Screening of Treg culture conditions using a novel scalable bioreactor

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- Regulatory T cells (Tregs) are pivotal in maintaining immune homeostasis and preventing autoimmunity, making them a promising therapeutic modality for a range of autoimmune and inflammatory diseases.
- Traditional methods for isolation and culture of Tregs have been labour-intensive, manual processes, which face limitations in scalability, cost and reproducibility. Innovative approaches are necessary (Hennessy et al., 2023).
- This study explores the use of novel bioreactors designed for the efficient and large-scale expansion of Tregs. These bioreactors incorporate advanced features such as precise control of the microenvironment and optimised nutrient delivery systems. Six conditions were tested to study the effect of agitation and seeding density on the proliferation and phenotype of Tregs in the MFX-12 bioreactors, with standard plasticware controls.
- Continuous monitoring of morphology, cell growth, viability and analytes (glucose and lactate) was performed for agitated and static bioreactors, along with controls at 250K cells/mL and 500K cells/mL seed density.
- Preliminary data demonstrate comparable growth and phenotype, with a higher viability in the MFX-12 compared to the control when seeded at 250K cells/mL. Data also highlights the need for a platform with online and inline analytics for monitoring morphology, growth, metabolic activity and phenotype of Tregs as a function of agitation and seeding density.
- Future work will focus on further optimising the bioreactor conditions and validating the therapeutic efficacy of bioreactor-expanded Tregs in preclinical and clinical settings.

Introduction

Ex Vivo expansion of Regulatory T cells (Tregs) is a fundamental unit operation in the provision of an associated cell based therapy. However, the process of isolating and expanding Tregs using traditional methods is currently labour-intensive, manual and faces challenges in terms of scalability, cost, and reproducibility. In addition, the ability to rapidly optimise complex culture strategies is hampered by the lack of scaled down models. Since cells are isolated in low numbers (Wolf et al., 2003), an expansion process requires a low starting volume with possibility for continuous increase in volume.

Here we describe work performed in collaboration with AstraZeneca, using a novel scalable bioreactor platform - the Cyto Engine - that explored different feeding strategies (media addition and media replacement), the effect of bi-directional agitation and of seeding density on Treg expansion in scaled down runs (1.5 - 12 ml).

The MFX-12 (Fig. 1a) is a precision controlled scalable bioreactor that offers a plug and play approach to high throughput screening in research or process development of cell therapies. The MFX-12 can either be operated manually or in an automated manner using the Cyto Engine START alpha prototype (Fig. 1b).

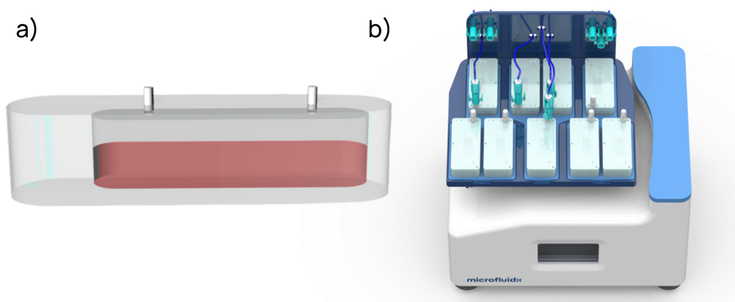


Fig.1: (a) MFX-12 bioreactor used for Treg expansion, allows precise control of media exchange and gas overhead (b) Cyto Engine START alpha prototype used for agitation

Methodology

Three Treg configurations were tested and compared over a 9 day period in MFX-12s agitated on the Cyto Engine START alpha prototype, MFX-12s static and controls (6 well plates moved to T12.5 flasks on Day 2). Figure 2 summarises the conditions and Figure 3 explains the experimental procedure. The gating strategy for flow cytometry is shown in Figure 4.

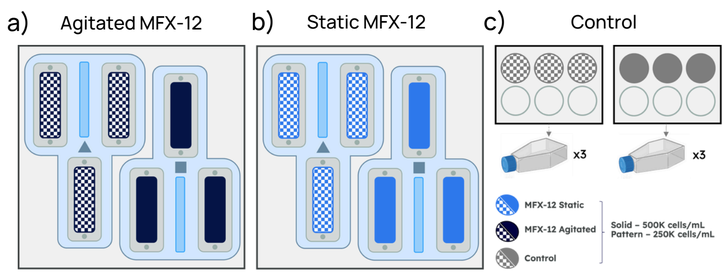


Fig.2: Exprimental conditions for (a) agitated MFX-12s, (b) static MFX-12s and (c) controls

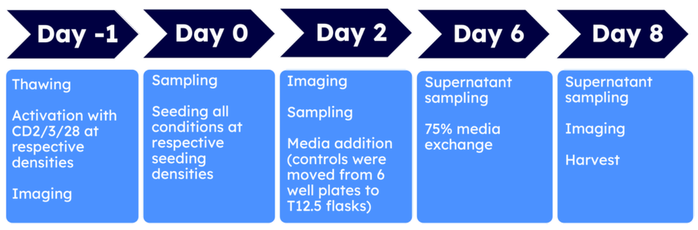


Fig.3: Experimental procedure for media addition and media exchange feeding strategies for an 8 day T-reg culture

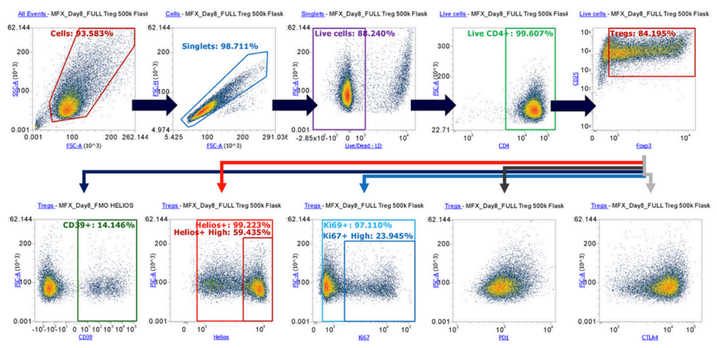
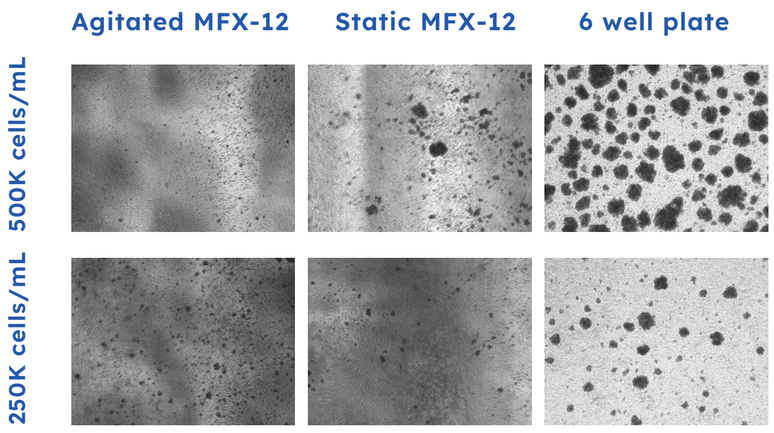


Fig.4: Gating strategy for flow cytometry

Results - growth & metabolomics

MICROSCOPY

a) DAY 2



b) DAY 6

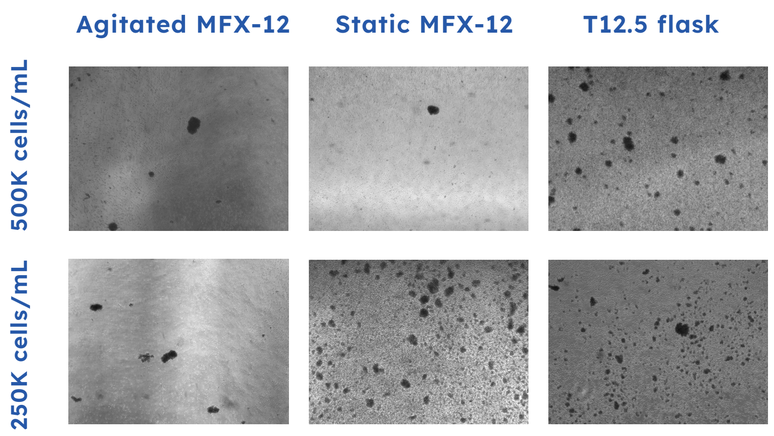


Fig.5: Treg morphology observed on a) Day 2 and Day 6 in agitated MFX-12s, static MFX-12s and T12.5 flasks with 250K and 500K cells/ml

- Microscopy showed smaller aggregates in the agitated MFX-12s, as opposed to static MFX-12s and flasks.
- More aggregates observed in static MFX-12s on Day 6 at 250K cells/mL as opposed to flasks and agitated MFX-12s.

CELL GROWTH

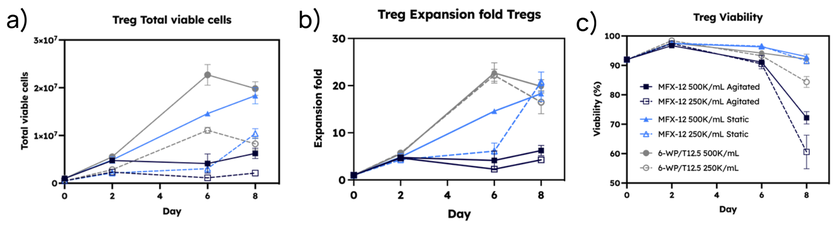


Fig.6: Expansion of Tregs in agitated MFX-12s, static MFX-12s and controls (a) Total viable cells, (b) expansion fold and (c) viability

- No significant difference in total viable cells, expansion fold and viability between static MFX-12s and controls on Day 8.
- Decrease in growth and viability was observed with agitation, potentially linked to less aggregates (Fig.5).
- When seeding at a lower cell density of 250K/mL the MFX-12 had a higher viability than the control condition at the same density.

ANALYTE ANALYSIS

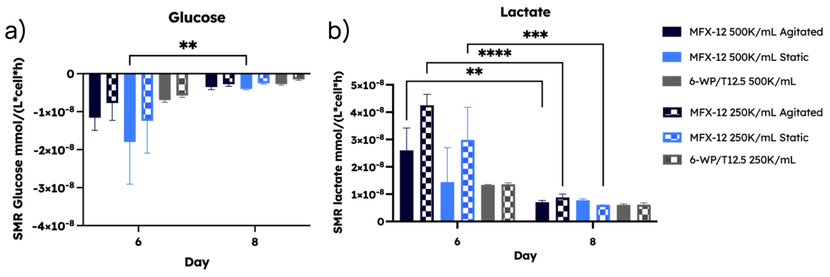


Fig.7: Metabolite analysis for (a) Glucose and (b) Lactate based on the specific metabolite rate (SMR) of Tregs on Day 6 and Day 8 in agitated MFX-12s, static MFX-12s and controls. SMR was calculated based on the specific growth rate (SGR). **P = 0.0014 - 0.0023,***P = 0.0004, ****P< 0.0001

- Higher metabolic activities (glucose and lactate) on Day 6 as opposed to Day 8. This aligns with specific growth rate (SGR) decreasing with time in all vessels.
- Higher metabolic activities in MFX-12s vs controls.
- These different activities observed between conditions demonstrate the need for a platform with online and inline analytics to facilitate adaptive feeding strategies.

Results - phenotype markers

FLOW CYTOMETRY

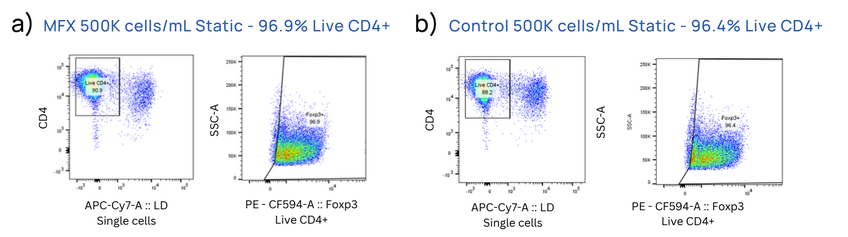


Fig.8: Comparable phenotype of Tregs (CD4+) in (a) static MFX-12s at 500K cells/mL, (b) control at 500K cells/mL, (c) static MFX-12s at 250K cells/ml and (d) control at 250K cells/mL.

- Treg phenotype (CD4+) in the Static MFX-12 is comparable to the control at each seeding density, suggesting no shift in regulatory cell phenotype.

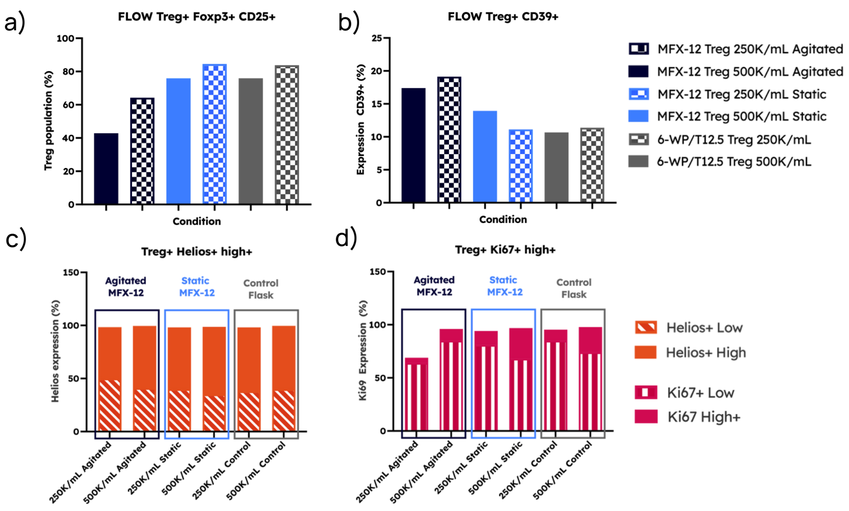


Fig.9: Comparable phenotype of Tregs (a) Foxp3, CD25+ and (b) CD39+ in static MFX-12s and controls at both 500K cells/mL and 250K cells/ml conditions. Comparable expression of (c) Helios+ and (d) Ki67+ in the Static MFX-12 and controls or their respective seeding densities.

- Comparable Foxp3+ and CD39+ expression in the Static MFX-12 is compared to the control Well/Flask for their respective seeding densities, suggesting no shift in regulatory cell phenotype.
- No loss of Helios+ high+ expression observed in all vessels, demonstrating stability (Lam et al., 2022)

Conclusions and future work

- Using Regulatory T cells (Tregs) we demonstrated:
  - Comparable expansion using media addition and exchange approaches in static MFX-12 vs controls.
  - Comparable phenotype (CD4+, Foxp3+, CD25+, CD39+) and expression (Helios+ and Ki67+) in static MFX-12s.
  - Higher cell viability in static-MFX-12s as opposed to controls when seeded at a lower seeding density
- Different metabolic activities observed between Day 6-8, supporting the need for feed a control loop, dependent on the seeding density and agitation rate.
- Agitation did not support Treg aggregates, perhaps linked to lower cell proliferation and viability observed
- The different morphologic and metabolic activities observed, demonstrate the power of utilising a platform with online and inline analytics to monitor cell growth and provide optimal conditions at the right time of expansion.

- Future work will:
  - further explore the impact of agitation on Treg expansion using intermittent strategies
  - Explore the use of this platform for optimising autologous processes with limited starting material, by applying statistically powered DoE to accelerate process development.

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