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Choosing the right type of serum for different applications of human adipose tissue–derived stem cells: influence on proliferation and differentiation abilities

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Abstract

Background aims. Adipose tissue–derived stem cells (ADSCs) are thought to have great potential in regenerative medicine. A xenoprotein-free culture and handling system is desirable. To date, there is only little and contradictory information about the influence of the different types of human serum on ADSC proliferation and differentiation. *Methods.* First, ADSCs were cultured in media containing regular human serum (HS plus) or fetal calf serum (FCS plus) with supplementation of growth factors for three passages. During passage 4, ADSC proliferative activity and adipogenic, osteogenic and chondrogenic differentiation ability was quantified. Second, ADSCs were cultured with three different human sera (regular human serum [HS], human serum from platelet-poor plasma [SPPP] or human serum from platelet-rich plasma [SPRP]) without supplementation of platelet-derived growth factor and assessed accordingly. The growth factor content of the different types of human sera was determined by means of multiplex protein assay and enzyme-linked immunosorbent assay. *Results.* The different sera did not affect ADSC doubling time significantly ($P < 0.05$). Specific glycerol-3-phosphat-dehydrogenase activity was significantly lower in cultures with SPRP ($P < 0.01$) compared with the other media compositions. Extracellular calcium deposition was significantly higher in cells differentiated in cultures with HS or SPPP compared with those with SPRP, HS plus or FCS ($P < 0.01$). Glycosaminoglycan content and collagen 2 were highest in cells cultured with SPRP ($P < 0.001$). *Conclusions.* Culturing ADSCs in human serum appears to be a reasonable and efficient alternative compared with FCS. With respect to the outcome of a sighted clinical application, it appears to be feasible to handle the cells in a serum suitable for the intended later use.

Key Words: *adipose tissue, differentiation, human serum, platelet-derived growth factor, proliferation, stem cells*

Introduction

Stem cells have become more and more important in regenerative medicine and can be isolated easily and reproducibly from subcutaneous adipose tissue (1–10). They have many advantageous capabilities such as easy and long-term proliferation, multi-lineage potential and tolerance toward hypoxic environments. Scavenging and applying stem cells from adipose tissue in diverse diseases and anti-aging treatments is a promising concept in regenerative medicine. Adipose tissue is an abundant and reliable source of stem cells and can be easily harvested under local anesthesia. Adipose-tissue derived stem cells (ADSCs) have vast therapeutic potential. On occasion, they have been applied clinically, for example, in maxillary reconstruction (11), critical limb ischemia (12) or insulin-dependent diabetes mellitus (13). However, for a standardized, safe and predictable therapeutic application, further research is needed (14).

The use of ADSCs in regenerative medicine requires a sound knowledge of optimized cell isolation and handling procedures as well as culture conditions because these can significantly affect their proliferative capacity, differentiation potential and gene expression. The use of fetal calf serum (FCS) has been shown to induce changes on the gene expression level in human mesenchymal stromal cells (15). Furthermore, sera from other species can be a source of infectious diseases and can trigger allergic reactions when applied to cells intended for clinical use. Hence, for safe application, a xenoprotein-free culture and

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handling system is desirable. The diverse human serum compositions mainly differ in their growth factor content. There is only little and contradictory information about their influence on ADSC proliferation and differentiation. One reason might be that the different types of human sera are not well characterized, which leads to a multitude of different preparations with consecutive differences in its growth factor and cytokine content. This is especially true for serum from platelet-rich plasma (SPRP) and, to a lesser extent, for serum from platelet-poor plasma (SPPP) and regular human serum (HS). To gather reliable information on the influence of different sera on cell types, it is crucial to determine their amounts of growth factors and cytokines.

In the present study, we analyzed the impact of three different human serum compositions in comparison to a standard culture system that was based on FCS in regard to the proliferation and differentiation capability of human ADSCs. First, FCS and HS were compared in a standard system with supplementation of platelet-derived growth factor (PDGF) and epidermal growth factor (EGF). Second, by focusing on PDGF, we analyzed the influence of the different growth factor levels by comparing HS, SPPP and SPRP in a medium system without PDGF supplementation. To further analyze the implemented differences, we determined the levels of the most important growth factors and cytokines in the different sera.

Methods

All chemicals, if not noted separately, were purchased from Sigma-Aldrich, Munich, Germany.

Donor specification

The present study was conducted under the guidelines and with the approval of the ethics committees of the University of Heidelberg and of the medical association of the district Baden-Württemberg, Germany. After informed consent was obtained, freshly excised or suctioned subcutaneous adipose tissue of six adults (two male, four female) with a range of age of 21–57 years (median age, 32.2 years) undergoing elective plastic surgery was used for isolation of the mesenchymal stromal cells.

Isolation of ADSCs

ADSCs were isolated from freshly excised subcutaneous adipose tissue or liposuction material through the use of a procedure modified from Hauner *et al.* (16). In brief, the adipose tissue was washed in 1% bovine serum albumin (BSA)/phosphate-buffered saline (PBS), minced, and digested enzymatically with the use of collagenase (collagenase CLS; 220 U/mg, Biochrom AG, Berlin, Germany; 1.5 mg/mL, in 1% BSA/Krebs-Ringer solution) for 45 min under constant shaking at 37°C. Mature adipocytes and connective tissue were separated by centrifugation (700*g*, 7 min at room temperature). The sedimented cells were resuspended, passed through a 100-μm mesh filter (Neolab, Heidelberg, Germany) and washed twice with 1% BSA/PBS. After erythrocyte lysis (3 min, 155 mmol/L ammonium chloride, 10 mmol/L potassium bicarbonate, 0.1 mmol/L ethylenediaminetetra-acetic acid [EDTA]) cells were washed again twice and plated at a density of 2×10^4 cells/cm^2 in an expansion medium (see below). After 24 h, the medium was changed to remove non-adhered cells.

Culture of ADSCs

Cells were first cultivated in expansion medium [60% Dulbecco's modified Eagle's medium (DMEM) low glucose (1 g/L D-glucose) (Invitrogen, Life Technologies, Darmstadt, Germany); 40% MCDB-201, 1 × insulin transferrin selenium (Becton Dickinson, Franklin Lakes, NJ, USA); 10^{-8} mol/L dexamethasone; 0.1 mmol/L ascorbic acid-2-phosphate; 100 U/mL penicillin (Biochrom); 0.1 mg/mL streptomycin (Biochrom); 10 ng/mL recombinant human EGF (Miltenyi, Bergisch Gladbach, Germany) and 10 ng/mL recombinant human PDGF-BB (CellSystems, Troisdorf, Germany)]. The medium was supplemented with 2% FCS or 2% regular HS to determine the influence of xenogenic versus allogenic serum. These media—additionally supplemented with EGF and PDGF—were named FCS plus and HS plus.

In the second part of the experiment, three different human sera were tested, differing in their growth factor content as the result of different manufacturing processes: the expansion medium was supplemented with 2% regular HS, SPPP or SPRP. Because we had found that EGF is crucial for a sufficient long-term culture of ADSCs in our culture system, 10 ng/mL recombinant human EGF (Miltenyi) was also added. To evaluate the influence of PDGF as the most important growth factor in serum, PDGF-BB was not supplemented to the expansion medium when comparing the three different human serum compositions.

The medium was changed every other day. Once the cells reached 70% confluence, they were detached with 0.25% trypsin-EDTA (Biochrom) and replated with 3.5×10^3 cells per cm^2. Cultures were incubated at 37°C with the use of 5% CO_2.