EVALUATING A GAS-PERMEABLE CULTURE SURFACE FOR THE GENERATION OF MEGAKARYOCYTES FOR IN VITRO PLATELET PRODUCTION

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The ability to generate large numbers of platelets for transfusions is limited by the challenges of (1) efficiently generating platelet-like-particles (PLPs) from megakaryocytes (Mks) and (2) producing many Mks from each input $CD34^+$ cell. Clinical-scale cell culture technologies for expanding $CD34^+$ cells and differentiating them into Mks must be able to handle large media volumes and cell numbers. Recently, the G-Rex membrane system has been used to expand large numbers of T-cells within a scalable closed system. The gas-permeable membrane provides efficient gas transfer from the incubator atmosphere to the cells. CO_2/O_2 diffusion is no longer dictated by the media height, which restricts media usage in standard tissue culture flasks and wells. Additionally, the system allows the use of various of cell densities and larger volumes of media without the need for numerous media exchanges. Although the G-Rex membrane system has shown extensive benefits for expansion of different T-cell populations and cell lines, it has not been evaluated for its impact on the expansion and differentiation of $CD34^+$ cells into Mks. In the bone marrow, megakaryopoiesis occurs over a gradient of oxygen tension – with hypoxic conditions near the bone and higher O_2 concentrations at the vasculature. Our lab has previously published a three-phase Mk expansion protocol that mimics the O_2 transition, as shown in Figure 1A. In this study we investigated the use of G-Rex membrane systems for Mk production from mobilized peripheral blood (mPB) $CD34^+$ cells. High initial cell surface densities (0.1×10^6 cells/cm^2 or greater) stunted the expansion and differentiation of cells through the first two phases of the process, whereas low surface densities (0.011×10^6 cells/cm^2) led to expansion comparable to the standard protocol. However, a starting surface density of 0.04×10^6 cells/cm^2 in the first phase of the culture resulted in a 2.3-fold increase in the number of Mks per input $CD34^+$ cell compared to the standard process (Figure 1B). A media dilution scheme on Day 5 promoted not only expansion in the G-Rex system, but also for the control protocol. The G-Rex system with a media dilution scheme led to 3.6-fold increase in the number of Mks produced per mL of media used, thus potentially reducing the cost of a scaled-up process (Figure 1C). Finally, Mks produced using the G-Rex system demonstrated normal maturation aspects such as polyploidization, proplatelet formation, and PLP generation ex vivo. While this study primarily focused on the starting cell surface densities and a dilution scheme, other variables that could be optimized include starting media volumes, cytokine concentrations, transferring cells to larger G-Rex systems at later phases, and even potentially lowering the oxygen levels in phase one. The results of this study demonstrate a new culture process for the generation of Mks ex vivo within a scalable technology that already has been shown to generate clinically relevant numbers of other cell types.

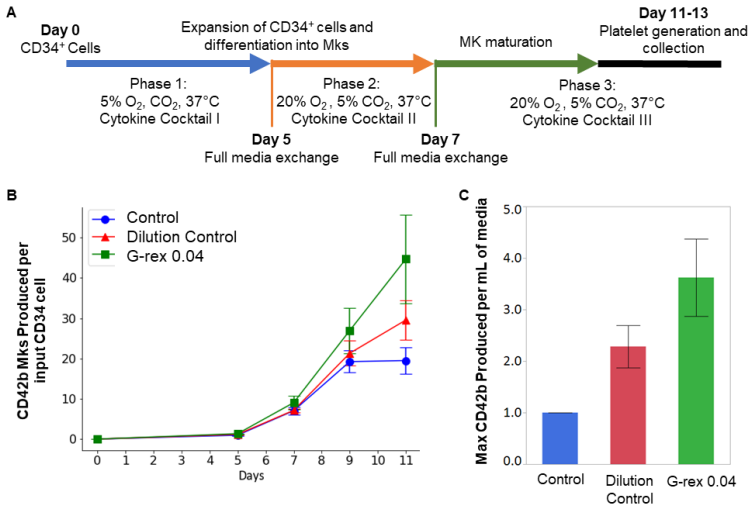

Figure 1 – (A) Three-phase protocol to generate Mks from CD34+ cells. (B) Expansion profiles of Mks for different conditions. (C) Mks produced per mL of media used.