

Therapy - The impact of centrifugation upon key product quality attributes

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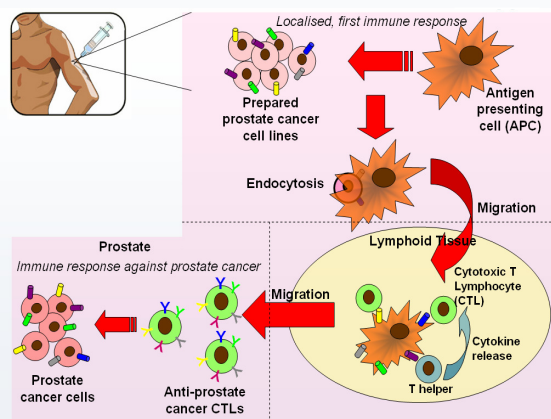
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Introduction

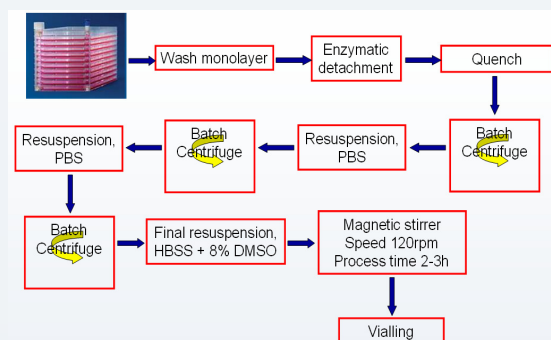
- It is ever more critical for cell based therapy to be delivered by a well-designed, data driven bioprocess
- A Quality by Design (QbD) approach allowed the combined effects of multiple process inputs to be evaluated at small scale for a selected unit operation – centrifugation.
- Linking an ultra scale-down 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 an immunogenic response, triggering a cascade effect which results in the creation and presentation of anti-prostate cancer cytotoxic T lymphocytes.

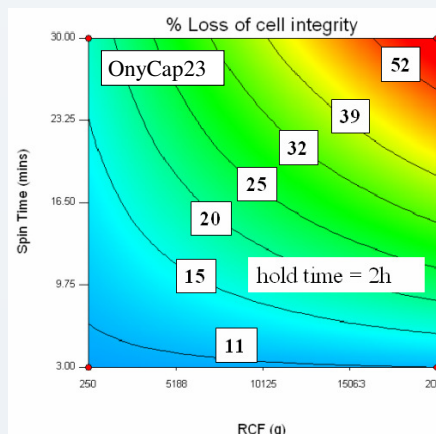
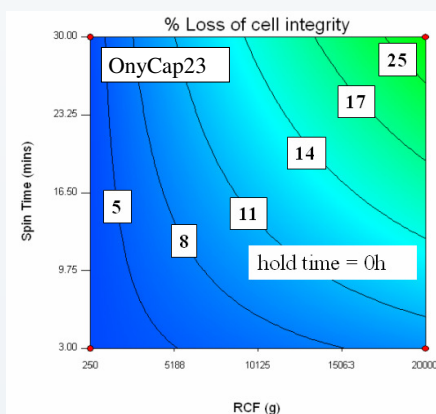
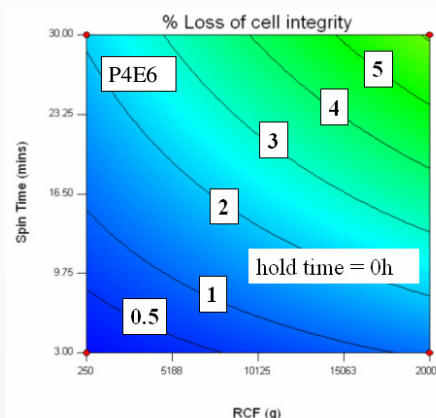
Product Process Flow Sheet



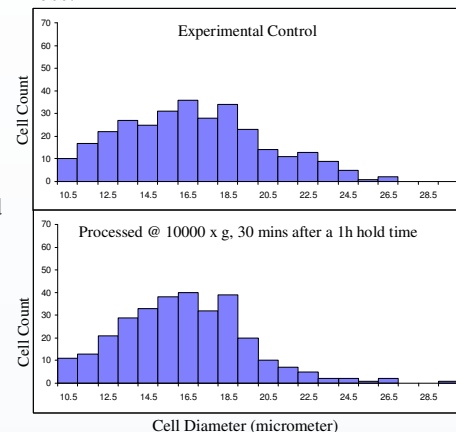
- Cell culture utilises cell factories for adherent monolayer growth, enzymatic harvest and numerous centrifugation driven wash stages.

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.

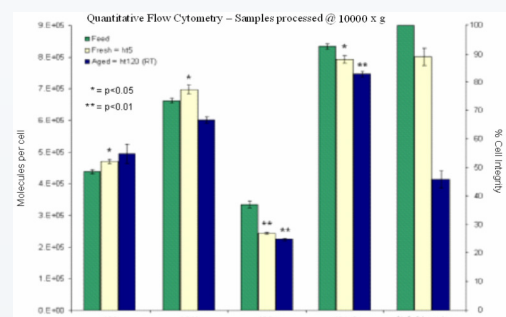


- Cell lines display an increase in cell membrane damage as both centrifugal force and spin time increase. The addition of a 2 hour pre-processing ambient hold time, such as may occur during large scale processing, results in an amplification of the membrane integrity loss.



- 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.