CHARACTERIZATION OF HUMAN LIMBAL STEM CELLS USING ACOUSTIC FOCUSING CYTOMETRY

Michael Olszowy[1], Langsdorf, Chris[1], Knollman, Paul[2], Bailey, Matthew[2], Dubbels, Bradley[1], Kuninger, David[2], Bradford, Jolene[1]

[1]Flow Cytometry Systems, Life Technologies, Eugene, OR, USA; [2]Primary Stem Cells, Life Technologies, Eugene, OR, USA

Abstract:
Limbal stem cells (LSCs) are found at the periphery of the cornea in the limbus and function to maintain and renew the corneal epithelium. *Ex vivo* expansion and transplantation of LSCs has been proposed as a treatment for various corneal diseases and injuries. In the present study we have developed a method to identify and characterize LSCs derived from primary human limbal tissue. Experimentation was focused on a model system composed of human corneal epithelial cells enzymatically released from normal corneal-sclera buttons and expanded in KSFM growth medium. Cells of various passage numbers were harvested and analyzed using multicolor acoustic-focusing cytometry. Previous work has demonstrated that stem cells specifically pump out the cell-permeant DNA-binding dye Vybrant® DyeCycle™ Violet via ABCG2-mediated efflux. This property enables the use of side-population technique to identify a sub-population of putative LSCs. The ABCG2 inhibitor Fumitremorgan C was shown to block the appearance of the corneal cell side-population. A549 human lung adenocarcinoma cells, which over express the ABCG2 transporter, were used to verify the action and inhibition of ABCG2. The LSC subpopulation was generally less than three percent of total cells, requiring analysis of large numbers of cells to generate statistically significant data. The extremely tight sample focusing provided by acoustic focusing cytometry enabled very high throughput while maintaining data integrity.