

Human Cells for Prostate Cancer Vaccine Therapy - The impact of centrifugation upon key product quality attributes



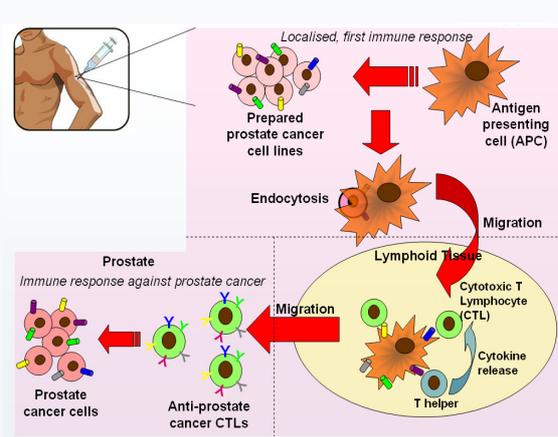
M.Delahaye¹, K.Lawrence¹, C.Chu¹, S. Ward² and M. Hoare¹

1. Advanced Centre for Biochemical Engineering, Department of Biochemical Engineering, University College London, Torrington Place, London, WC1E 7JE, UK
2. Onyvax Ltd, St. Georges Hospital Medical School, London, SW17 0RE, UK

Introduction

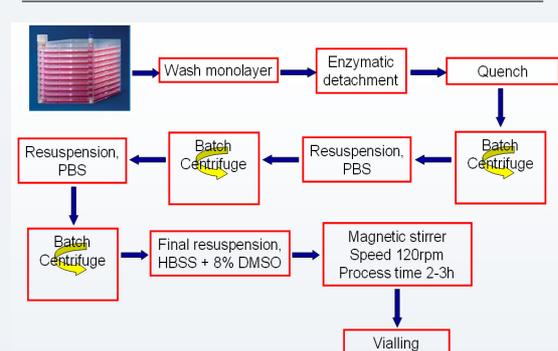
- It is ever more critical for cell based therapy to be delivered by a well-designed, data driven bioprocess
- A Quality by Design (QbD) approach allowed the combined effects of multiple process inputs to be evaluated at small scale for a selected unit operation – centrifugation.
- Linking an ultra scale-down approach with the manufacturing scale process allows the feasibility of the cell-product production method to be evaluated.
- An array of scaled-down engineering tests and biological analyses were performed on a prostate cancer vaccine cell candidate with a view to reducing cell-growth constraints and determine cell responses to processing.

Principles of whole cell based vaccine therapy



- A number of immortalised prostate cell lines, once harvested and irradiated, are administered to the patient.
- These cells stimulate and immunogenic response, triggering a cascade effect which results in the creation and presentation of anti-prostate cancer cytotoxic T lymphocytes.

Product Process Flow Sheet

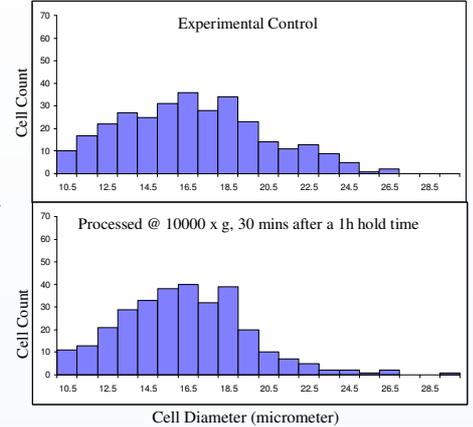


- Cell culture utilises cell factories for adherent monolayer growth, enzymatic harvest and numerous centrifugation driven wash stages.

Experimental Findings

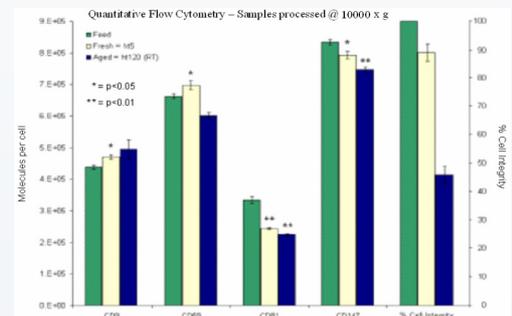
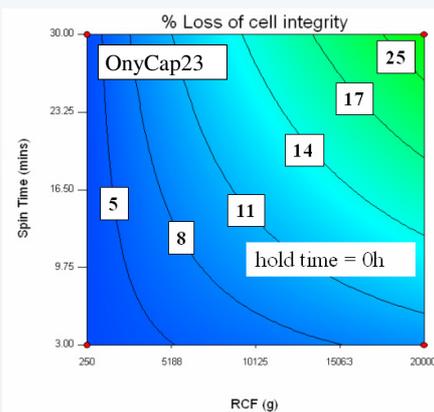
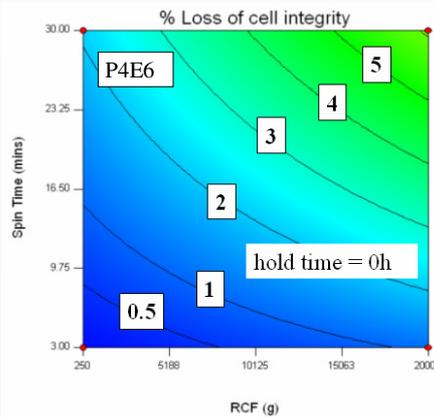
- A Design Of Experiments (DOE) study was created to establish the key process inputs and their subsequent effects on the target cells within batch centrifugation.
- The use of this approach offered an insight into parameter interactions, offering a more in depth analysis with the need for far less experimental runs.
- Goal – to establish a window of operation with relation to the three processing parameters; Relative Centrifugal Force (RCF), Spin Time and Pre-processing Hold Time, for the efficient and effective bioprocessing of whole cell therapies.
- Selection of cell line is key to achieving high yields during bioprocessing.

- Cell lines display an increase in cell membrane damage as both centrifugal force and spin time increase. The addition of a 2 hour pre-processing ambient hold time, such as may occur during large scale processing, results in an amplification of the membrane integrity loss.



- The effect of applied RCF for a given spin time is less evident within size distribution plots taken pre and post processing.

- Small decreases of less than 10% were observed in cell size evaluation, with intense cell compaction forces thought to be responsible



- Surface marker phenotype analysis by quantitative flow cytometry suggests that the application of extreme centrifugation conditions does have a significant effect on the selected cell surface marker profile, although small in comparison to cell integrity loss.

- The above examples are for extremes outside the normal operating window. This is deliberately to help evaluate cell properties and aid in cell line selection.

- Processing conditions that are sufficient to recover cells but lead to a loss of integrity, especially if the cells have been held, have little or no effect on cell size and surface marker phenotype.

Conclusions

- The impact of centrifugation upon a whole cell vaccine has been quantified, identifying key critical process parameters (CPPs) associated with key cell quality attributes.

- CPP interactions are displayed within this study type, allowing the construction of a design space for centrifugation based processing.

