ENABLING LARGE-SCALE EX VIVO PRODUCTION OF MEGAKARYOCYTES AND PLATELETS FROM CD34+ CELLS USING GAS-PERMEABLE SURFACES AND MICROFLUIDIC BIOREACTORS

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Patients suffering from acute or sustained thrombocytopenia require platelet transfusions, which are entirely donor-based and limited by challenges related to storage and fluctuating supply. Developing cell-culture technologies enables ex vivo and donor-independent platelet production. However, the ability to generate large numbers of platelets is limited by the challenges of (1) producing many megakaryocytes (Mks) from each input CD34+ cell and (2) efficiently generating platelet-like-particles (PLPs) from Mks. To address the first challenge, we evaluated Mk production from mobilized peripheral blood CD34+ cells cultured on a commercially available gas-permeable silicone membrane, which provides efficient gas exchange, and investigated the use of fed-batch media dilution schemes. Starting with a cell surface density of 4×10^4 CD34+ cells/cm^2 (G4), culturing cells on the membrane for the first 5 days and employing media dilutions yielded 38 ± 25 Mks per input CD34+ cell by day 11 – a 2.2-fold increase compared to using standard tissue culture surfaces with full media exchanges. By day 7, G4 conditions generated 1.5-fold more CD34+ cells due to greater CD34 retention. Media dilution schemes for G4 and standard tissue culture surfaces improved culture viability, leading to a 3.6-fold increase in Mks produced per mL of media for G4 and 2.8-fold for tissue culture compared to controls. G4-Mks exhibited lower mean ploidy yet the number of high-ploidy Mks was equal to or greater than controls. Finally, G4-Mks produced proplatelets and PLPs that activated and aggregated upon stimulation. Further optimization is required to take full advantage of the gas-permeable system for Mk production. Additional studies would include refining surface densities, adjusting cytokine concentrations and initial media volumes and evaluating cord blood CD34$^+$ cells.

To address the second challenge, we applied computational fluid dynamics (CFD) modeling to assess published microfluidic platelet bioreactors and used those results to develop an improved 7-µm slit bioreactor with well-defined flow patterns and uniform shear profiles (USRB-7µm). The slits mimic fenestrations in endothelial cells lining sinuses in the bone marrow through which Mks extend cytoplasmic projections, called proplatelets (proPLTs), that are sheared off (50-200 s^{-1}) into platelets. In the USRB-7µm, a center channel flow pushes Mks into 7-µm slits, with shear rates of 5000 s^{-1}. Two outside flows converge at the slits exerting near uniform shear rates (250-350 s^{-1}) to fragment extending proPLTs, similar to the in vivo process. The USRB-7µm permits real-time visualization of proPLT formation and the rapid-release of individual platelet-like-particles (PLPs), which has been observed in vivo, but not previously reported for bioreactors. Collected PLPs exhibited characteristics similar to fresh blood platelets. Surprisingly, using only the center flow without the outside flows led to a 6-fold increase in PLP production. Based on this, we scaled-up the USRB-7µm using only a single flow to carry Mks into high-shear slit regions, mimicking in vivo observations of Mks being processed directly into platelets within the lung capillary bed (>2600 s^{-1}). The new lung-USRB retained uniform shear rates with a 93-fold capture area increase to allow more Mks to be processed into PLPs. The USRB-7µm and lung-USRB will be useful tools for the analysis of proPLT/PLP formation to further understanding of how to increase ex vivo platelet production.

These results highlight distinct improvements in Mk cell-culture and demonstrate how new technologies and techniques are needed to enable clinically-relevant production of Mks for platelet generation and cell-based therapies.