The role of perfusion in maintaining high density T-cell cultures

Intellectual Property Notice: The Biopharma business of GE Healthcare was acquired by Danaher on 31 March 2020 and now operates under the Cytiva™ brand. Certain collateral materials (such as application notes, scientific posters, and white papers) were created prior to the Danaher acquisition and contain various GE owned trademarks and font designs. In order to maintain the familiarity of those materials for long-serving customers and to preserve the integrity of those scientific documents, those GE owned trademarks and font designs remain in place, it being specifically acknowledged by Danaher and the Cytiva business that GE owns such GE trademarks and font designs.
The role of perfusion in maintaining high density T-cell cultures

A Marenghi, M Janas, C Nunes, V Sauvage, B Davies, A Bajaj and A Burns

ML Janas (michelle.janas@ge.com), is a senior scientist and C Nunes, A Marenghi and V Sauvage are scientists at GE Healthcare, Cell Therapy Technologies, Cardiff, UK.

B Davies, A Bajaj and A Burns are scientists at the GE Global Research Centre, Niskayuna, NY.

Abstract
T-cell therapies are a rapidly growing field of personalised medicine, attracting the interest of venture capitalists and pharmaceutical companies alike. They exploit the T-cell’s innate ability to protect against pathogens, as well as seek and destroy cancerous cells. Although there are many different forms of T-cell therapies currently being trialled, they follow a common protocol. T-cells are isolated from the patient, modified and expanded in the laboratory and then infused back into the patient ready to fight disease.

Introduction
Manufacturing for cell therapies has adopted many of the principles of the bioprocess industry, including the use of bioreactors for cell cultivation. Rocking platform bioreactors are one example, where the combination of rocking agitation and perfusion media exchange allows high cell concentrations to be reached. To understand the impact of media perfusion on high-density T-cell cultures in detail, we used the Xuri™ Cell Expansion System W25, a rocking bioreactor, to grow primary T-cells with and without media perfusion. The impact of perfusion on cell growth and viability was analysed as was the role of perfusion in controlling the key metabolites and growth factors.

Materials and Methods
Activation and Expansion of T cells
For each bioreactor inoculate, 5 x 10^6 frozen PBMCs were thawed, washed twice, and cultured in T25 flasks at 1 x 10^6 cells/ml. T cell expander CD3/CD28 beads (Life Technologies) were added to the culture at a ratio of 3:1 beads:CD3+T cells. Cells were cultured in a humidified incubator at 37°C. After 3 days, cells were counted and maintained at 0.5 x 10^6 cells/ml by media addition for a further 2 days.

On Day 5 of expansion, cells were transferred to a 2 L Xuri Cellbag™ perfusion disposable bioreactor (GE Healthcare), at 0.5 x 10^6 cells per ml. Xuri Cellbag bioreactors were placed on the Xuri Cell Expansion System W25 set at 37°C /5% CO2 with a rock rate of 15 rpm and a rock angle of 6°. Cells were maintained at 0.5 x 10^6 cells per ml until a maximum working volume of 1 L was obtained. Continuous perfusion was initiated when the cultures reached a cell density of 2 x 10^6 cells/ml, and perfusion rate was increased according to cell concentration for the remainder of the expansion to day 14.

Results

Figure 1 Media perfusion (rates illustrated in green) supports T-cell growth

Figure 2 Media perfusion (rates illustrated in green) supports T-cell viability

Figure 3 Media perfusion removes unwanted waste metabolites from the bioreactor

Figure 4 Media perfusion provides critical growth factors to T cells grown in a bioreactor

Conclusions
- Without perfusion T cell growth arrest occurs and viability decreases.
- Media perfusion removes unwanted metabolites such as lactate and ammonia from culture.
- Perfusion provides crucial factors such as glucose and IL-2 to the culture.

GL, CardiOeasy and Cellbag are trademarks of General Electric Company.
Cellbag, Xuri™, XuriCell™, and CellXpress™ are trademarks of General Electric Company or one of its subsidiaries.


Performance of an IVD device or its outcome depends on the appropriate individual testing approach for clinical applications.

All authors are employees of General Electric Healthcare (GE Healthcare, Munich, Germany) and may have a financial relationship with the company. All authors have reviewed the manuscript and approved the final version.

Drug manufacturers & clinicians are responsible for obtaining the appropriate IND/BLA/NDA approvals for clinical applications.