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Overcoming the bottleneck of platelet lysate supply in large-scale clinical expansion of adipose-derived stem cells: A comparison of fresh versus three types of platelet lysates from outdated buffy coat–derived platelet concentrates

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Abstract

Background. Platelet lysates (PL) represent a promising replacement for xenogenic growth supplement for adipose-derived stem cell (ASC) expansions. However, fresh platelets from human blood donors are not clinically feasible for large-scale cell expansion based on their limited supply. Therefore, we tested PLs prepared via three methods from outdated buffy coat–derived platelet concentrates (PCs) to establish an efficient and feasible expansion of ASCs for clinical use. *Methods.* PLs were prepared by the freeze-thaw method from freshly drawn platelets or from outdated buffy coat–derived PCs stored in the platelet additive solution, InterSol. Three types of PLs were prepared from outdated PCs with platelets suspended in either (1) InterSol (not manipulated), (2) InterSol + supplemented with plasma or (3) plasma alone (InterSol removed). Using these PLs, we compared ASC population doubling time, cell yield, differentiation potential and cell surface markers. Gene expression profiles were analyzed using microarray assays, and growth factor concentrations in the cell culture medium were measured using enzyme-linked immunosorbent assay (ELISA). *Results.* Of the three PL compositions produced from outdated PCs, removal of Intersol and resuspension in plasma prior to the first freezing process was overall the best. This specific outdated PL induced ASC growth kinetics, surface markers, plastic adherence and differentiation potentials comparable with PL from fresh platelets. ASCs expanded in PL from fresh versus outdated PCs exhibited different expressions of 17 overlapping genes, of which 10 were involved in cellular proliferation, although not significantly reflected by cell growth. Only minor differences in growth factor turnover were observed. *Conclusion.* PLs from outdated platelets may be an efficient and reliable source of human growth supplement allowing for large-scale ASC expansion for clinical use.

Key Words: *adipose-derived stem cells, cell proliferation, cell therapy, gene expression, growth factors, platelet lysate*

Introduction

Mesenchymal stromal cells (MSCs) from bone marrow and adipose tissue have been tested in a wide variety of indications due to their immunomodulatory, regenerative and trophic properties [1,2]. Approximately 500 clinical trials, currently ongoing or completed, have focused primarily on alleviating bone and cartilage, heart, graft-versus-host, neurological and autoimmunologic diseases [3–6]. *Ex vivo* expansion is often necessary because of the relatively low number of MSCs at donor sites [7,8]. For *ex vivo* expansion, the standard growth supplement (GS) remains fetal bovine serum (FBS) [9,10], which is associated with risks of xeno-immunization and the transmission of bovine pathogens. To circumvent these drawbacks, non-xenogeneic alternatives have been thoroughly investigated. In particular, human platelet lysates (PLs) produced from platelet concentrates (PCs) have been repeatedly identified as superior or comparable with

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FBS in promoting MSC expansion [11–21]. Most studies have been conducted using PLs from fresh donor platelets; however, such PLTs are a scarce blood component and are in high demand by clinicians for direct patient care [22,23].

In a previous clinical trial, we found that high-dose enrichment of fat grafts with *ex vivo* expanded adipose-derived MSCs (ASCs) significantly improved fat graft survival [24]. To implement the use of clinically relevant ASC doses, upscaling the cell expansion process is necessary. In fact, in an ongoing clinical trial of fat grafting to the female breast with high doses of ASCs, at least 5000 mL of PLs for cell expansion per patient is required [25]. Thus, an alternative to the previous use of PLs prepared from fresh buffy coat units is necessary to avoid depleting the limited supply of PLs, which represents a bottleneck step in large-scale ASC expansion for clinical use.

Although fresh platelets (PLTs) are scarce, outdated PLTs are discarded as biological waste on a regular basis. PCs are only kept available for transfusion for 5 to 7 days before being discarded because storage impairs the *in vivo* function of PLTs and increases the risk of bacterial contamination [26,27]. Thus, despite strict inventory planning, 13%–17% of manufactured PCs expire prior to clinical use [28,29]. In the production of PL, the *in vivo* platelet function is not required, but rather the cellular content of growth factor and cytokines. Therefore, outdated PCs might cover the demand for PLs in cell expansion by using otherwise discarded blood components from voluntary donors.

Although there are only limited studies on PLs prepared from outdated PCs, the results have been contradictory and studies have only been performed for the expansion of bone marrow–derived MSCs [30,31],which has been shown to differ from ASCs in terms of immunophenotype and differentiation ability [8,32,33]. Furthermore, in Europe, preparing PLs from expired PCs is a challenge because the favored method for PC production is the buffy coat method, which usually involves the storage of PLTs in a platelet additive solution (PAS) [34,35]. The use of PAS instead of plasma in the final growth medium may influence ASC growth either due to the presence of PAS or the lack of plasma components. However, centrifugation of outdated PCs followed by resuspension in plasma may lead to a loss of variety of valuable platelet-derived growth factors that are released from platelets during the storage period. Therefore, we tested three different methods to produce PLs from outdated PCs, as described in the Materials and Methods section.

In the present study, we compared PLs prepared from outdated PCs versus fresh PCs for ASC expansion with respect to their proliferative and differentiation capacities and their surface marker expression and gene expression profiles. Additionally, we compared the concentrations of growth factors in the culture media during ASC expansion. We chose low passage ASCs (P1) because the current recommendations for MSCs in clinical trials focus on keeping the number of passages/population doublings as low as possible to avoid cellular senescence and the risk of chromosomal aberrations [36,37].

Materials and methods

Isolation of the stromal vascular fraction from adipose tissue and ASC expansion

The stromal vascular fraction (SVF) was isolated from the lipoaspirate of six healthy female donors, aged 25–42 years, who underwent abdominal liposuction.

Approvals were obtained from the National Danish Ethics Committee and the Danish Health and Medicines Authority (EudraCT no. 2014-000510-59) [25].

After liposuction, SVF cells were manually isolated by enzymatic digestion in standard grade collagenase NB4 (SERVA Electrophoresis GmbH) suspended in Hank's balanced salt solution (HBSS x 1,Ca^{2+}, Mg^{2+}; Gibco, Life Technologies), as previously described [11]. The isolated SVF cells were seeded at a density of 5000 cells/cm^2, and non-adherent cells were removed by changing the media after 2 days of cell culture. ASCs in the primary passage (P0) were harvested using the dissociation agent TrypLe Select (Gibco, Fisher Scientific) at near confluence, and the cells were subsequently seeded at a density of 1000 ASCs/cm^2 in passage 1 (P1). ASCs in P1 were used for all assays in the present study.

In addition to either 10% or 20% compositions of the various PLs, as specified below, the culture medium was composed of 1 g/L glucose Dulbecco's modified Eagle's medium (DMEM) (Gibco, Life Technologies), 2 U/mL heparin (LEO Pharma) and 1% penicillin-streptomycin (10 000 units/mL; Gibco, Life Technologies).

Production of platelet lysate

Whole blood units were collected from blood donors who were regular members of the voluntary blood donor corps of the Capital Region of Denmark. Blood donations were tested for infectious disease markers in accordance with Danish law.

PLs from freshly derived buffy coats and plasma (F-PL) were produced according to the procedure described by Schallmoser *et al.* [38] with minor modifications, as previously specified [11]. In short, four buffy coat units were pooled with 1 U of plasma into 1 U of platelet-rich plasma (PRP), which was stored at –40°C until the ordinary release criteria were fulfilled. Thereafter, 10 U of PRP were thawed in a 37°C plasma water bath (Plasma Thawing System, Hemler), pooled