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Combined platelet and plasma derivatives enhance proliferation of stem/progenitor cells maintaining their differentiation potential

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Abstract

Background aims. Platelet derivatives have been proposed as alternatives to animal sera given that for cell therapy applications, the use of fetal bovine/calf serum (FBS/FCS) is subjected to severe limitations for safety and ethical concerns. We developed a cell culture medium additive obtained by the combination of two blood-derived standardized components. *Methods.* A platelet lysate (PL) and a platelet-poor plasma (PPP) were produced in a lyophilized form. Each component was characterized for its growth factor content (platelet-derived growth factor-BB/vascular endothelial growth factor). PL and PPP were used as single components or in combination in different ratio at cumulative 5% final concentration in the culture medium. *Results.* The single components were less effective than the component combination. In primary cell cultures (bone marrow stromal cells, adipose derived adult stem cells, osteoblasts, chondrocytes, umbilical cord–derived mesenchymal stromal cells, lymphocytes), the PL/PPP supplement promoted an increased cell proliferation in respect to the standard FCS culture in a dose-dependent manner, maintaining the cell functionality, clonogenicity, phenotype and differentiative properties throughout the culture. At a different component ratio, the supplement was also used to support proliferation of a cell line (U-937). *Conclusions.* The PL/PPP supplement is an efficient cell culture medium additive that can replace FCS to promote cell proliferation. It can outdo FCS, especially when adopted in primary cultures from tissue biopsies. Moreover, the dual component nature of the supplement allows the researcher to determine the more appropriate ratio of the two components for the nutritional and functional requirements of the cell type of interest.

Key Words: *differentiation, fetal calf serum, platelet lysate, platelet-poor plasma, proliferation, stem progenitor cells*

Introduction

Cells are usually cultured *in vitro* in media containing animal serum that provides growth factors, proteins, vitamins, trace elements and hormones, essential for their growth and maintenance. The present gold standard is fetal calf serum (FCS), used as medium supplement in a 10% to 20% concentration range. The availability and ease of storage of FCS [1], together with its high content of growth factors and the low antibodies level [2] in comparison with other animal derived sera, have led to its adoption as the conventional medium additive. However, several scientific, technical and ethical concerns were raised by the scientific community regarding the use of FCS, especially for the culture of cells intended for clinical applications.

There are high qualitative and quantitative variations between the different FCS batches [3–5], and these differences could be responsible for the lack of consistency sometimes observed when comparing the results obtained in different laboratories or in the same laboratory at different times. For this reason, each laboratory must select the "best" batch of FCS for its specific culture needs by expensive and time-consuming testing of different lots. However, the amount of serum for each batch is limited and the testing of different lots must be repeated again and again. Safety concerns are related to the fact that the bovine serum is a potential source of contamination by viruses, mycoplasmas and prions. The zoonotic infection risk is represented by the transmission of viruses such as bovine viral diarrhea virus and bovine

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parvovirus [6–9] and of prions responsible for bovine spongiform encephalopathy (BSE) in cows and for its human equivalent variant, Creutzfeldt-Jacob disease [10,11]. Moreover, ethical concerns have been raised about the suffering of the unborn calves during the blood harvest for the serum production [12–14].

The European Commission released a note for "Guidance on Minimizing the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Human and Veterinary Medical Products" (EMA/410/01) that clearly states that the use of material of non-animal origin should be preferred in the cell culture. Hence, human alternatives to FBS are being tested in several laboratories. The use of human serum as a source of essential nutrients, adhesion factors and growth factors for cell growth was reported to be controversial on mesenchymal stromal cells (MSC) [15–18]. Platelet derivatives have been extensively tested as FCS alternatives in different formulations and concentrations. The rationale behind the use of platelet-derived components is the presence of a "cocktail" of growth-promoting factors entrapped in platelets. Under physiological conditions, growth factors are released by platelet activation and this release can be mimicked *in vitro* to prepare growth factor–rich fluids. Different procedures were proposed that can exert significant impacts on the platelet, leucocyte and plasma protein content [19] in the final fluid concentrate and ultimately on the concentration and type of growth factor released. In one method, growth factors are released through alternating phases of freezing and thawing [20–22]. Other methods propose the use of platelet sonication [23] or the platelet activation by thrombin/$CaCl_2$ treatment [24–28] to obtain the growth factor release. The contents of fibrinogen and fibronectin are higher in platelet lysates (PL) obtained by the freeze-thaw procedure compared with those produced by activation with trombin [29]. When the PL is used as cell culture medium supplement, high fibrinogen levels require the addition of anticoagulants, such as heparin, to avoid clotting due to the conversion of fibrinogen into fibrin [30,31].

In the present study, we developed a dual component platelet and plasma derivative to be used as cell culture supplement to replace FCS. It was derived from pools of discarded blood donation units and was standardized in its content of growth factors. The dual constitution of the supplement allowed us to establish the more appropriate ratio of the two components for the nutritional and functional requirements of the cell type of interest. We used the new additive both for large-scale expansion of human primary cultures derived from several tissues and for the culture of established cell lines. Optimization of the cell proliferation in the two types of cultures required different PL concentrations. Primary cultures required higher PL concentrations than cell lines.

Methods

Dual-component cell culture medium supplement preparation

PL was produced starting from buffy coat samples obtained from the whole blood of healthy donors at the Blood Transfusion Center of the San Martino-IST Hospital, Genoa. All the procedures described were performed in a closed-bag system. Blood donors were screened according to the Italian law for the blood banks and were negative for HIV, HBV, HCV and syphilis. Five to 10 buffy coat bags were connected by means of sterile junctions. The pooled buffy coats were centrifuged at 1100 rpm for 10 min.

After the centrifugation, the fraction including the plasma and the buffy layer—the platelet-rich plasma (PRP)—was transferred to a new blood bag. A complete blood count (CBC) was performed with the use of an electronic hematology analyzer (Siemens ADVIA 2120, Siemens Healthcare). The PRP was then centrifuged at 2600 rpm for 20 min to sediment the platelets. After the centrifugation, the upper phase, the platelet-poor plasma (PPP), was transferred to a new blood bag and centrifuged at higher speed to remove the debris. The platelet concentration of the pellet was adjusted by addition of PPP until a final platelet count of 10×10^6 plt/μL. The PRP bag and the PPP bag were then frozen and stored at −20°C until further processing. To break the platelet membranes and release the platelet content, the PRP bag was exposed to three freeze-thawing cycles. After the freeze-thawing cycles the PRP was centrifuged at high speed for 20 min at room temperature (RT) to precipitate the broken platelets membranes and the supernatant—the PL—was recovered in a new blood bag and, if not immediately processed, stored at −20°C.

For the final processing, a sodium-heparin solution (Epsoclar, Biologici Italia Laboratories S.r.l.) was added to both the PL and the PPP at the final concentration of 40 U/mL to prevent gel formation when they were used as supplement in the cell culture medium. The PL and PPP components were then divided into aliquots, lyophilized and stored at −20°C. The platelet derivatives lyophilization was carried out with the Heto LyoPro 3000 (Analytical control De Mori) for 20 h and the vials containing the lyophilized products were closed under vacuum