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Serum-free, xeno-free culture media maintain the proliferation rate and multipotentiality of adipose stem cells *in vitro*

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Background aims

Human adipose stem cells (ASC) are an abundant, readily available population of multipotent progenitor cells that reside in adipose tissue. ASC have been shown to have therapeutic applicability in pre-clinical studies, but a standardized expansion method for clinical cell therapy has yet to be established. Isolated ASC are typically expanded in medium containing fetal bovine serum (FBS); however, sera and other culturing reagents of animal origin in clinical therapy pose numerous safety issues, including possible infections and severe immune reactions.

Methods

To identify optimal conditions for ex vivo *expansion of ASC, the effects of seven serum-free (SF) and xeno-free (XF) media were investigated with both FBS and allogeneic human serum (alloHS; as a control media). Surface marker expression, proliferation, morphology and differentiation analyzes were utilized for investigating the effects of media on ASC.*

Results

The proliferation and morphology analysis demonstrated significant differences between ASC cultured in SF/XF culture media compared with serum-containing culture media, with medium prototype StemPro® MSC SFM XenoFree providing significantly higher proliferation rates than ASC cultured in media containing serum, while still maintaining the differentiation potential and surface marker expression profile characteristic of ASC.

Conclusions

Looking forward, fully defined XF media formulations will provide the means for the development and approval of safer clinical cell therapy treatments. However, to fully recognize the capacity of these XF culture media, further pre-clinical safety and efficacy studies must be performed.

Keywords

adipose stem cells, fetal bovine serum, flow cytometry, human serum, multipotentiality, serum-free, xeno-free.

Introduction

Adipose stem cells (ASC) are an attractive and abundant stem cell source with therapeutic applicability in pre-clinical studies in diverse fields, but standard expansion methods have not yet been established for cells aimed at clinical use. Importantly, unlike the human bone marrow stromal/stem cells (BMSC) that are present at low frequency in the bone marrow (BM), ASC can be retrieved in high numbers from either liposuction aspirates or subcutaneous adipose tissue fragments, and can easily be expanded *in vitro* [1]. ASC display properties similar to those observed in BMSC and, upon induction, undergo adipogenic, osteogenic,

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chondrogenic, neurogenic and myogenic differentiation *in vitro* [2–11].

In addition, ASC exhibit immunoprivileged properties [12,13]. ASC do not express HLA-DR (major histocompatibility complex class II; MHC II) and suppress proliferation of activated allogenic lymphocytes *in vitro* [12,14–16]. Furthermore, *in vitro* and *in vivo* results show that ASC promote engraftment and prevent or treat severe graft-versus-host disease (GvHD) in allogeneic stem cell transplantation [17–20].

Nevertheless, expansion of ASC is necessary prior to performing clinical studies. Currently, standard *in vitro* cell expansion techniques utilize fetal bovine serum (FBS) and other reagents such as trypsin, serum albumin and growth factors of animal origin as part of the ASC cultivation workflow. Because of critical safety issues, use of animal-derived reagents in clinical cell therapy applications is not a preferred option. Transplantation of human cells exposed to animal-derived products transfers xenogeneic antibodies, such as Neu5GC, into the human body and may trigger a severe immune response [21–23]. Severe anaphylaxis and immune reactions [24,25] have been reported to be induced in patients transplanted with human cells exposed to animal-derived reagents. Other possible risks include viral and bacterial infections, prions and as yet unidentified zoonoses [25,26].

Several criteria are set for the application of adult stem cells in human subjects: safety, reproducibility and quality. Yet contradictory results regarding the replacement of FBS with pooled allogeneic human serum (alloHS; as a control media) [27,28], platelet-rich plasma [27] and human platelet lysate [29] have been reported. Furthermore, serum by composition shows some lot-to-lot variability that may affect reproducibility [30–33]. Autologous serum, on the other hand, has been reported to make BMSC proliferate faster and differentiate less rapidly [13,34], which also appears to be the case for ASC (B. Lindroos and S. Miettinen, unpublished observations), but autologous serum availability may be limited.

Clinical stem cell therapy studies using ASC are under way [35,36], which calls for a strong focus on the safety, reproducibility and quality of transplanted *in vitro*-expanded stem cells. By replacing FBS and other animal-derived cell culture reagents with allogeneic human serum (HS) or a defined serum-free (SF), xeno-free (XF) media formulation, the safety and quality of the transplanted stem cells may be enhanced significantly [27,33,37–42].

Earlier reports have published the characterization of ASC grown in culture media containing FBS and HS [27,33,43]. To compare the effect of culture media containing FBS or HS with SF or XF culture media, we analyzed the cell proliferation frequency, multilineage differentiation capacity and surface marker expression profiles. We carried out comparative flow cytometric analysis of ASC cultured in completely XF and SF/XF conditions. Furthermore, proliferation studies and differentiation assays were performed in SF and XF conditions, which have not been reported previously. To our knowledge, this is the first extensive study conducted on ASC grown in SF or XF conditions.

Methods

The study was conducted in accordance with the ethics committee of the Pirkanmaa Hospital District (Tampere, Finland). ASC were isolated from adipose tissue samples collected from female donors ($n = 24$, age 52 ± 12 years) undergoing elective surgical procedures in the Department of Plastic Surgery, Tampere University Hospital (Tampere, Finland).

Isolation of ASC from adipose tissue samples was carried out using a mechanical and enzymatic method as described previously [10,44]. Briefly, the adipose tissue was minced manually into small fragments and digested with 1.5 mg/mL collagenase type I (Life Technologies, Paisley, UK; catalog number 17100-017) in a water bath at 37°C under shaking conditions. The digested tissue was centrifuged and filtered in sequential steps to separate the ASC from the surrounding tissue. The isolated cells were then expanded in Dulbecco's modified Eagle medium (DMEM)/F-12 1:1 (Gibco Life Technologies, Paisley, UK; reference number 21331) supplemented with 1% L-analyl-L-glutamine (GlutaMAX I; Life Technologies; catalog number 35050-061), 1% antibiotics/antimycotic (a/a; 100 U/mL penicillin, 0.1 mg/mL streptomycin and 0.25 µg/mL amphotericin B; Life Technologies; reference number 15240) and serum from either 10% FBS (Invitrogen, Paisley, UK; catalog number 10108-165) (Medium FBS) or 10% alloHS (PAA Laboratories GmbH, Pasching, Austria; catalog number C15-021, lot number C02106-1878) (Medium HS) (Table I). ASC from the donors were isolated into both Medium FBS and Medium HS. ASC isolated and expanded in Medium FBS (10%; Invitrogen; catalog number 10108-165) were detached using 1% trypsin (Lonza/Biowhittaker, Verviers, Belgium) (Table I). and ASC isolated and expanded in