Generation of HLA Class I Deficient Platelets from CD34+
Hematopoetic Stem/Progenitor Cells
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Source of cells for generating ex vivo platelets could come from CD34+ hematopoetic progenitor/stem cells from bone marrow biopsy, umbilical cord blood or mobilized peripheral blood. The possibility of manufacturing ex vivo cultured blood cells (blood pharming) would lead to improvements in transfusion practice. Ex vivo generated platelets should be compatible as the term universal applies (low risk of rejection), infection free and readily available (off the shelf). This study aims to upscale the in vitro generated platelets number and to decrease the HLA class I expression to overcome rejection. CD34+ hematopoetic stem/progenitor cells with genetically silenced HLA Class I expression were differentiated to Megakaryocytes(MKs) using cytokine cocktail. MKs generated from CD34+ cells were comparable to in vivo megakaryocyte in terms of morphology with the protection against in vitro antibody mediated cytotoxicity. Yet the number of terminally differentiated platelets recovered is low. Alternate source of cell comes from megakaryoblastic leukemic cell line CMK and MKPL1 with hematopoetic progenitor potential. These cell lines express stemness marker CD34. They also express TPO receptor and markers for late stage of MK maturation. Gene expression analysis revealed these cell lines were committed to MK lineage. MKs and platelets can be generated in vitro from both cell lines. Addition of Nicotinamide or Ciliobrevin D induced the increment of late stage MK maturation and TPO receptor marker as early as day 5 differentiation. The generated platelets were functional and expressed markers commonly found in blood platelets (CD61 and PECAM-1). Higher platelets number obtained with addition of Nicotinamide and Ciliobrevin D.

Keywords: Megakaryocyte, Platelets, In Vitro Differentiation, HLA class I silencing