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# Pooled human lysate versus fetal bovine serum—Investigating the proliferation rate, chromosome stability and angiogenic potential of human adipose tissue–derived stem cells intended for clinical use

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**Abstract**

*Background aims.* Because of an increasing focus on the use of adipose-derived stem cells (ASCs) in clinical trials, the culture conditions for these cells are being optimized. We compared the proliferation rates and chromosomal stability of ASCs that had been cultured in Dulbecco's modified Eagle's Medium (DMEM) supplemented with either pooled human platelet lysate (pHPL) or clinical-grade fetal bovine serum (FBS) ($DMEM^{pHPL}$ versus $DMEM^{FBS}$). *Methods.* ASCs from four healthy donors were cultured in either $DMEM^{pHPL}$ or $DMEM^{FBS}$, and the population doubling time (PDT) was calculated. ASCs from two of the donors were expanded in $DMEM^{pHPL}$ or $DMEM^{FBS}$ and cultured for the final week before harvesting with or without the addition of vascular endothelial growth factor. We assessed the chromosomal stability (through the use of array comparative genomic hybridization), the expression of ASC and endothelial surface markers and the differentiation and angiogenic potential of these cells. *Results.* The ASCs that were cultured in pHPL exhibited a significantly shorter PDT of 29.6 h (95% confidence interval, 22.3–41.9 h) compared with those cultured in FBS, for which the PDT was 123.9 h (95% confidence interval, 95.6–176.2 h). Comparative genomic hybridization analyses revealed no chromosomal aberrations. Cell differentiation, capillary structure formation and cell-surface marker expression were generally unaffected by the type of medium supplement that was used or by the addition of vascular endothelial growth factor. *Conclusions.* We observed that the use of pHPL as a growth supplement for ASCs facilitated a significantly higher proliferation rate compared with FBS without compromising genomic stability or differentiation capacity.

**Key Words:** *adipose tissue–derived stem cells, chromosomal stability, fetal bovine serum, mesenchymal stromal cells, pooled human platelet lysate*

## Introduction

The use of mesenchymal stromal cells (MSCs) in clinical research and regenerative therapy has gained considerable attention over the previous decade. MSCs are multipotent stem cells that can differentiate into different cell types, such as endothelial cells, adipocytes, osteocytes and myocytes (1–3). MSCs can be isolated with relative ease from a wide range of tissues, including bone marrow, adipose tissue and umbilical cord stroma. Thus far, the MSCs that have been the most thoroughly characterized are bone marrow MSCs (4).

Adipose tissue–derived stem cells (ASCs) are a relatively newly described and promising population of adult MSCs (5,6). These cells are located in the stromal vascular fraction (SVF) of adipose tissue, from which they can be isolated by enzymatic digestion. An attractive feature of ASCs, compared with bone marrow–derived MSCs (BM-MSCs), with which they share a considerable number of phenotypic characteristics, is their relative abundance (7). The regenerative potential of ASCs is believed to be mediated partly by engraftment and differentiation, a process that replaces injured cells, and partly by a paracrine effect that is facilitated by soluble mediators. (8–10). With respect to the latter, another potential advantage of ASCs in the context

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of regenerative medicine is that these cells contain higher levels of endogenous vascular endothelial growth factor (VEGF) than do BM-MSCs (11).

Currently, *ex vivo*—expanded, autologous ASCs are being investigated in the context of regenerative treatments for various diseases, such as anal fistulas (12,13) and ischemic heart disease (14). Within the field of plastic surgery, ASCs have the potential to increase the use of fat as an autologous filler. Accordingly, a number clinical trials have investigated the use of fat for correcting depressed scars (15) and breast and facial deformities (16,17).

Angiogenesis and neovascularization are key processes in regenerative therapies for various diseases, such as ischemic heart disease (18), limb ischemia (19) and ischemic stroke (20). These two processes are also critical in tissue transplantation procedures, for which swift neovascularization is necessary for graft survival (21). Previous research on the induction of neovascularization has demonstrated that VEGF may stimulate MSCs toward an endothelial-like phenotype (22,23).

The successful implementation of ASCs in clinical settings requires compliance with current Good Manufacturing Practices (CGMP) and the development of standardized protocols for the *ex vivo* expansion of ASCs. The goal of such protocols should be consistent and reliable cell growth and proliferation that maintain the desired phenotype without compromising safety. Fetal bovine serum (FBS) has traditionally been used as a growth supplement for culturing ASCs and other MSC tissue populations. However, FBS has several disadvantages, including significant batch-to-batch variation (24), possible contamination with known and unknown animal pathogens and the risk of xenoimmunization (25). To overcome these disadvantages, some researchers have attempted to replace FBS with human-derived alternatives, such as human serum or platelet lysate (PL) (26).

The aim of the present study was to facilitate compliance with CGMP by comparing the growth, differentiation potential and safety of ASCs cultured in growth media supplemented with either pooled human platelet lysate (pHPL) or FBS. In the current report, we present the results of that research and an ASC cultivation protocol that may meet the demand for rapid, high-dose clinical treatments.

## Methods

### *Isolation and culture of ASCs*

Adipose tissue was obtained from four healthy female patients after the approval of the Danish Research Ethics Committee (Protocol No. H-C-FSP-2012—043). The patients were undergoing cosmetic liposuction of the abdomen and the inner thighs with the use of the Vibrasat device (Möller Medical GmbH & Co., KG, Fulda, Germany).

Shortly after the liposuction was performed, the lipoaspirate was washed with phosphate-buffered saline (PBS) (BioWhittaker, Walkersville, MD, USA) and centrifuged for 5 min at 300*g* at room temperature. To isolate the SVF, the supernatant was incubated and enzymatically digested with Collagenase NB 4 Standard Grade (SERVA, Heidelberg, Germany) for 45—60 min at 37°C with constant rotation. The enzymatic activity was neutralized by addition of a cell culture expansion medium supplemented with either 10% FBS or 10% pHPL, according to the groups described below. The suspension was filtered through a 100-μm filter and centrifuged for 10 min at 1200*g* at room temperature. The cell pellet was resuspended in the appropriate culturing medium in 50-mL tubes and was filtered through a 70-μm filter. Last, the cell suspension was centrifuged for 10 min at 1200*g* at room temperature, and the cells in the pellet that contained the SVF were counted with the use of a Countess Automated Cell Counter (Invitrogen, Taastrup, Denmark). The latter step was performed to measure the total concentration of viable cells that were obtained by processing the lipoaspirate.

The SVF was seeded in 175-$cm^2$ flasks (NUNC, Thermo Fisher Scientific, Roskilde, Denmark) at a density of approximately 85 000 cells/$cm^2$. The culture medium consisted of Dulbecco's modified Eagle's medium (DMEM) (PAA Laboratories, Pasching, Austria), 1% penicillin-streptomycin (GIBCO-Invitrogen, Taastrup, Denmark), 1% GlutaMAX (GIBCO-Invitrogen) and 10% of either FBS or pHPL. In addition, 2 IU/mL preservative-free heparin (LEO Pharma, Ballerup, Denmark) was added to the pHPL-supplemented medium. The two culture media are hereafter referred to as $DMEM^{FBS}$ and $DMEM^{pHPL}$, respectively. CGMP-approved FBS from a specific lot (A51210—2424), which was batch-tested against five other lots for its ability to support ASC proliferation, was used throughout the experiment. The primary cultures (P0) were incubated for 4 days in a humidified atmosphere that contained 95% air and 5% $CO_2$ at 37°C. After the first day of cultivation, the non-adherent cells were discarded, the cell culture flasks were carefully rinsed with PBS and the medium was replaced.

### *pHPL production*

The pHPL was manufactured as described by Schallmoser *et al.* (26), with minor modifications. Briefly, after informed consent was obtained, whole blood units were collected from blood donors, all of whom were regular members of the voluntary, unpaid