USING COMPUTATIONAL FLUID DYNAMICS (CFD) TO DESIGN AND CHARACTERIZE A MICROFLUIDIC BIOREACTOR FOR RAPID RELEASE OF CULTURE-DERIVED PLATELETS

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Platelet transfusions are entirely dependent on human volunteer donors, and these methods are limited by platelet storage at room temperature, a 5-day platelet shelf life, and differences in donor/recipient immunology. Much progress has been made in generating large numbers of culture-derived megakaryocytes (Mks, the precursor cells to platelets). However, much remains unknown about what initiates and regulates platelet formation, so stimulating a high percentage of Mks to undergo terminal maturation and platelet release in vitro remains a major challenge. Methods of in vitro platelet production have typically yielded less than 10 platelets/Mk, compared to >1,000 in vivo. In vivo, platelets are formed when bone marrow Mks extend long, cytoplasmic projections, called proplatelets (proPLTs), into the sinusoid where shear forces accelerate proPLT elongation and release platelets into circulation. Recent studies have demonstrated the utility of shear forces to enhance platelet release from cultured Mks in vitro. We are exploring the production of platelet-like particles (PLPs) within a microfluidic bioreactor that utilizes shear forces on Mks to generate proPLTs and PLPs. Microfluidic devices have emerged as a valuable tool for cell culture studies. Advantages include low input cell requirements, the ability to screen multiple conditions in parallel, compatibility with time-lapse imaging, and tight control of microenvironment conditions. In addition, device fabrication is straightforward and inexpensive using soft photolithography. In this study, we performed a computational fluid dynamics (CFD) analysis of several published platelet microbioreactor systems, and used the results to develop a new bioreactor system. Through CFD simulations and microfluidic device fabrication, a design – test – build methodology was used to develop a dual-flow microfluidic bioreactor system with uniform shear stress at levels similar to those found in the bone marrow niche. Experimental studies were conducted to validate the simulations in terms of streamline profiles and flow patterns with and without cell capture. Furthermore, the design of the bioreactor allows for a wide physiological shear rate range, and fits within the stage of a fluorescent microscope housed in an incubator that allows for real-time analysis of proPLT formation and PLP release. The videos and images captured within our system show that the new bioreactor not only promotes the prototypical proPLT formation process with beadson-a-string morphology, but also supports rapid release of individual PLPs – which has been observed in vivo, but not previously reported for platelet bioreactors. In addition, we demonstrate that step increases in the shear forces within the microbioreactor system can be used to enhance proPLT and PLP formation. Bioreactor-derived PLPs exhibit functional activity, as evidenced by CD41a and CD42b surface marker expression, CD62P translocation from granules to the surface in response to thrombin agonist activation, and morphological/ cytoskeletal changes upon binding to fibrinogen – before and after activation. The system can be further scaled, for example, through parallelization of reactors.