Optimization of Microcarrier-based Culture of Muscle Precursor Cells

Shaun Burer [1], Wendy Tra [1], Jenny Ann Prange [2], Rosa Angelica Alves de Sousa [2], Rens Roosloot [1], Ruud Das [1], Joost de Bruijn[1,3,4]
1. Scinus Cell Expansion BV, Bilthoven, The Netherlands
2. Laboratory for Urologic Tissue Engineering and Stem Cell Therapy, Department of Urology, University Hospital Zurich, Switzerland
3. Twente University, Enschede, The Netherlands
4. Queen Mary, University of London, London, United Kingdom

INTRODUCTION

Stress urinary incontinence (SUI) affects over 200 million people worldwide. A novel approach to treat SUI is to locally administer autologous muscle precursor cells (MPCs) into the defective sphincter muscle. For the treatment of one patient millions of cells are required. Therefore the production of these MPCs needs to be scaled up.

Scinus Cell Expansion BV developed a novel bioreactor technology using a microcarrier based expansion process which provides a safe and (cost-) effective procedure for the clinical upscale of MPCs. Here, we present the optimization of a microcarrier based culture of MPCs, using a downscaled model of the SCINUS technology.

MATERIALS AND METHODS

MPC isolation

Human MPCs were isolated from an *m. soleus* biopsy by enzymatic digestion and were used for microcarrier culture in spinner flasks (Figure 1).

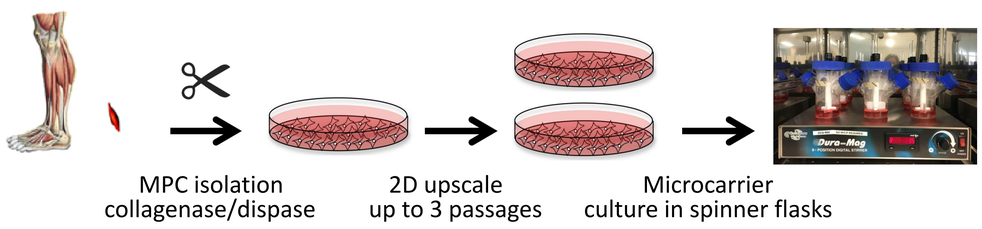

Figure 1 Schematic representation of the microcarrier culture of MPCs

Microcarrier selection

MPCs were cultured for 4 days in spinner flasks on dissolvable microcarriers (Corning Life Sciences) with two types coating: 1) Denatured collagen (DC) and 2) Vitronectin (Synthemax-II, SM2). Cell adhesion was determined 24 hours after of seeding by a cell count. Visual inspection and cell count were performed daily to determine cell proliferation, distribution and cell morphology.

Spinner flask parameters	Value
Volume (mL)	60
Microcarrier concentration (g/L)	1
Seeding density (cells/cm^2)	5000
Rotations per minute (RPM)	35
Agitation regime	1 h static interval / 8h

Expansion optimization; design of experiments (DOE)

For microcarrier based expansion, cells need to migrate from one microcarrier to another. In this process, it is necessary to maintain a homogenous distribution of cells on microcarriers in order to reach high cell yields. To optimize this expansion step, a V two-level factorial design was set up (Stat-Ease Design Expert software) to investigate the influence of cell concentration, microcarrier concentration and agitation interval of the spinner flask on the distribution of cells on microcarriers.

DOE Factors	Range
1: Cell concentration	5.000 – 25.000 cells/mL
2: Microcarrier concentration	1 – 5 g/L
3:Agitation interval	3 – 8 breaks / 24h

MPCs were seeded on DC microcarriers in spinner flasks according to the DOE design. The available surface area was increased threefold on day 3, while keeping the microcarrier concentration constant. Cell concentration, distribution and population doubling time (PDT) were determined at the end of the culture on day 6.

RESULTS

High adherence of MPCs to DC microcarriers

Cell adhesion was determined 24 hours after seeding. MPCs adhered better to DC microcarriers compared to SM2 microcarriers.

	DC	SM2
Total cell number (day 0)	1.50*10^6	1.50*10^6
Total cell number (day 1)	1.17*10^6	4.79*10^5
Adherent cells (%)	**77**	**32**

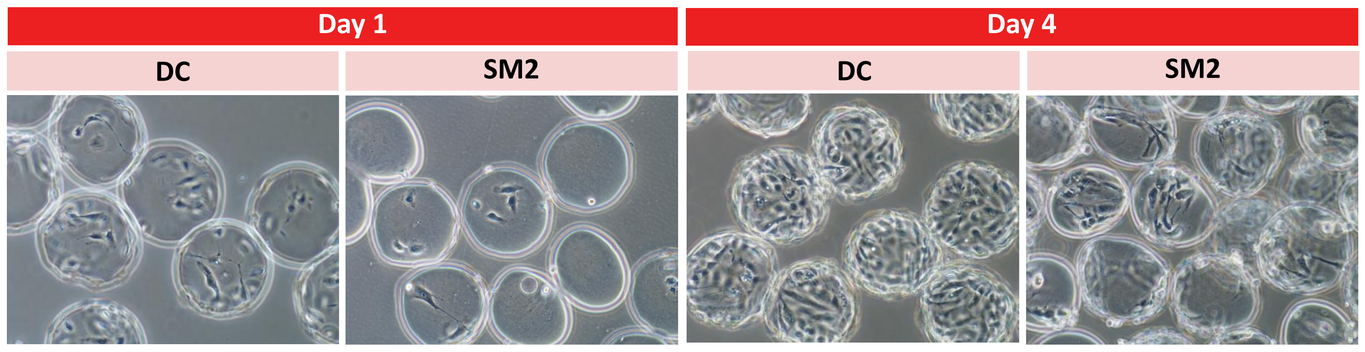

Figure 2 Visual inspection of MPCs cultured on DC and SM2 microcarriers, sampled from spinner flasks at different time points

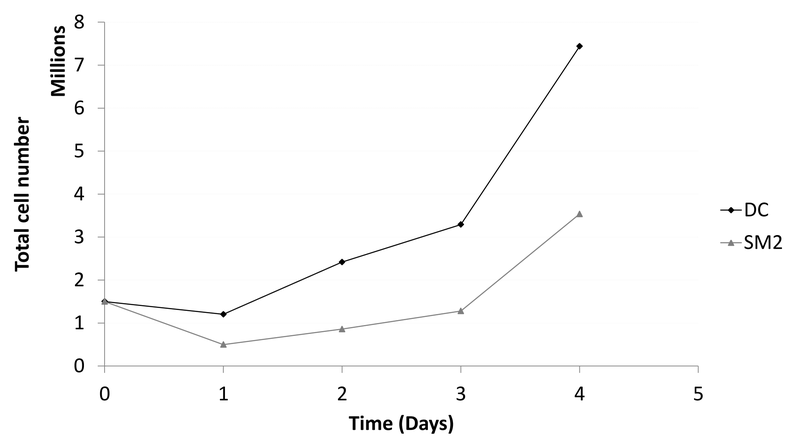

Figure 3 Total cell number in the spinner flask was determined daily. MPCs proliferate in both conditions, reaching between 3.5 and 8.5 million cells at the end of the culture

Proliferation and homogenous distribution of MPCs on DC microcarriers

Cell proliferation and cell morphology of MPCs cultured on both microcarriers were determined daily. DC microcarriers outperformed the SM2 microcarriers regarding cell proliferation (PDT of 27h for DC and 45 h for SM2), distribution and cell morphology (Figures 2 and 3).

Significant DOE model for expansion optimization of MPCs

A significant model was obtained using a Two way ANOVA ($p < 0.05$). There is a interaction between cell concentration and microcarrier concentration that influences the distribution of MPCs cultured on DC microcarriers when cultured in spinner flasks. Low cell concentrations in combination with high microcarrier concentrations result in a low distribution percentage and vice versa (Figure 4). Furthermore, an effect of cell concentration on PDT of MPCs was found. Despite the fact that the agitation interval on its own did not influence the PDT, it has some effect when higher cell concentrations are used. The results from this DOE did not include biological effects such as medium limitations.

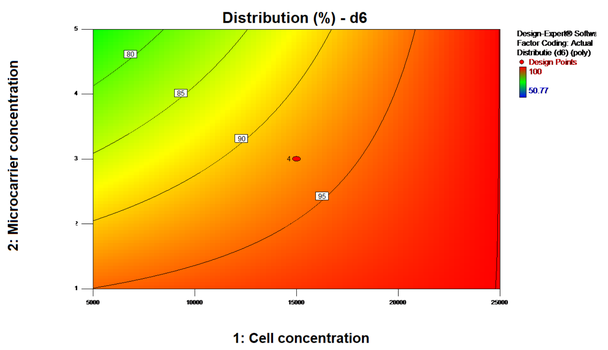

Figure 4 Contour plot of the interaction of cell concentration and microcarrier concentration on distribution. The X1-axis and X2-axis show cell concentration and microcarrier concentration respectively. The distribution (%) on day 6 of culture is showed by the contour line.

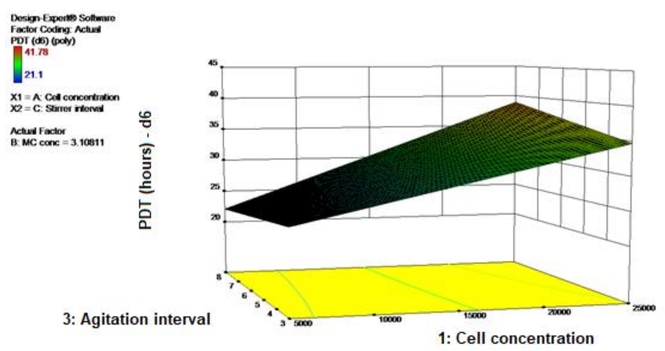

Figure 5 3D surface plot of the interaction of cell concentration and stirrer interval on PDT. The X1-axis and X2-axis show cell concentration and stirrer interval respectively. The PDT (hours) on day 6 of culture is showed on the z-axis.

CONCLUSION AND DISCUSSION

MPCs were successfully cultured on microcarriers. Denaturated collagen-coated microcarriers outperformed vitronectin-coated microcarriers based on cell adhesion, distribution and proliferation. Furthermore, MPC were able to migrate to freshly added microcarriers. This process of bead-to-bead transfer is affected by cell and microcarrier concentration. In conclusion, microcarrier culture of MPCs is possible and the use of the SCINUS Cell Expansion system for upscaling MPCs to clinically relevant numbers seems promising.

Acknowledgements: This project has received funding from the European Union's Horizon 2020 research and innovation programme under *grant agreement* nº [731377].

MUS.I.C. Multisystem Cell Therapy for Improvement of Urinary Continence