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The effect of platelet lysate fibrinogen on the functionality of MSCs in immunotherapy

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ABSTRACT

Human platelet lysate (PL) represents an attractive alternative to fetal bovine serum (FBS) for the *ex vivo* expansion of human mesenchymal stromal cells (MSCs). However, there is controversy whether MSCs propagated in unfractionated PL retain their immunosuppressive properties. Since fibrinogen can be a major component of PL, we hypothesized that the fibrinogen content in PL negatively affects the suppressor function of MSCs. Pools of outdated plateletpheresis products underwent a double freeze–thaw centrifugation and filtration to produce unfractionated platelet lysates (uPL), followed by a temperature controlled clotting procedure to produce a fibrinogen depleted platelet lysate (fdPL). Fibrinogen depletion affected neither the mitogenic properties of PL or growth factor content, however fdPL was less prone to develop precipitate over time. Functionally, fibrinogen interacted directly with MSCs, dose dependently increased IL-6, IL-8 and MCP-1 protein production, and compromised the ability of MSCs to up-regulate indoleamine dioxygenase (IDO), as well as, mitigate T-cell proliferation. Similarly uPL expanded MSCs showed a reduced capability of inducing IDO and suppressing T-cell proliferation compared to FBS expanded MSCs. Replacing uPL with fdPL largely restored the immune modulating effects of MSCs. Together these data suggest that fibrinogen negatively affects the immunomodulatory functions of MSCs and fdPL can serve as non-xenogenic mitogenic supplement for expansion of clinical grade MSCs for immune modulation.

1. Introduction

Despite the rarity of MSCs in the bone marrow, their pro-angiogeneic [1], immune modulatory features [2] and their expandability *ex vivo* makes them clinically useful [3]. Presently there have been over 200 registered clinical trials using MSCs worldwide (http://clinicaltrials.gov, search was performed using most known names of MSCs). Most of these studies targeted immune-related disorders (multiple sclerosis, graft versus host disease, Crohn's disease), cardiovascular conditions (myocardial infarction, ischemia) and orthopedic reconstruction, but pulmonary and neurological conditions are also under investigation.

In manufacturing MSCs, the majority of laboratories utilize serum-like supplements (whether human or animal origin) as a critical component for their *ex vivo* expansion. However with the potential for zoonotic transmission [4], reports of increased immunogenicity [5] and documented cases of anaphylactoid responses after implantation [6] the use of xenogeneic material, like fetal bovine serum (FBS), to clinically expand MSCs has spurred the development of alternatives. One such alternative is to expand human MSCs in human platelet lysate [7]. Platelets are small irregularly shaped enucleated (containing no DNA) cell fragments, derived from precursor megakaryocytes. Platelets are present in blood at high concentrations (150,000–50,000/μL, representing 0.1–0.25% (w/w) of the blood), normally persist approximately 5–9 days in the circulation and are primarily responsible for hemostasis. Platelets are also the primary source of a number of growth factors [8,9], attachment factors, and enzymes found in serum. In the past decade numerous studies have shown that platelet lysate is superior to FBS and serum for expanding MSCs *in vitro* [7,10–12],

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however there are conflicting data regarding the impact platelet lysate has on MSCs functionality. Several investigators have shown that MSCs grown in PL retain their immunosuppressive capabilities [11,13], whereas others have suggested that platelet lysate expanded MSCs have inferior immunosuppressive qualities [14,15]. In surveying the platelet lysate manufacturing protocols, we found that in studies where PL was generated from buffy coats and suspended in AB serum, PL expanded MSCs retained their immunosuppressive capabilities. Conversely in those MSCs where immunosuppressive capabilities were compromised, PL was derived from platelet concentrates which typically use donor plasma and 10% acid citrate dextrose (ACD) as part of the suspension medium.

Given that serum is essentially the liquid remnant of clotted plasma, we hypothesized that PL generated from platelet concentrates contain elements that are detrimental to MSCs. Based upon the literature [16] we reasoned that fibrinogen could be a candidate plasma component that might negatively affect the immunosuppressive properties of PL. Fibrinogen is a 340 kDa glycoprotein, primarily synthesized by hepatocytes. Fibrinogen circulates as a component of blood at a concentration of approximately 7 uM with a half-life of around 100 h [16]. Fibrinogen and its cleavage products have well described roles in hemostasis where fibrin forms a clot limiting blood loss and provides a key substrate of the provisional matrix which is vital for normal repair. Fibrinogen and its cleavage products are also recognized as being capable of altering vasoconstriction, angiogenesis, cell migration and proliferation in fibroblasts, smooth muscle cells and lymphocytes [17–21]. Therefore the presence of fibrinogen in platelet lysate generated from platelet concentrates has the potential to impact the behavior of *ex vivo* expanded hMSCs. In this study we hypothesized that depletion of fibrinogen from platelet lysate would yield a superior product for expanding human MSCs for use in immunomodulation therapy. To test this hypothesis we optimized a method to deplete fibrinogen from platelet concentrates/plateletpheresis products (fdPL) which restored the immunosuppressive activity of *ex vivo* expanded MSCs compared to non-fibrinogen depleted PL (uPL) and does so without compromising the desirable growth promoting characteristics of PL.

2. Materials and methods

2.1. Manufacturing of platelet lysate

At the Emory University Hospital blood bank plateletpheresis products are purchased from the American Red Cross (ARC) and meet all AABB & FDA regulatory requirements for sterility and infectious disease screening for transfusion products. Upon outdating of plateletpheresis products, for human infusion, we have obtained American Red Cross consent and an Emory IRB waiver to use these products to generate platelet lysate. To generate human phPL we employed a freeze–thaw procedure to ensure proper fracturing of platelet membranes for intracellular growth factor release. For each lot of PL, approximately five outdated plateletpheresis products were removed from the freezer and thawed at 4 °C. Each unit (approximately 200–250 mL) was then aliquoted into smaller volumes (approximately 20–25 mL) for re-freezing at −80 °C. Thawed platelet units were individually filtered through a 40 μm *PALL* blood transfusion filter (PALL BIOMEDICAL, INC USA) and allowed to pool into a collection bag. Pooled, filtered lysate was equally aliquoted into labeled 250 ml conical tubes (Corning® Lowell, MA USA) and centrifuged for 20 min at 4000 × *g* at room temperature. Spun lysate was again filtered (40 μm), aliquoted into 250 ml conical tubes, and either mixed with $CaCl_2 \pm 2$ U/ml heparin in their respective concentrations (fdPL) or progressively filtered using a 0.2 μm pore size (uPL). $CaCl_2 \pm$ heparin supplemented lysate was incubated for 1 h at 37 °C/CO_2/

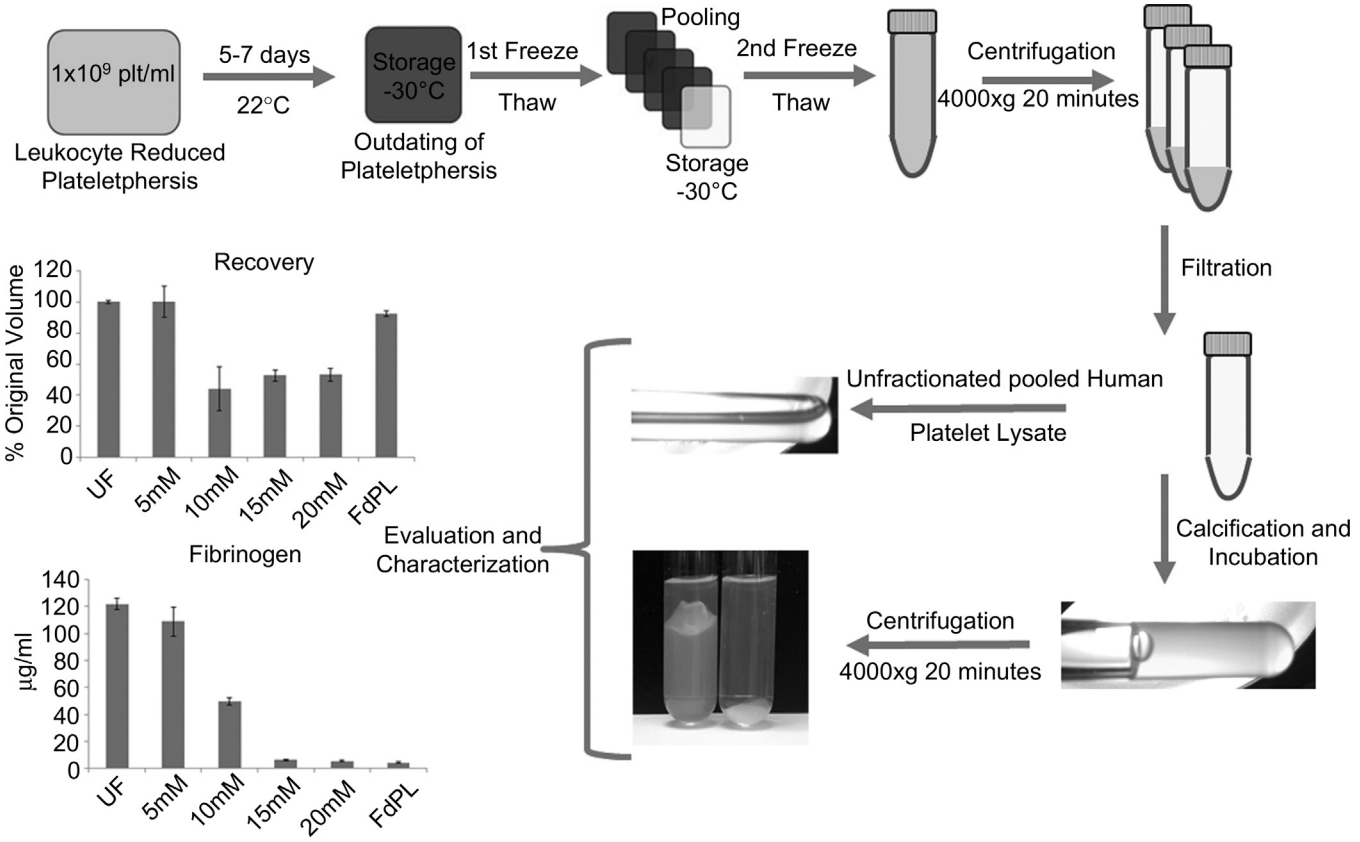

Fig. 1. Schematic representation of manufacturing process for generation of platelet lysate. Outdated plateletpheresis units are frozen at −30 °C thawed then pooled with 5–7 additional units and refrozen. After a second thawing, cell membranes and debris are removed by centrifugation and filtration to 0.2 μm. Unfractionated pooled human platelet lysate then underwent a series of calcium and temperature dependent clotting procedures which yielded varying degrees of supernatant recovery and fibrinogen depletion. $N = 4$ per group; $^{\#}p < 0.05$; NS = $p > 0.05$.