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

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

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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.

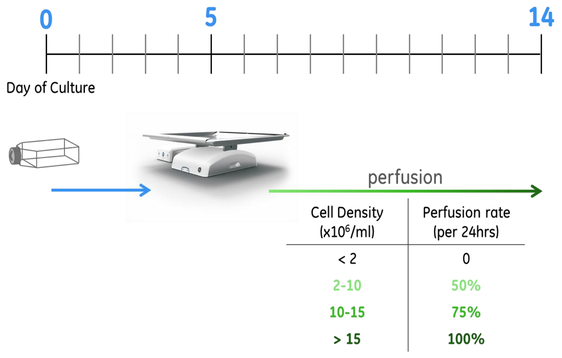
Materials and Methods

Activation and Expansion of T cells

For each bioreactor inoculate, 5 × 10^7 frozen PBMCs were thawed, washed twice, and cultured in T225 flasks at 1 × 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 × 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 × 10^6 cells per ml. Xuri Cellbag bioreactors were placed on the Xuri Cell Expansion System W25 set at 37°C /5%CO_2 with a rock rate of 15 rpm and a rock angle of 6°. Cells were maintained at 0.5 × 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 × 10^6 cells/ml, and perfusion rate was increased according to cell concentration for the remainder of the expansion to day 14.

Experimental Protocol



Cell Density (x10^6/ml)	Perfusion rate (per 24hrs)
< 2	0
2-10	50%
10-15	75%
> 15	100%

Results

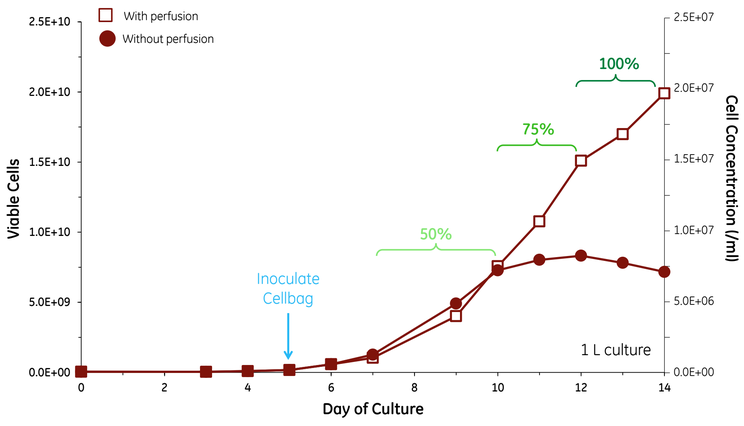


Figure 1 Media perfusion (rates illustrated in green) supports T-cell growth

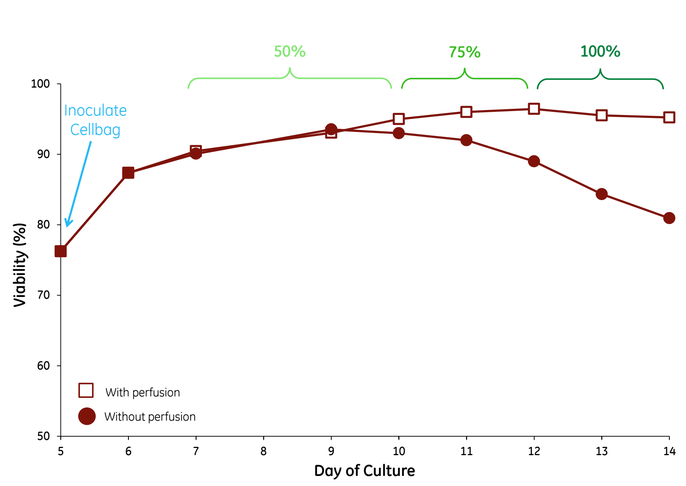


Figure 2 Media perfusion (rates illustrated in green) supports T-cell viability

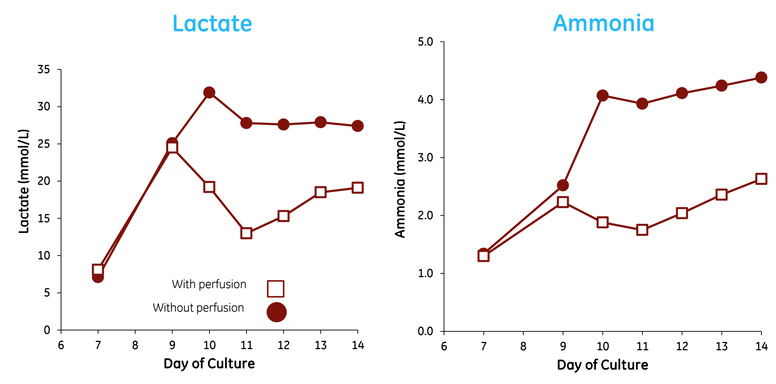


Figure 3 Media perfusion removes unwanted waste metabolites from the bioreactor

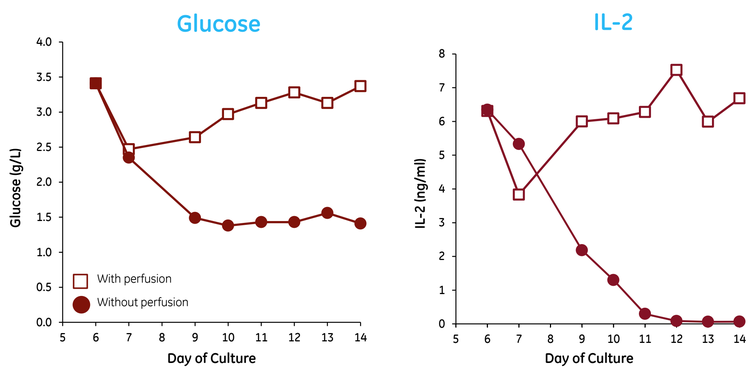


Figure 4 Media perfusion provides critical growth factors to T cells grown in a bioreactor

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.