Ultra-scale down studies of human cell bioprocessing for a prostate cancer vaccine therapy - The impact of capillary shear

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Introduction

• Processing of human cells at large scale demands an understanding of the effects of the engineering environment on cell quality.

•The ability to work with small quantities of cells at an early stage of development and predict the effect of the engineering environment will greatly speed the design of large scale operations.

•An array of engineering and biological tests were performed to understand how processing parameters could affect product quality.

•Two cell lines, OnyCap23 and P4E6, which are part of a multi-cell prostate cancer vaccine were tested in this study.

Process Flow Sheet

Wash monolayer → Enzymatic detachment → Quench → Batch Centrifuge → Resuspension, PBS → Batch Centrifuge → Resuspension, PBS → Batch Centrifuge → Final resuspension, HBSS + 8% DMSO → Magnetic stirrer Speed 120rpm Process time 2-3h → Vialling

•The production process of whole cell therapies starts with cell growth, either adherent or suspension.

•Cells suffer different types of hydrodynamic stresses according to specific unit operations.

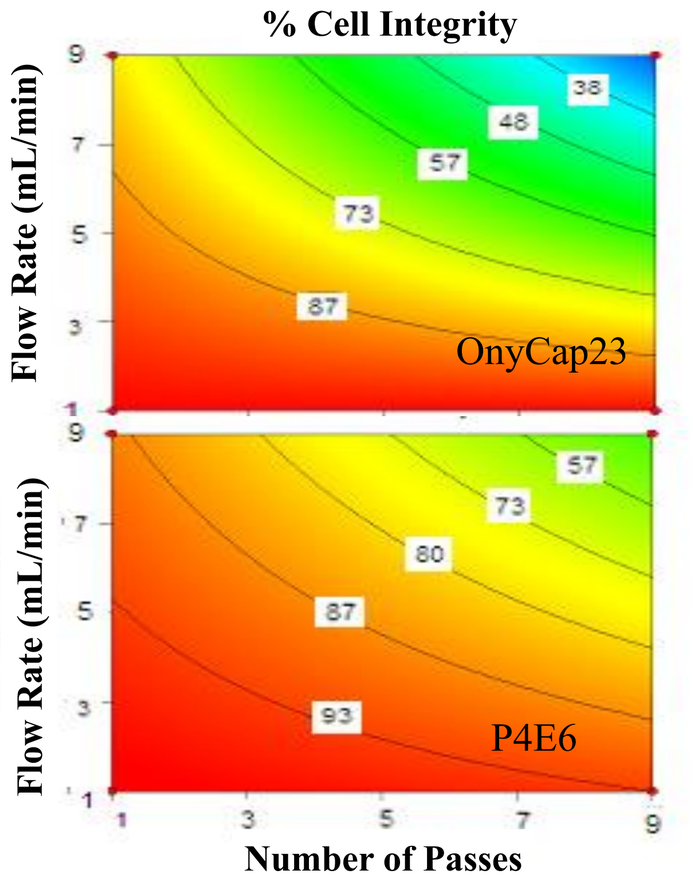
•The engineering environment will define product quality. In the case of whole cell cancer vaccines, the material is presented to the patient's immune system.

•The correlation between process limits and the properties of individual cell lines will define equipment selection and optimal operating strategies.

•This study focuses mainly on the stress suffered during re-suspension, formulation, vialling and injection.

Experimental Protocol: Capillary Device

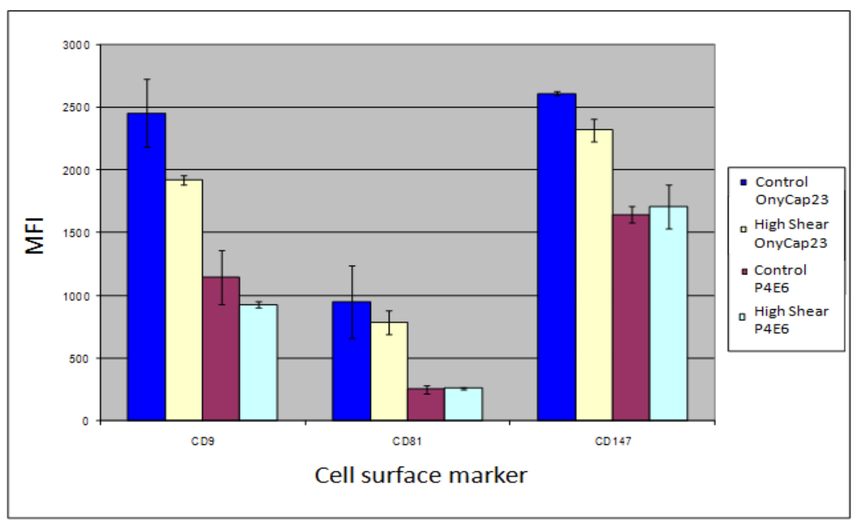
•An ultra scale-down approach using capillary shear devices allowed the rapid characterization of human cell lines in terms of their resistance to hydrodynamic stress. To understand the cell response to hydrodynamic stress critical quality attributes like cell membrane integrity, cell size and surface phenotype were measured.



•A DoE approach was used to study the effect of multiple process inputs on the final cell integrity. The relative sensitivity observed for the three variables studied was: flow rate 1.0, number of passes 0.26 and capillary length 0.08.

•Computational fluid dynamics were used to analyze the capillary entry region to allow the properties of the cells to be characterized in terms of a critical stress below which there is no significant loss of membrane integrity or characteristic surface markers.

•The results suggested that the OnyCap23 and P4E6 cell lines are resistant to damage below critical elongational shear stress values of 235 N/m^2 and 275 N/m^2 respectively.

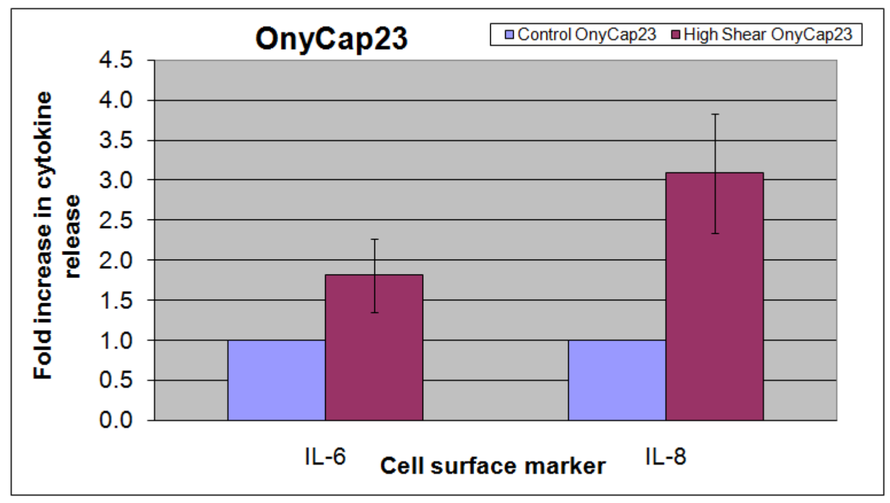


•The effect of capillary shear on the cell surface markers was also measured by flow cytometry.

•Mean fluorescent intensity results suggest that after one pass, capillary shear has a negative effect on OnyCap23 only. CD9>CD147>CD81

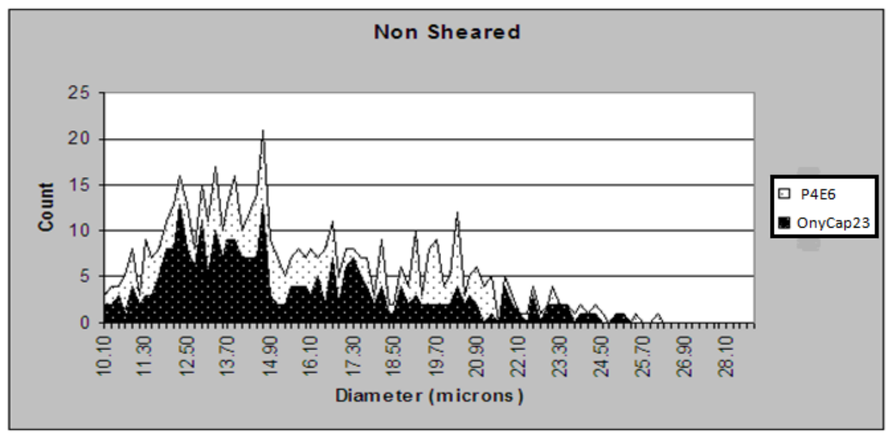
•A biopotency co-culture assay showed an up-regulation of INFγ release by immune cells in response to sheared cells.

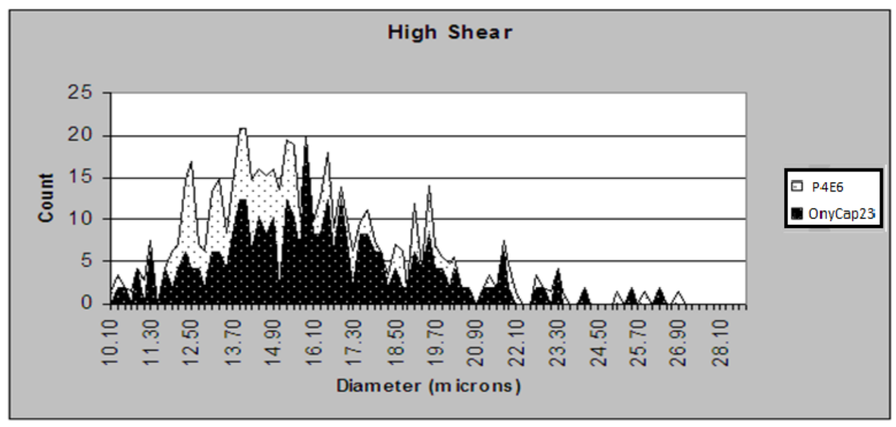
•A Luminex cytokine multiplex assay showed an increase of IL-6 and IL-8 concentrations for both cell lines after shear. These cytokines are indicative of cell stress.



•The effect of capillary shear on the cell size was measured using an automated image analysis tool.

•After one pass at high shear rates it is possible to see an increase in the average size of the cell population and a reduction in the total cell number of OnyCap23 cells at high shear.





Conclusion

•An experimental methodology has been developed in order to study the impact of the hydrodynamic environment upon several biological characteristics of two cell lines.

•P4E6 cell line was shown to be more resistant to the hydrodynamic stress for all the critical quality attributes measured.

•Future work aims to move this technology to an automated platform in order to study the relationship of the critical elongational stress values with process stages such as pumping, vialling, etc.

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