Culture of Adipose-derived Stem Cells on microcarriers

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INTRODUCTION

Adipose-derived stem cells (ASCs) can be isolated from fatty tissue. Similar to MSCs isolated from bone marrow, ASCs have multi-lineage potential and can be used as a potential source in regenerative medicine. Additionally, fat tissue is more accessible than bone marrow, and larger volumes can be obtained. For the production of cells for cell therapy in patients, an upgrade to clinical large scale culture (> $200x10^6$ cells) is necessary. Clinical scale cultures require a reproducible and efficient process. Therefore, a process for culturing of large quantities of ASCs using microcarriers (MCs) was developed.

MATERIALS AND METHODS

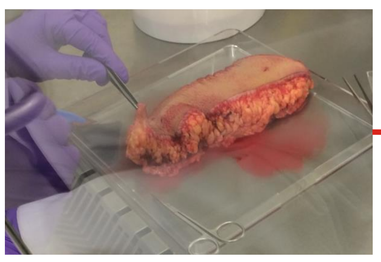
Experimental procedure

Stem cells were isolated from 25 gram fatty tissue obtained from abdominoplasty. After enzymatic digestion with collagenase-I, the stromal vascular fraction (SVF) was obtained and ASCs were isolated using plastic adherence. Next, ASCs were seeded onto 4 g/L microcarriers (Enhanced Attachment, Corning) in spinner flasks. All experiments were done in triplo

Three different microcarrier expansion regimes were tested:

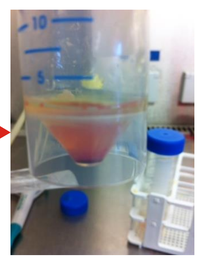
- Condition 1: Daily expansion by increasing the microcarrier concentration and volume with 33%
- Condition 2: Bi-daily expansion by increasing the microcarrier concentration and volume with 70%
- Condition 3: Bi-daily expansion by increasing the microcarrier concentration and volume with 100%

Cells were counted every 2-4 days. Culture medium and microcarriers were added based on cell counts and lactate measurements.

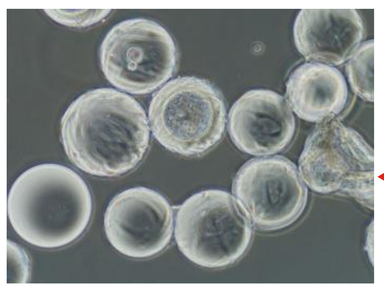


Fatty tissue from abdominoplasty

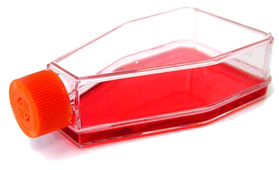
Digestion and centrifugation



Stromal vascular fraction



Monitoring growth on microcarriers



Monolayer culture (1 passage)

Flow cytometry

Spinner flask

Monolayer control

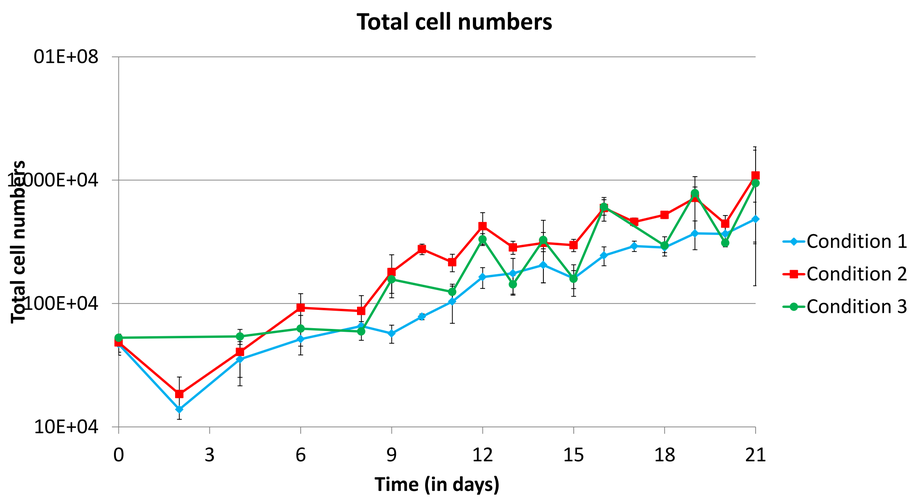
Harvest cells and stain for flow cytometry

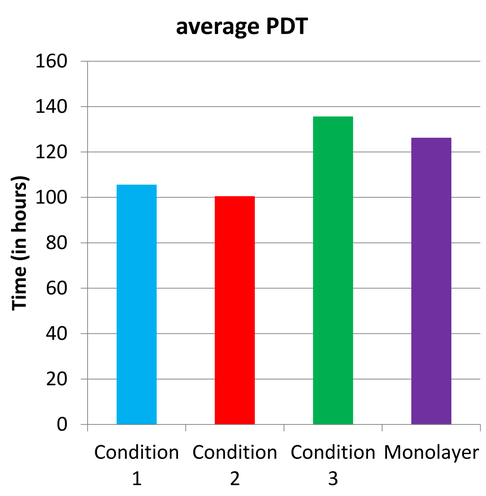
Positive	Negative	Isotype
CD73	CD45	IgG1 FITC
CD90	CD80	IgG1 PE
CD105	CD34	IgG2 PE
	CD3	
	CD31	
	HLA-DR	

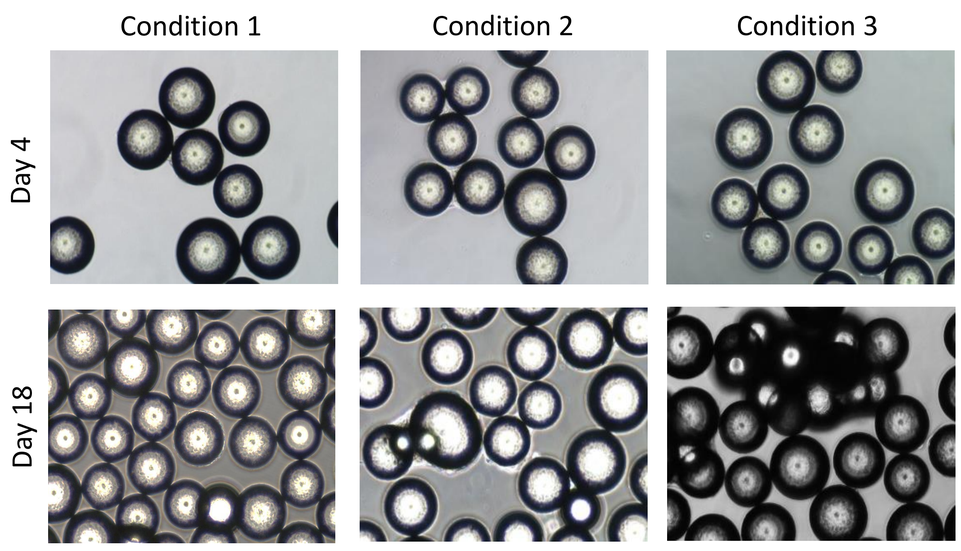
RESULTS

ASC culture on microcarriers

ASCs were counted throughout the spinner flask culture, and the results showed substantially higher cell numbers in condition 2 and 3 when compared to condition 1. Calculation of the population doubling time showed that the PDT was clearly lower for cells cultured with expansion regime of condition 2. The results were supported by the visual inspection. As shown here, there are considerably less cells visible on the microcarriers in condition 1 when compared to condition 2 and 3. However, in condition 3 large cell/microcarrier aggregates were observed.



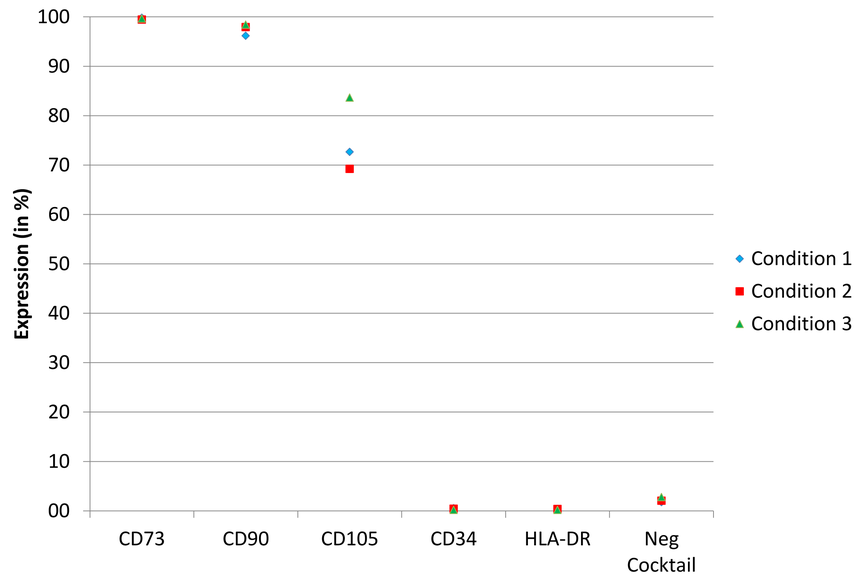




Top left: ASCs cultured with condition 2 and 3 grew to 11 and 9.5 million cells, respectively.
Top right: Population doubling time calculated from the log phase and was 105 h for condition 1, 100 h for condition 2, 135 h for condition 3, and 126 h for monolayer.
Bottom left: Visual inspection showed less cells in condition 1, and formation of large aggregates in condition 3.

ASC characteristics

ASC harvested from Enhanced Attachment microcarriers displayed the correct surface marker profile. Cells were positive for CD73, CD90 and CD105, while being negative for CD45, CD34, CD11b, CD19, and HLA-DR. No significant differences in the surface markers between the three expansion regimes was observed.

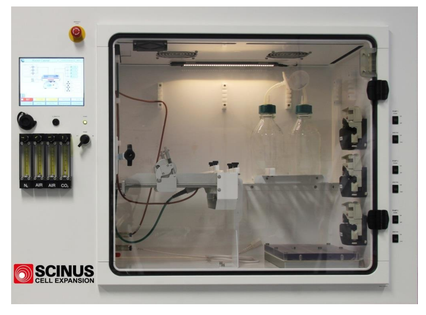


ASC harvested from Enhanced Attachment MCs in spinner flasks displayed correct surface marker expression. (A) CD90, (B) CD105, (C) CD73, (D) Negative cocktail , (E) CD34, and (F) HLA-DR. No considerable differences between the three conditions were observed

CONCLUSION AND DISCUSSION

ASC cultures were expanded using three expansion regimes. Based on the cell count and visual inspection, we conclude that 33% microcarrier expansion every other day is the most optimal for the culture of ASCs on Enhanced Attachment microcarriers. We showed that ASCs could be harvested efficiently with retention of ASC characteristics.

This culture process can be combined with closed bioreactor technology such as the Scinus bioreactor (right), an advanced closed bioreactor, for the production of GMP-compliant cells for cell therapy.



The Scinus Cell Expansion system, a closed bioreactor for cell therapy production

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