BIOMANUFACTURING OF PLATELET-LIKE CELLS AND CELL MICROPARTICLES FOR CELL-THERAPY APPLICATIONS

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Megakaryocytes (Mks) are large polyploid cells derived from hematopoietic stem/progenitor cells (HSPCs) triggered by thrombopoietin (Tpo). During differentiation and maturation from HSPCs, Mks migrate from bone marrow toward blood vessels, and give rise to proplatelets (PPTs) and platelets (PLTs) released into blood circulation. We have shown that Mks also shed megakaryocytic microparticles (MkMPs) (1), which are 0.1 to 1 micron extracellular vesicles (EVs). These MkMPs specifically target HSPCs *in vitro* and induce them into Mk differentiation in the absence of Tpo by delivery of cargo such as proteins and RNA (1,2). PLTs (collected from donated blood) are an expensive cell product in limited supply due to their short life time (4-5 days at room temperature; freezing is not possible) and large needs in Transfusion Medicine for patients with thrombotic deficiencies. Culture-derived PLTs has been shown to have functional activity as PLTs and hold a great potential for providing abundant PLT supply. In this study, we examined biomanufacturing issues of cells or cell derived particles for potential use in lieu of collected PLTs.

First, we examined the possibility that MkMPs may be used in lieu of collected PLTs. To examine this, we tested the hypothesis that human MkMPs (huMkMPs) might interact with murine HSPCs and promote Mk and PLT biogenesis *in vivo*. If this hypothesis is correct, it would suggest that huMkMPs can be used in Transfusion Medicine in lieu of PLTs, especially because huMkMPs would interact more efficiently with huHSPCs than with muHSPCs and also because huMkMPs can be stored frozen. To test this hypothesis, we investigated the interaction of huMkMPs with huHSPCs, both *in vivo* and *in vitro*. Injection of huMkMPs to wild-type mice enhanced PLT levels by up to 49%, while reticulated (newly synthesized) PLTs increased from 11.8 % to 15.9 % (a substantial and statistically significant increase). Furthermore, huMkMPs were able to rescue the PLT levels of antibody-induced thrombocytopenic mice by up to 52% Taken together, these data show that huMkMPs target murine HSPCs to enhance PLT biogenesis *in vivo*.

How would one then optimize Mk, PLT and MkMP biomanufacturing for practical applications at large scale? Difficulties regarding PLTs yield per Mk or per input HSPC and PLT functionality remain unsolved. We have shown that shear forces enhance Mk maturation, and the production and function of PPTs, PLTs and MkMPs (1). To achieve metrics suitable for biomanufacturing of PLTs and MkMPs, we improve at the late stage of Mk culture. Cultures under mixing conditions imposing increased biomechanical forces in different culture vessels were carried out. PPTs, PLPs, and MkMPs production under mixing condition were enhanced by ≥ 4-6 fold. Furthermore, PLTs and MkMPs generated under increased biomechanical forces maintained their biological functionality. These data suggest that biomanufacturing of these PLTs and MkMPs (and other cell types and EVs) produced under optimized culture condition engaging optimal biomechanical forces show great potential for serving as PLTs substitutes in Transfusion Medicine, and, more broadly, as agents for novel cell therapies.

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