Analysis RNAseq and morphometrics to establish the predictive value of clone metrics within and between patients is in progress. The Cell X™ platform provide effective tools for automated assessment and processing of the heterogenous cells and colonies that serve as the starting materials for MSC manufacturing to reduce variation and reproducibility of manufacturing processes.

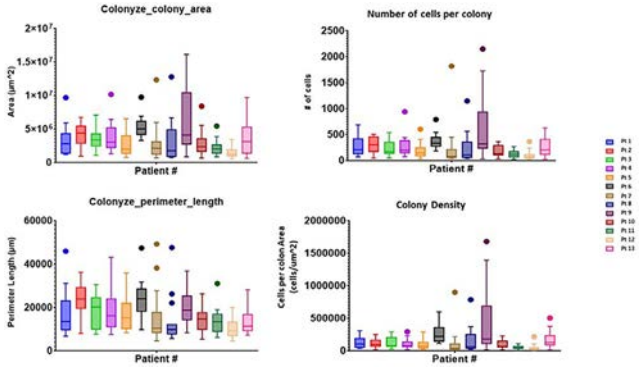

Fig 1 (abstract 145). Colony Founding Cell morphological metrics measured with ImageJ software showed wide variation in morphological attributes (area, perimeter, circularity, Feret Diameter) of different founding progenitor cells within and between patients.

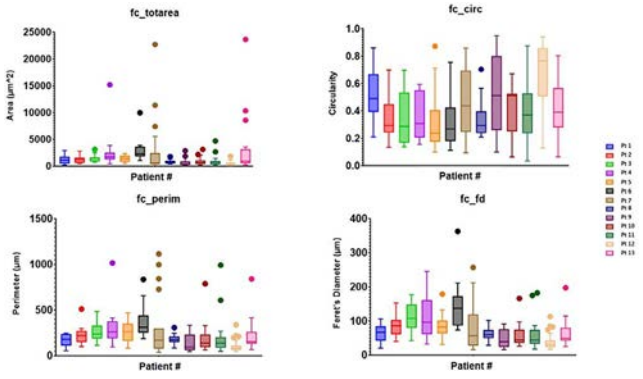

Fig 2 (abstract 145). Colony morphological metrics (colony area, colony perimeter, cells per colony, cell density) measured with Colonyze™ software, on day 9-10, at the time of PBS selection, reconfirms with variation among single cell-derived colonies, within and between patients.

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Mesenchymal Stem/Stromal Cells

ADVANCE BIOPROCESSING WITH CUSTOMISED MICROCARRIERS: ENHANCING ANCHORAGE DEPENDENT CELL YIELDS

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Keywords: Microcarriers, Biodegradable, Mesenchymal Stromal Cells.

Background & Aim: Microcarriers, essential in bioprocessing since the 1960s, are microscale beads used in bioreactors for cell growth. Despite their widespread use, innovation in this sector has been limited, leading to challenges in industrial scaling. Primary issues include particulates and residuals from construction materials, non-biodegradability, and a lack of customizability. Customizability is crucial as different cell types require specific physical and chemical substrate properties; an aspect inadequately addressed in current microcarrier technology. Research has extensively shown that cell growth, secretion, and differentiation are significantly influenced by substrate properties. We have applied this knowledge to develop adaptable microcarriers.

Methods, Results & Conclusion: To replicate physiological conditions, Smart MCs utilized microfluidics technology to create 150-micron diameter microcarriers with adjustable physical and chemical properties, such as stiffness and surface charges. This study focuses on the X1 series of microcarriers in three stiffness levels: X1S (soft, 16.67 kPA), X1M (medium, 37.9 kPA), and X1H (hard, 77 kPA). Experimental data showed a strong correlation between microcarrier stiffness and cell proliferation (Fig. 1A).

The study involved multiple cell types, including C2C12 mouse myoblasts, L929 mouse fibroblasts, and MARC-145 monkey kidney cells, to assess the impact of microcarrier stiffness on cell behaviour. C2C12 and L929 were seeded at 4 cells/microcarrier and harvested on day 5, whereas MARC-145 was seeded at 16 cells/microcarrier and harvested on day 3. C2C12 preferred X1S microcarriers, while L929 and MARC-145 favoured X1M. (C2C12 has 20.5 folds increase in 5 days, L929 has 20.3 folds increase in 5 days and MARC-145 has 6.5 folds increase in 3 days, Fig. 1B, C). The higher cell yields from X1 microcarriers compared to competitors were attributed to enhanced cell proliferation, their biodegradable nature enabling better cell harvesting, and differentiation capability to muscle cells (Fig. 1D).

In conclusion, the bespoke microcarriers represent a significant breakthrough in bioprocessing technology. Aligning microcarrier characteristics with specific requirements ensured improved cell growth. This innovation promises to surmount traditional microcarrier limitations, setting new standards for efficiency and versatility in the field.

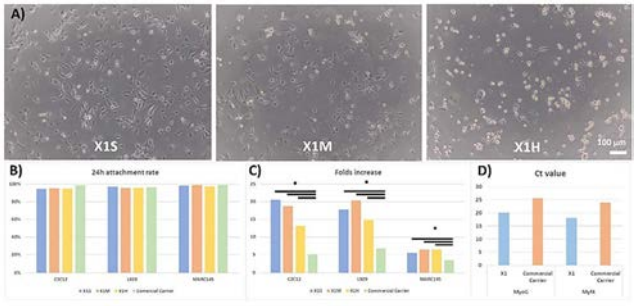

Figure 1: Cellular Outcomes on Microcarriers of Varied Stiffness. A) C2C12 elongate more on X1S substrate compared to X1M and X1H after 3h of seeding. **B)** This panel illustrates the cell attachment rate, which is consistently high across all microcarrier types. This finding suggests that material choice or surface chemistry plays a more pivotal role in cell binding than stiffness does. **C)** Depicted here is the fold increase in cell numbers after a 5-day culture period, highlighting each cell type's preference for a specific microcarrier stiffness. C2C12 and L929 cells demonstrated a remarkable 20-fold increase over this duration, significantly outperforming commercial carrier, which only achieved a 5-fold expansion, despite identical media volumes. In the case of MARC-145 cells, there was a 6.4-fold increase, compared to just 3.4-fold with commercial carriers ($p<0.05$). **D)** This section shows the gene expression levels of muscle-related proteins in C2C12 cells after on-microcarrier differentiation. Under identical culture conditions, cells on X1 microcarriers exhibited higher expression of muscle-related proteins (MyoG, Myf4) than those differentiated on commercial carrier's. This enhanced expression indicates a superior differentiation environment provided by the X1 microcarriers.

Fig. 1 (abstract 146). Cellular Outcomes on Microcarriers of Varied Stiffness.

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Mesenchymal Stem/Stromal Cells

ENHANCED TRANSFECTION OF HUMAN BONE MARROW-DERIVED MESENCHYMAL STROMAL CELLS WITH MESSENGER RNA

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Keywords: MSC, Transfection, RNA.

Background & Aim: Human bone marrow-derived mesenchymal stromal cells (MSCs) have been investigated as a therapy for a wide