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Serum-converted platelet lysate can substitute for fetal bovine serum in human mesenchymal stromal cell cultures

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Abstract

Background aims. Fetal bovine serum (FBS) is commonly used as a serum supplement for culturing human mesenchymal stromal cells (hMSCs). However, human cells grown in FBS, especially for extended periods, risk potential exposure to bovine immunogenic proteins and infectious agents. To address this issue, we investigated the ability of a novel human platelet serum supplement to substitute for FBS in hMSC cultures. *Methods.* Platelet lysate-serum (PL-serum) was converted from platelet lysate-plasma (PL-plasma) that was manufactured from pooled platelet-rich plasma (PRP) apheresis units. Growth factor levels and the number of residual intact platelets in PL-serum and PL-plasma were compared with enzyme-linked immunosorbent assays and flow cytometry, respectively. Proliferation responses of hMSCs cultured in PL-serum, PL-plasma, or FBS were assessed with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, the immunophenotype of harvested hMSCs was evaluated by flow cytometry and tri-lineage differentiation potential was evaluated by assessing adipogenic, osteogenic and chondrogenic development. *Results.* Selected growth factor levels in PL-serum were not significantly different from PL-plasma ($P > 0.05$). hMSC cultures supplemented with PL-serum had comparable growth kinetics to PL-plasma, and hMSC yields were consistently greater than with FBS. hMSCs harvested from cultures supplemented with PL-serum, PL-plasma or FBS had similar cell surface phenotypes and maintained tri-lineage differentiation potential. *Conclusions.* PL-serum, similar to PL-plasma, can substitute for FBS in hMSC cultures. Use of PL-serum, in contrast to PL-plasma, has an added advantage of not requiring addition of a xenogeneic source of heparin, providing a completely xeno-free culture medium.

Key Words: *blood platelets, cell proliferation, growth factors, mesenchymal stromal cells, platelet-rich plasma*

Introduction

Fetal bovine serum (FBS) is derived from clotted whole blood or plasma from bovine fetuses and is widely used as an *in vitro* medium supplement for cultures of animal and human cells. FBS is a rich source of growth factors and contains other proteins needed for cell growth (1,2). A major disadvantage of using FBS is that human cells grown in FBS, especially for extended periods, carry a risk of exposure to potentially immunogenic residual bovine proteins and possible contamination with infectious agents (e.g., the prion causing mad cow disease or bovine spongiform encephalopathy) (3,4). Another disadvantage of using FBS is the lot-to-lot differences in activity secondary to variable concentrations of factors contained within FBS that facilitate cell growth and differentiation (5). When culturing a given cell type, it is recommended that several FBS lots be tested and pre-selected to identify batches of FBS with comparable activities (2).

Despite the inherent disadvantages of FBS, it continues to be a key component in manufacturing clinical-grade hMSCs. There are >200 phase I/II clinical trials evaluating the safety or efficacy of hMSCs for treating various acute and chronic diseases (204 known status, 125 open studies, according to www.clinicaltrials.gov as of March 25, 2013). hMSCs are isolated from numerous sources; however, bone marrow and adipose tissue are primary sources of hMSCs for clinical trials. Regardless of the source of hMSCs, these cells occur at a very low frequency, and *ex vivo* expansion is necessary to generate the numbers required for clinical applications (6). hMSC expansion culture strategies typically involve passage of cells twice over a period of 3–4 weeks using culture medium that traditionally contains FBS as a growth supplement.

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Although FBS successfully supports hMSC expansion cultures, the use of FBS in human cell cultures for stem cell therapies has been prohibited since 2001 in Germany, and similar regulatory recommendations are foreseen throughout the European Union and in the United States (7). Consequently, with the anticipated requirement for large numbers of clinical-grade hMSCs, there is a clear need to find a suitable FBS replacement and to develop off-the-shelf reagents to facilitate consistent production of hMSCs compliant with current Good Manufacturing Practices in a xeno-free system.

One approach to achieve a xeno-free system is to develop defined serum-free, growth factor-supplemented media that support expansion of hMSCs. Several formulations are evolving and are being optimized (8–13). However, hMSCs grown in serum-free or completely defined media appear to be smaller in size and have lower population doubling times. hMSCs grown in serum-free conditions also reach senescence at an earlier passage and have lower alkaline phosphatase activity after induction of osteogenic differentiation than hMSCs grown in media supplemented with FBS (11,14). Most defined media for hMSCs also lack the molecules needed for hMSC attachment and require pretreatment of culture vessels with fibronectin or collagen (11,12).

Another approach is to replace FBS with human serum (3,15–18). However, results from studies using pooled human serum are contradictory. Some studies show successful expansions of hMSCs (16,18), whereas others indicate that pooled human serum and serum from elderly individuals interfere with the proliferation or differentiation capacity of hMSCs (3,15,17).

A third strategy is to replace FBS with platelet lysate-plasma (PL-plasma), which is derived from platelet-rich plasma (PRP). PL-plasma shows promise as an alternative substitute for FBS in expansion cultures of bone marrow-derived hMSCs (19–27), partly owing to the growth factors released from platelets when PRP undergoes one or several freeze/thaw cycles to manufacture PL-plasma. Among the growth factors released are platelet-derived growth factors (PDGF), basic fibroblast growth factor (bFGF), endothelial growth factor (EGF), vascular endothelial growth factor (VEGF) and transforming growth factor (TGF)-β (9,19,28). When hMSCs are grown in PL-plasma, it has been shown that the cells display morphologies, proliferative capacities, phenotypes, immunomodulatory properties and tri-lineage differentiation potential comparable to hMSCs grown in FBS (19–27). However, a major drawback of PL-plasma is that it contains fibrinogen and other coagulation factors. Its use as a culture supplement requires the addition of heparin to prevent coagulation and clot formation (16). Commercially available heparin is manufactured primarily from porcine sources, and although it is approved for human use, hypersensitivity to heparin is reported (29,30). Efforts to remove a xenogeneic serum supplement by replacing FBS with PL-plasma are nullified by requirements to use a xenogeneic source of heparin.

To develop a completely xeno-free culture medium for hMSCs, we investigated the feasibility of converting PL-plasma to PL-serum. In this study, we present a simple manufacturing approach to convert PL-plasma to PL-serum and show the impact of serum conversion on residual platelet counts and growth factor concentrations in PL-serum. We also report on the relative abilities of PL-serum, PL-plasma and FBS to support hMSC proliferation and tri-lineage differentiation.

Methods

Preparation of PL-plasma and PL-serum

Expired single-donor platelet apheresis (i.e., PRP) units, collected by the American Red Cross, were shipped within 3 days post-expiration to the Cell Therapy and Regenerative Medicine facility at the University of Utah. A lot comprised five to six pooled single-donor apheresis units (total volume of about 1.3 L per lot), and platelet counts were performed using a Sysmex XE-5000 Automated Hematology System (Sysmex America, Mundelein, IL, USA).

Pooled PRP was frozen, thawed and processed to obtain PL-plasma and PL-serum. PL-plasma was produced from pooled PRP that was frozen at −80°C or snap-frozen at −196°C and thawed at 4°C or 37°C. After thawing, pooled PRP was centrifuged at 4000*g* for 20 min, and the supernatant was collected to obtain PL-plasma. PL-serum was manufactured by adding calcium chloride (20 % w/v) to thawed pooled PRP at a ratio of 1:100 and mixing the product. After allowing the product to form a clot overnight at 4°C, the coagulated product was centrifuged at 4000*g* for 20 min, and the supernatant was collected to obtain PL-serum.

Growth factor analyses

A quantitative solid-phase sandwich enzyme-linked immunoassay was performed to determine growth levels for PDGF-AA, PDGF-AB, PDGF-BB, EGF, bFGF, insulin-like growth factor (IGF), TGF-β and VEGF (Quantikine kit; R&D Systems, Minneapolis, MN, USA). The assays were performed according to the manufacturer's instructions. Briefly, 50–100 µL of PL-plasma or PL-serum at optimized dilutions was added in duplicate to micro-plate wells pre-coated