

Can the extent of adipose tissue washing impact the therapeutic efficacy of autologous stromal vascular fraction (SVF) cell therapies?

Adam J. Katz, Gabriela Aguilo-Seara, Hulan Shang, Scott Northrup, Ramon Llull
Department of Plastic and Reconstructive Surgery, Wake Forest School of Medicine, Winston-Salem, NC

INTRODUCTION

Adipose-derived SVF cell therapies have entered Phase 3 FDA testing. A major challenge for point-of-care autologous cell strategies is the inherent variability that exists when using primary cells derived from a variety of patients. SVF is composed of numerous cell types, including mesenchymal stromal cells and blood derived cells (BDCs). Individual patient/donor characteristics, tissue harvest methods, and tissue processing/cell isolation methods all present possible sources of SVF variability, and tissue processing/cell isolation is likely the most easily modifiable.

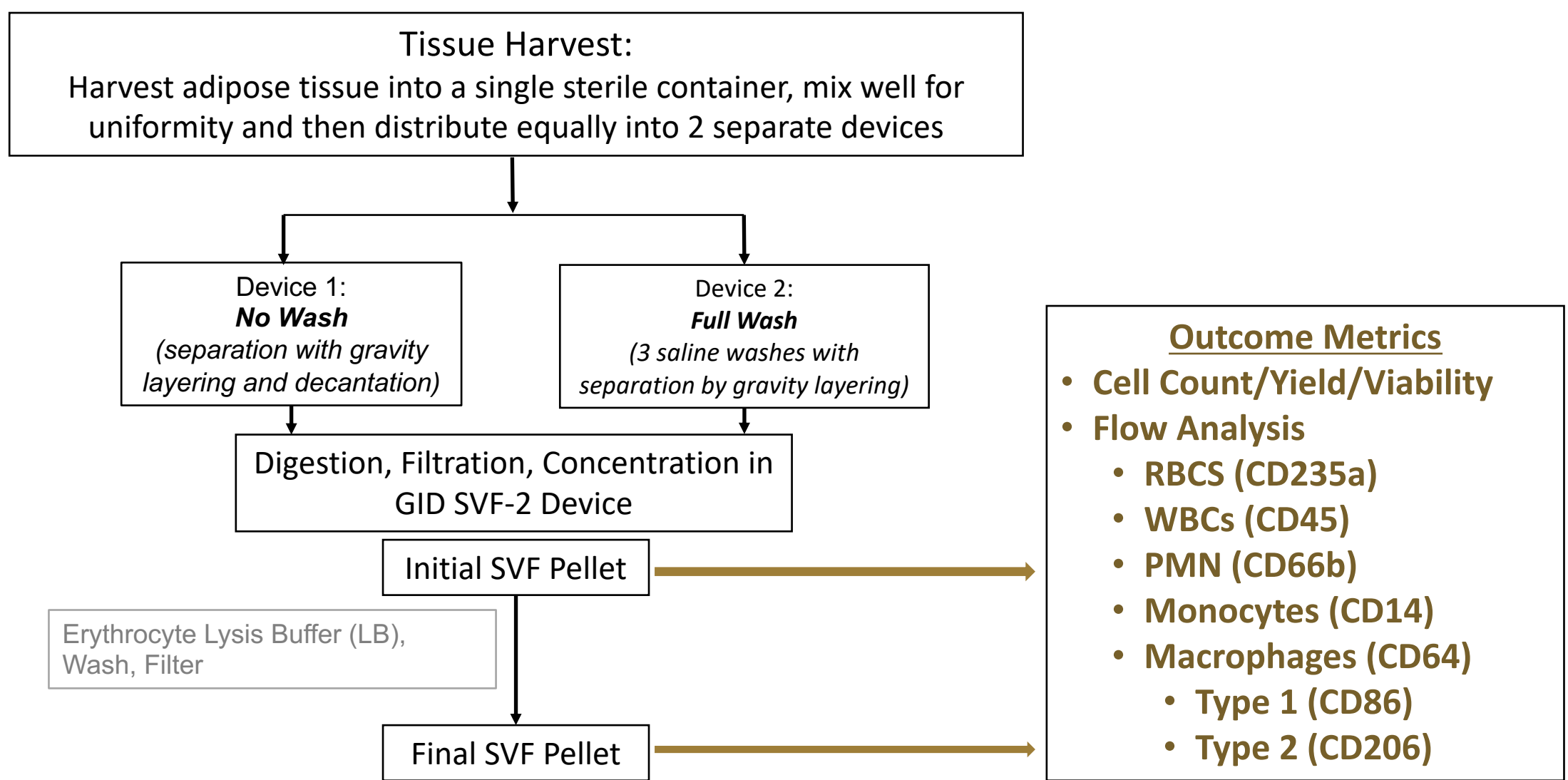
Cell preparations typically include some degree of erythrocyte (RBC) “contamination.” Most protocols utilize a RBC lysate step, despite the fact that RBC contents are known to be inflammatory. The impact of these steps on cell viability and biological activity remain unclear.

Our central hypothesis is that 1) the extent of adipose tissue washing influences the number of blood derived cells (BDCs) in SVF preparations, and 2) BDCs within SVF preparations confer modifiable effects on SVF bioactivity.



METHODS

Aim 1: Fresh LA was washed and/or treated with lysing buffer. The resulting isolated SVF was evaluated by flow cytometry for cell yield, viability, and presence of BDC populations, as described in the diagram.



Aim 2: RBCs isolated from fresh lipoaspirate (LA) were divided and suspended in equal volumes of either DMEM with 10% fetal bovine serum (D10) at three dilutions or ACK (ammonium-chloride-potassium) lysing buffer (LB). RBCs undergoing lysis were then centrifuged, and the supernatant was diluted in D10 in similar fashion. Freshly isolated SVF cells and second passage ASCs (control) were cultured in the media solutions for up to 7 days. Conditioned medium was collected on days 0, 3, and, 6/7 and evaluated for RBC derivatives, hemin and hemoglobin, using ELISA. Cell proliferation was evaluated after 6 or 7 days using Presto Blue Assay.

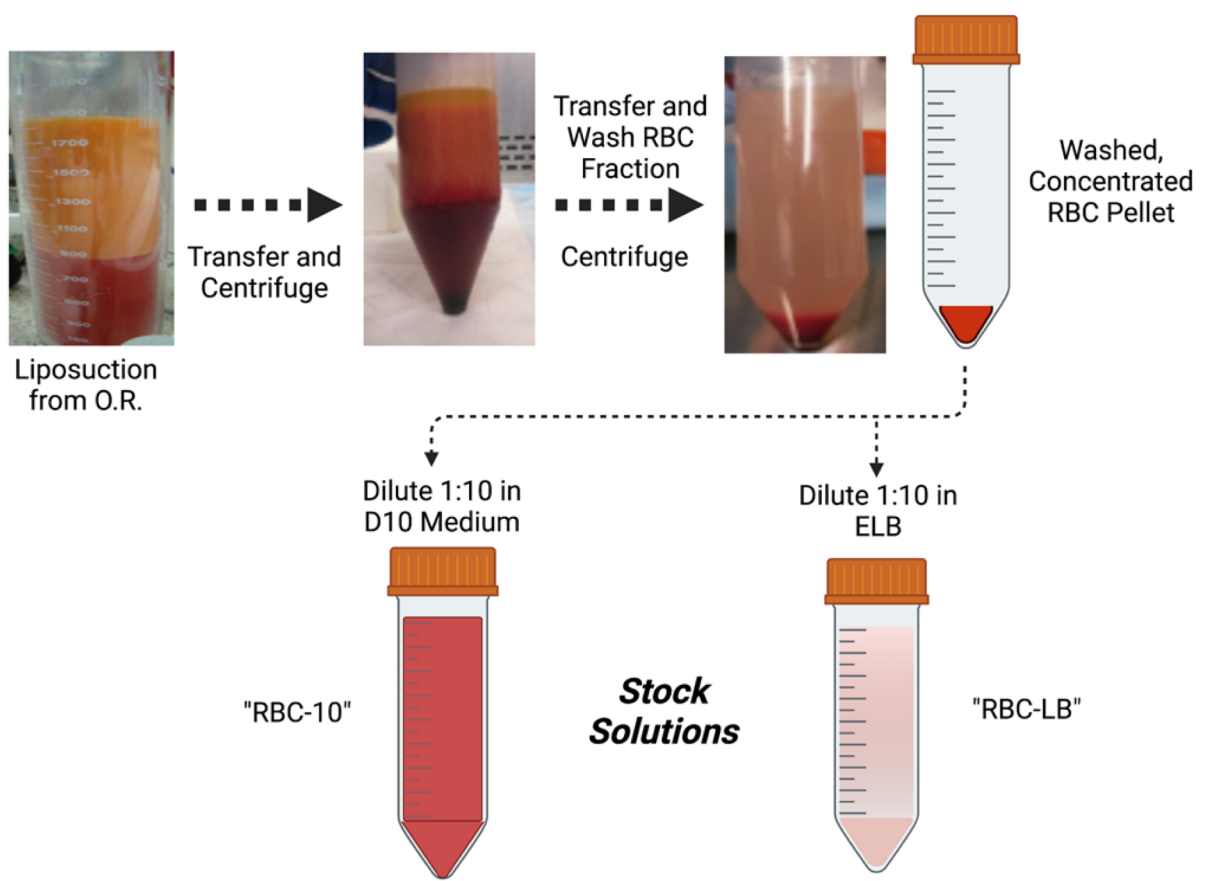


Figure 1 describes the preparation of stock solutions for culture, where an RBC pellet is washed and concentrated by centrifugation and then diluted in media with or without LB.

Medium	Stock	Diluent	Dilution	Medium	Stock	Diluent	Dilution	Medium	Stock	Diluent	Dilution
RBC-10 1:5	RBC10D10	D10	1:5	RBC-LB 1:5	RBC LB	D10	1:5	ELB 1:5	ACK LB	D10	1:5
RBC-10 1:10	RBC10D10	D10	1:10	RBC-LB 1:10	RBC LB	D10	1:10	ELB 1:10	ACK LB	D10	1:10
RBC-10 1:50	RBC10D10	D10	1:50	RBC-LB 1:50	RBC LB	D10	1:50	ELB 1:50	ACK LB	D10	1:50

Table 1 describes the 9 different culture conditions at dilutions of 1:5, 1:10, and 1:50 for stock solutions containing RBCs alone, RBCs with LB, and LB alone. All solutions were diluted in D10 medium, which also served as the control.

RESULTS

Aim 1: Washing of LA reduced RBC burden 3.7x compared to unwashed samples and those treated with lysing buffer alone. Washing also decreased CD45+ BDCs 1.7x, including CD66b+ neutrophils by 3.5x, while yielding higher CD206+ macrophage (type 2) counts.

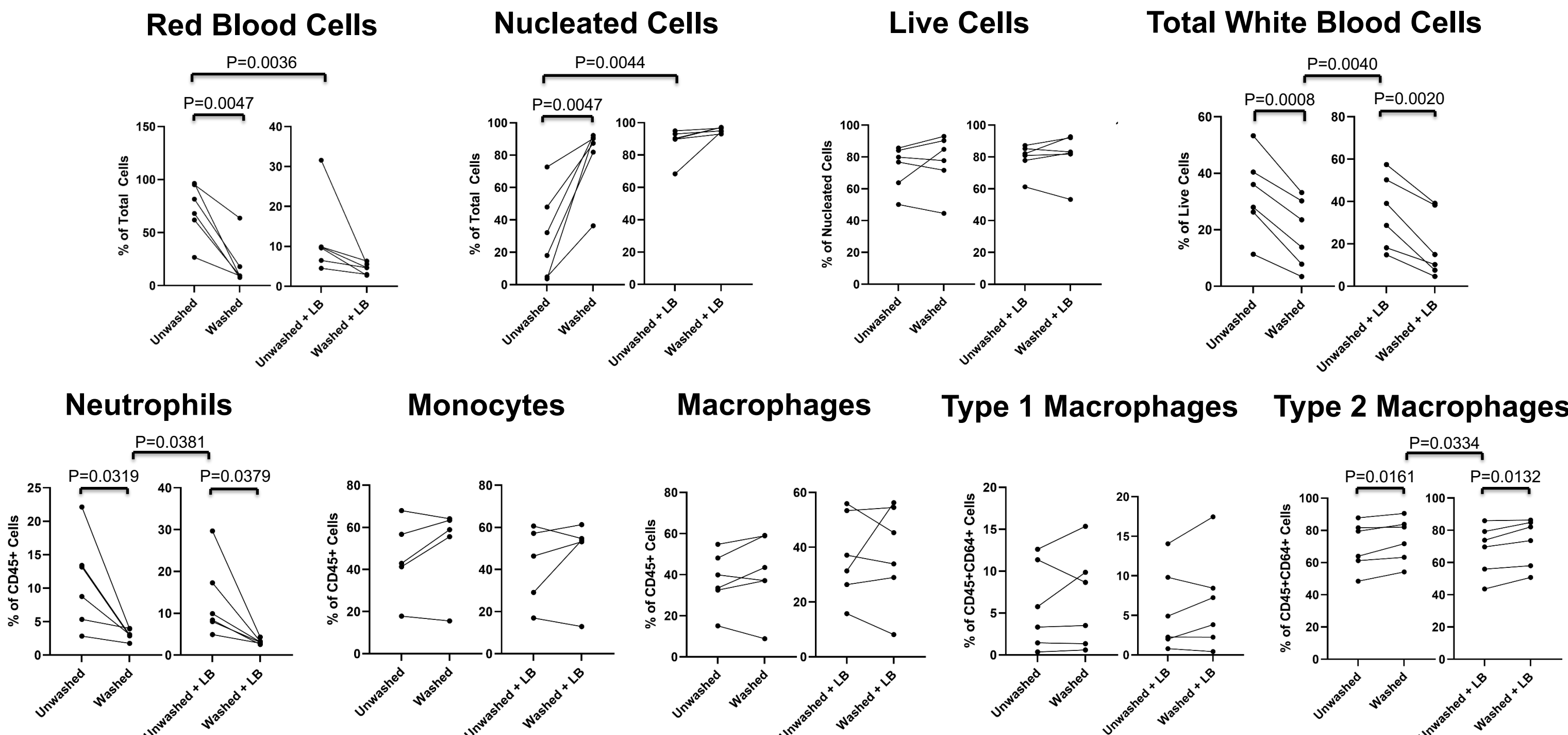


Figure 2 depicts the flow cytometry results for the selected blood derived cells and their statistical significance.

Aim 2: SVF cultured with all three RBC concentrations proliferated 2.5-4x more than cells grown in control D10 (p=0.0013, p=0.0003 for the two highest RBC concentrations) and 3.2-3.7x more than lysate cultures (p=ns). This pattern was consistent in ASC (control) culture. The degree of cell proliferation was inversely related to levels of free hemoglobin and hemin detected in medium.

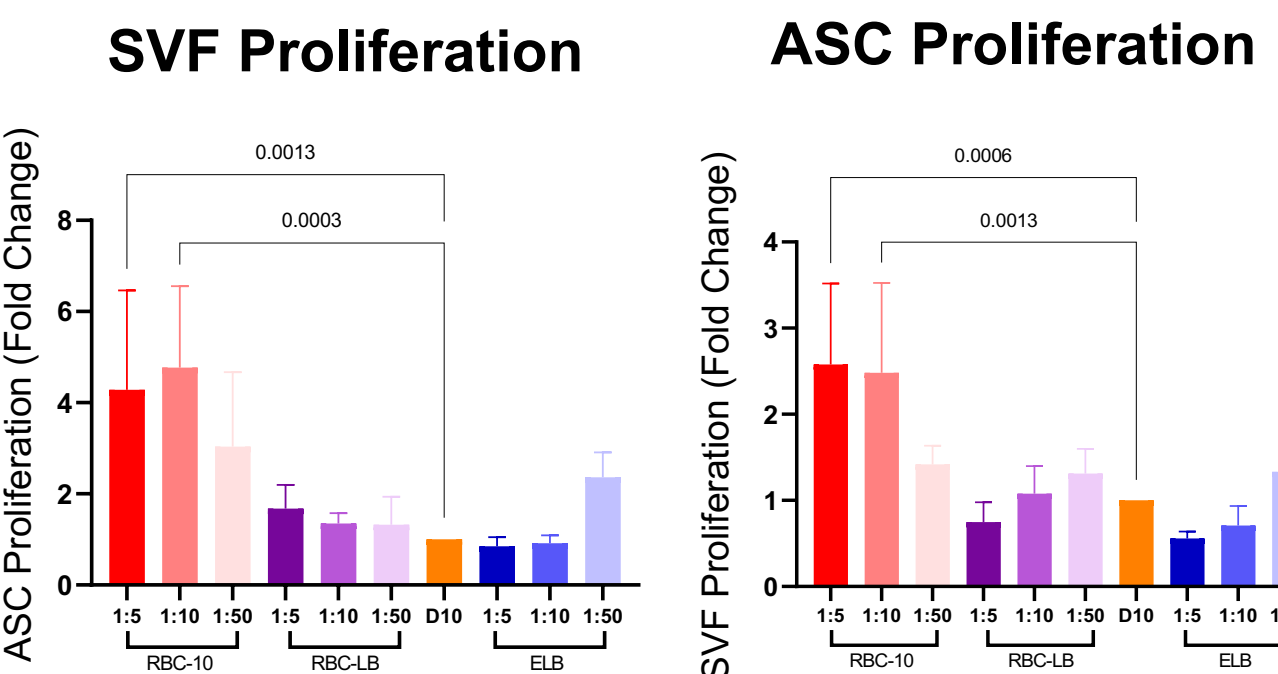


Figure 3 shows the results of SVF and ASC proliferation of solutions described in Table 1, as measured by Presto Blue Assay at day 7 of culture.

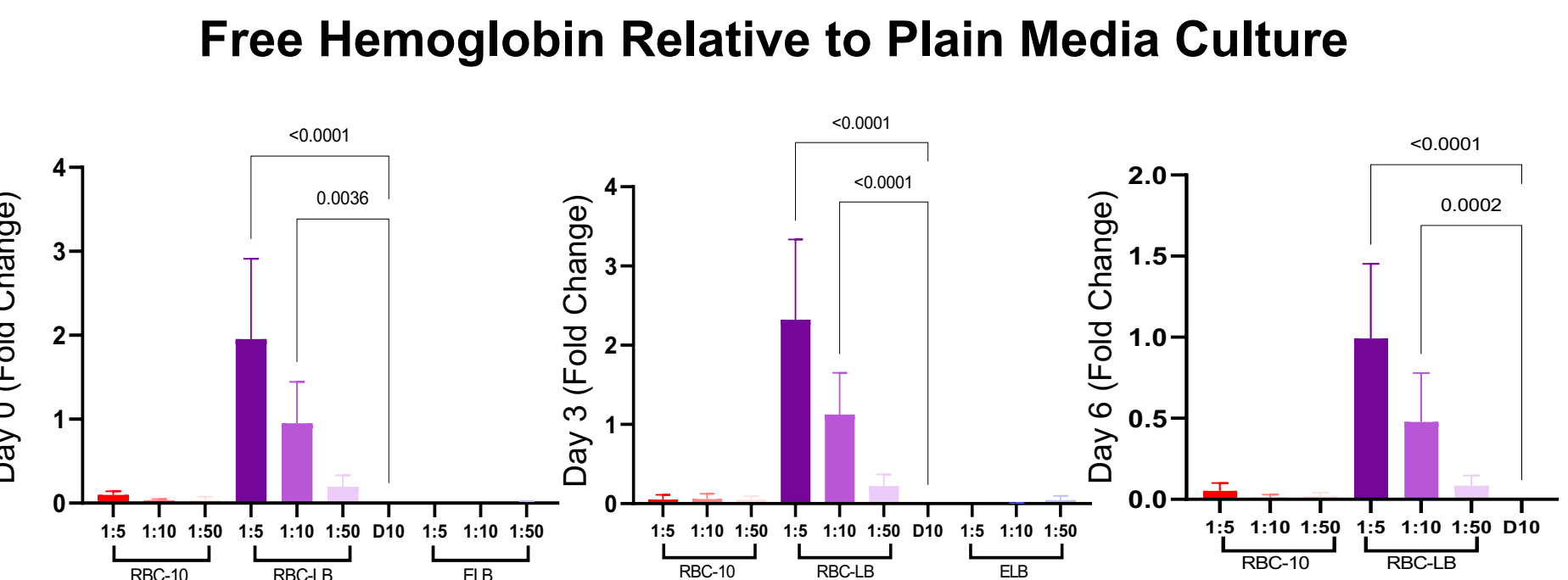


Figure 4 shows the fold change of free hemoglobin as measured by ELISA for culture conditions described in Table 1 on days 0, 3, and 6 of culture.

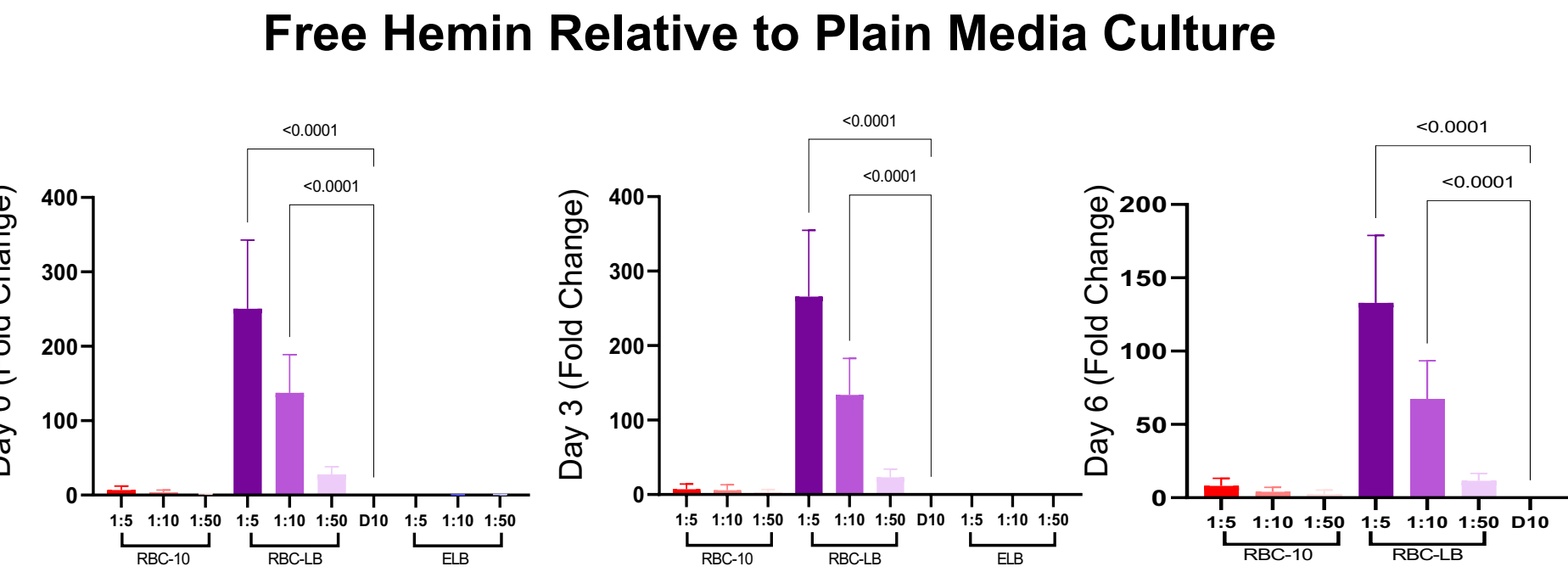


Figure 5 shows the fold change of free hemin as measured by ELISA for culture conditions described in Table 1 on days 0, 3, and 6 of culture.

CONCLUSIONS

Tissue processing methods impact SVF cell composition, and the extent of washing specifically, impacts the quantity of BDCs through decreasing RBC burden and altering WBC composition, primarily by decreasing the presence of pro-inflammatory cells. Furthermore, SVF cell composition, specifically the presence of RBCs in culture, impacts SVF biological activity *in vitro*. Future studies should include further exploration of how BDCs affect SVF biological activity both in-vitro and in-vivo.

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