We have evaluated and characterised this new automated vessel design and ambr250 modular system capability in application to cell therapy processes by demonstrating scale down potential for industrial processes using hMSCs, expansion of T cell populations relevant to patient dosing and generation of detailed and accurate models of the dynamic bioprocessing environment to illustrate the manufacturing potential of dynamic culture in cell therapy manufacture.

Characterisation of a dynamic modular automated system for the scale-up of allogeneic and autologous cell therapy products

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[2] TAP Biosystems (part of the Sartorius Stedim Biotech Group)

Overview

- There is a growing requirement for automated, scalable expansion systems that support suspension-based autologous and 3D microcarrier culture for allogeneic cell therapies.
- TAP Biosystems (part of the Sartorius Stedim Biotech Group) has developed ambr 250 modular, a small scale, single-use stirred tank bioreactor. In partnership with the Cell and Gene Therapy Catapult, a new ambr 250 modular vessel design was evaluated for cell therapy scale-up and scale-out applications.
- We have evaluated and characterised this new automated vessel design and ambr® 250 modular system capability in application to cell therapy processes by demonstrating scale-down potential for industrial processes using hMSCs, expansion of T cell populations relevant to patient dosing and generation of detailed and accurate models of the dynamic bioprocessing environment to illustrate the manufacturing potential of dynamic culture in cell therapy manufacture.

Allogeneic Cell Expansion

- Current strategies for allogeneic cell therapy manufacture typically explore the scale up route to achieve product dose demands for emerging products. Mesenchymal stem cell products make up a large portion of the phase II clinical trial subset, and thus were selected to provide a representative example of an allogeneic cell therapy.
- One of the routes to effective scale-up of human mesenchymal stem cells (hMSCs) is 3D culture using microcarriers in stirred tank reactors (STR). The ambr 250 modular platform (TAP Biosystems, UK) has the potential to provide a representative scale-down tool for allogeneic platform process development and cell culture optimisation due to its low volume and representative control options.
- The aim was to perform proof-of-concept work to assess the potential of the ambr 250 modular system use for bone marrow derived hMSC (RoosterBio, US) culture on microcarriers. The system was used to carry out hMSCs culture on collagen (Pall, UK) microcarriers at 15g/L. The study assessed cell growth performance, metabolite profiles, critical quality attributes of the final cell product as well as operational aspects of the system.
- Previous work carried out at Cell and Gene Therapy Catapult provided bases for microcarrier, medium and cell selection used for these studies. A bench-scale single-use bioreactor system (3 L) was also selected to provide a scalability assessment basis for the system performance comparison.

Fig. 1

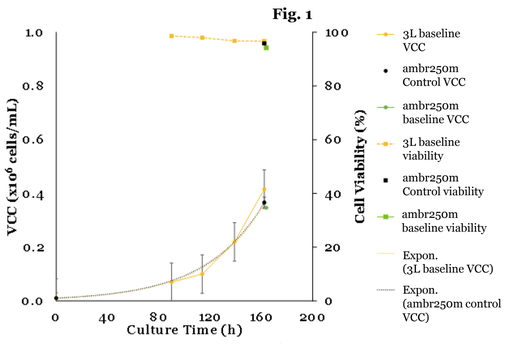


Fig. 2

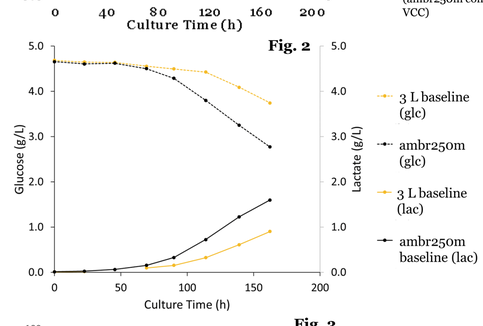


Fig. 3

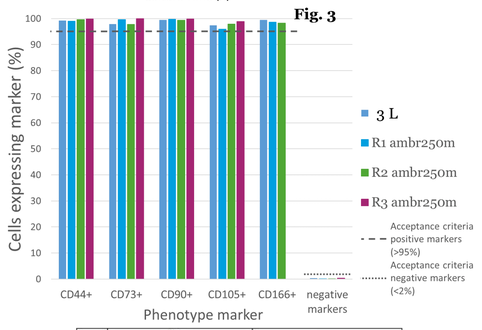


Fig. 4

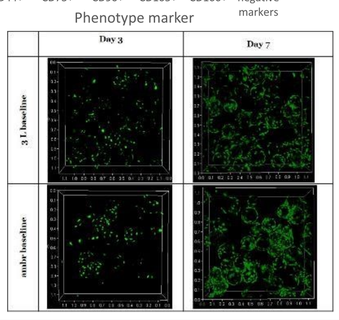
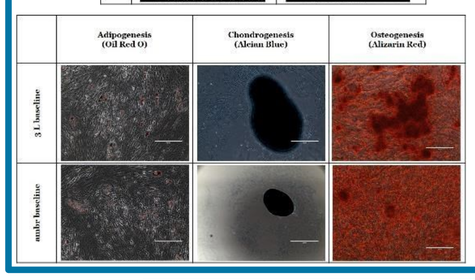


Fig. 5



- Potential microcarrier damage and maintenance of micro-carriers in suspension (N_{JS}) was investigated prior to expansion studies. 150 rpm was set within ambr 250 modular.
- hMSCs were expanded in 2D cell factories (RoosterBio medium used throughout) and used for the inoculation of both the 3 L and ambr systems.
- Cell counts were performed for the 3 L daily from day 4, with final harvest counts only from ambr (Fig. 1). The average of 3 expansions in the 3 L system ranged from 22-54 fold ex-pansion, compared with 30-36 fold within ambr 250 modular.
- Metabolite samples were taken daily from both systems, with elevated glucose consumption and lactate production observed with ambr when compared with the 3 L data (Fig. 2).
- Cell phenotype of the harvested populations were confirmed by flow cytometry (Fig. 3), with comparable expression of the positive marker panel (ISCT).
- Confocal microscopy was used to qualitatively assess cell coverage of the microcarriers (Fig. 4), with comparative images produced from both expansion systems.
- Harvested cell populations were also evaluated for their differentiation potential, with both systems demonstrating adipogenesis, osteogenesis and chrondrogenesis (Fig. 5).

Autologous Cell Expansion

- Current manufacturing solutions for autologous cell therapies consist of stationary vessels (flasks or bags), requiring a high level of manipulation and costly scale-out throughout the product expansion phase. Agitated vessels, such as stirred-tank reactors (STR) and lateral movement culture systems which are more amenable to scalability, have enhanced control of the culture environment, and provide automation of culture maintenance and analysis.
- In partnership with TAP Biosystems we wished to evaluate performance of their ambr 250 modular prototype when used in an autologous suspension cell therapy scale-up and scale-out application. The prototype system contained two separate bioreactor stations (denoted, BR) under individual control by the system software, and single-use bioreactor vessels with integrated reagent reservoirs.
- A cell bank of lymphocytes was generated for use within system testing and used to evaluate the prototype bioreactor in supporting T-cell expansion and how it compared with both static bags and lateral movement alternatives.
- Cultures were expanded (in triplicate) for a total of eight days (Fig. 6), with cell count and viability analysis, pH, glucose and lactate measurement and cell identity evaluation from the three systems.

Fig. 6

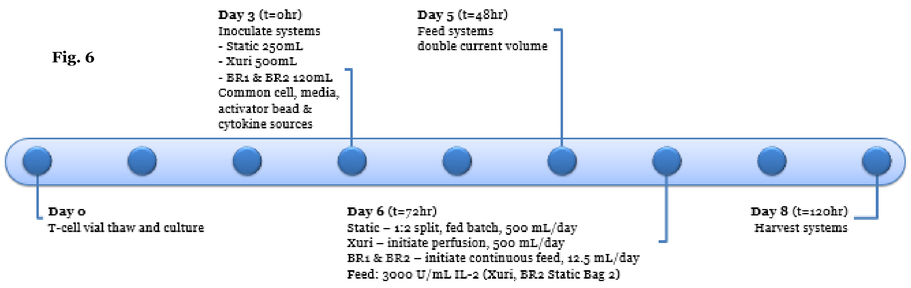
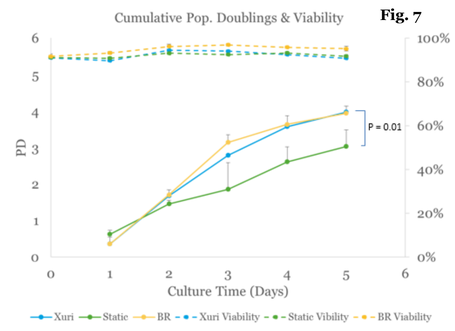
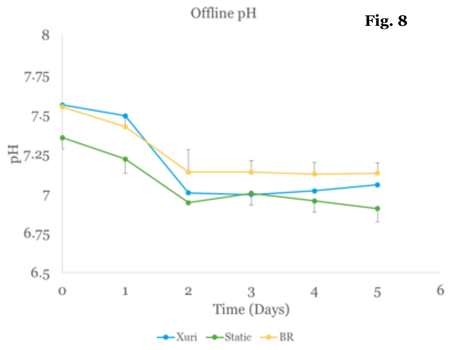


Fig. 7



- Population doubling and viability data was generated using an automated cell counter (Trypan blue exclusion), with both Xuri (GE Healthcare, USA) and ambr 250 modular BR systems supporting significantly higher levels of cell proliferation in comparison with the static alternative (Fig. 7)

Fig. 8



- pH was compared offline across all 3 systems, with a high level of control maintained within the ambr 250 modular BR around the 7.2 set-point (Fig. 8)
- Metabolites were samples daily, with slightly elevated glucose consumption and lactate production observed within ambr 250 modular BR compared with the Xuri and static alternatives (Fig. 10)
- Cell identity (CD3+/CD4+/CD8+) was confirmed across inoculate and harvested samples (Fig. 9)

Fig. 9

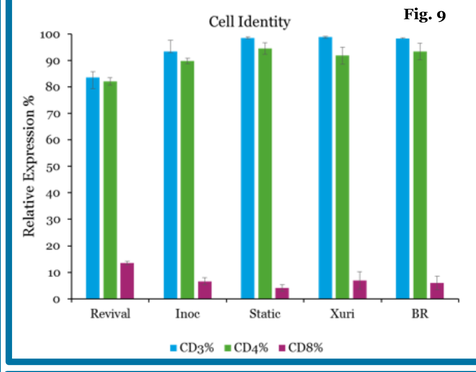
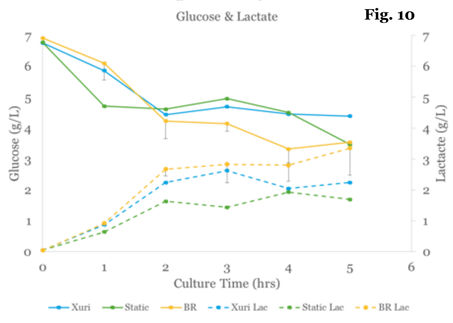


Fig. 10



Vessel Characterisation

- In addition to both the T Cell and hMSC expansion studies, further investigation was undertaken with the aim of providing a detailed understanding of the dynamic fluid structures within the vessel in order to assist the future development of the system.
- This understanding was facilitated by the generation of a computer model for the fluid within the vessel, driven by the rotating impeller. The computer model was a numerical simulation carried out using a commercial computational fluid dynamics package (CFD) called Flo (CFX, Ansys, US) (Fig. 11).
- In simulation, a rotating impeller was created by dividing the mesh into two domains: a stationary domain originating at he vessel wall and extending towards the impeller, and a rotating domain encasing the impeller (Fig. 11).
- In order to validate this numerical model, an experimental model of the ambr250 mod was constructed to facilitate a laser imaging study (particle image velocimetry or PIV), resulting in the generation and evaluation of high resolution velocity fields (Fig. 12)
- The PIV and CFD results showed high similarity in the model displayed (Fig. 13 – CFD the left hand portion of the paired images, PIV the right hand portion), thus validating the simulation strategy. Similar trends in dynamic fluid structures were observed.
- This capability will facilitate the capacity to predict stresses present within automated expansion systems, providing foresight into the potential success for future cell therapy applications.

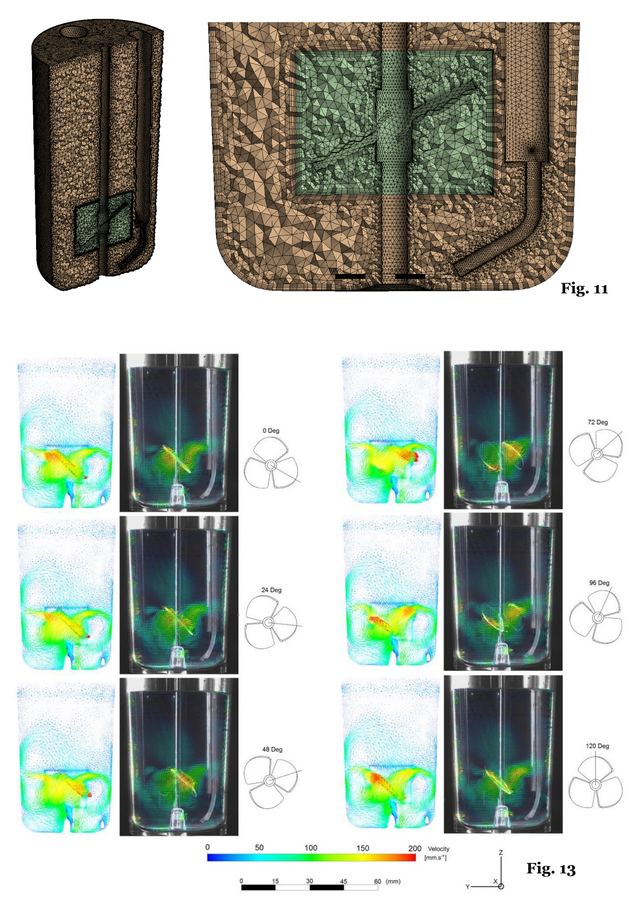
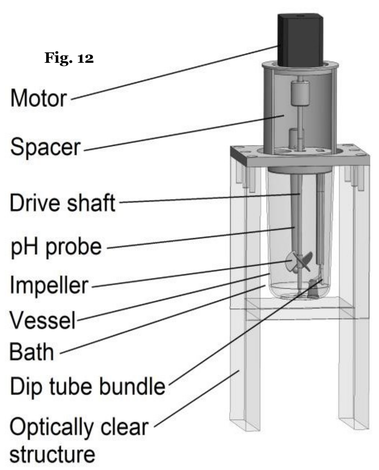
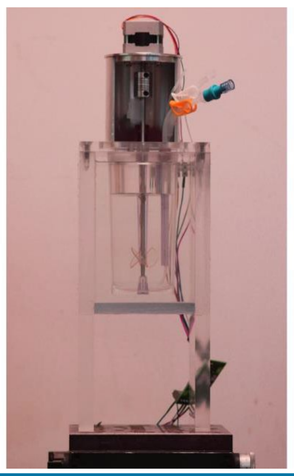


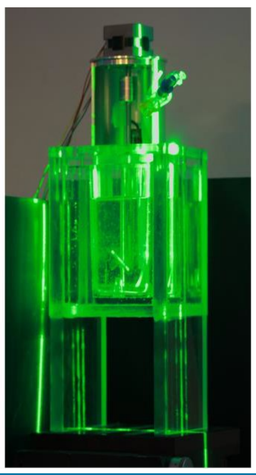
Fig. 11

Fig. 13

Fig. 12







We work with Innovate UK

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