluconate solution (Zentiva), which is mainly used in preparing the alginate matrix of Drug Delivery Systems. The detailed procedure will be the subject of a patent.

Characterization of hADSCs incorporated in 3-D alginate systems

Scanning electron microscopy (SEM)

The hADSCs-laden alginate hydrogels, maintained in culture for 2 and 7 days, were washed twice with PBS and fixed for 6 h in a 24-LSC Martin Christ laboratory freeze dryer. Then, the samples were coated with gold and imaged using a FEI Quanta Inspect F with field emission gun. The microscope was driven with an acceleration voltage of 30 kV and a working distance of 10 mm detecting secondary electrons.

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