Culture of Adipose-derived Stem Cells on Microcarriers using the Scinus Cell Expansion bioreactor

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INTRODUCTION

Adipose-derived stem cells (ASCs) can be isolated from fat tissue obtained after abdominoplasty. Advantages of fat tissue over bone marrow as a source for stem cells include the easier accessibility, and availability of larger volumes.
For the production of stem cells for cell therapy in patients, an upgrade to clinical large scale culture (> 200x106 cells) is necessary. Clinical scale cultures require a reproducible and efficient process. For this, a microcarriers based culture is a very suitable method. Within the Scinus Cell Expansion system (see Figure 1) adherent cells can be cultured on microcarriers in a closed environment under GMP conditions. A process for culturing large quantities of ASCs using microcarriers (MCs) using the Scinus Cell Expansion system was developed.

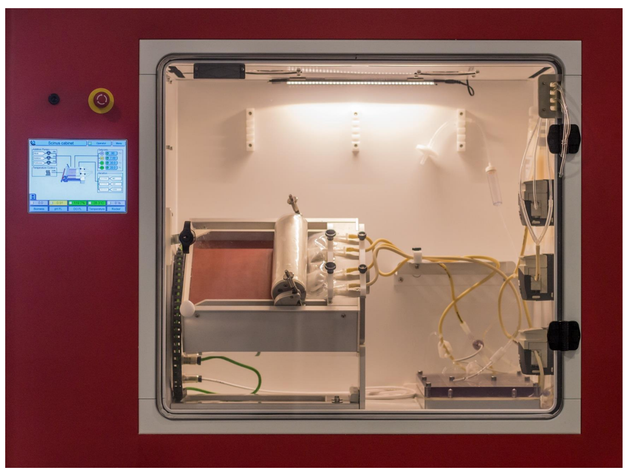


Figure 1 The Scinus Cell Expansion system, a closed bioreactor for cell therapy production

MATERIALS AND METHODS

Isolation of ASCs

Human ASCs were isolated from abdominal fat by enzymatic digestion using collagenase (see Figure 2). The stromal fraction was seeded onto T-flasks, and the adherent cells were used during the experiments.

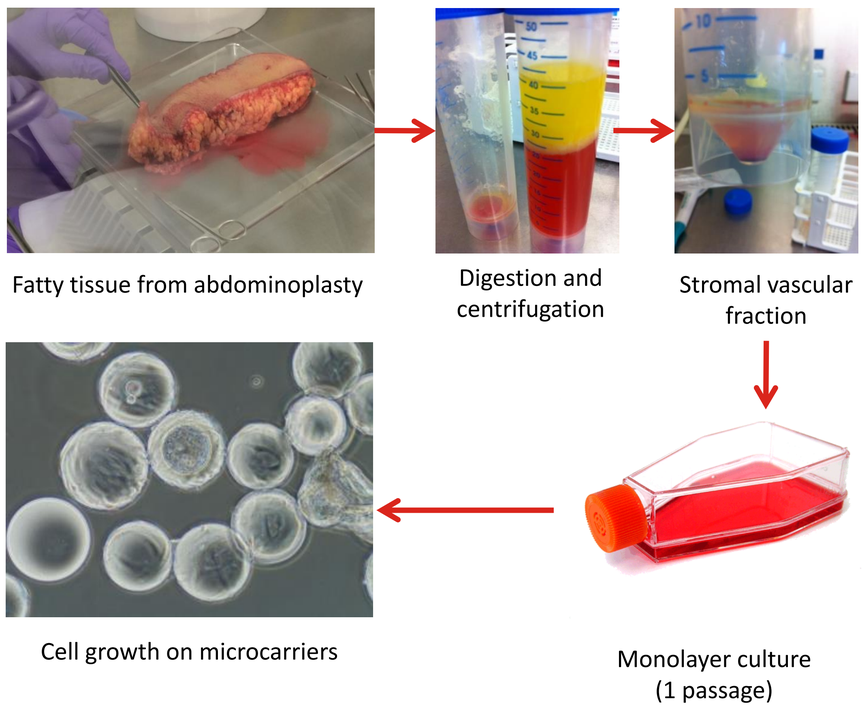


Figure 2 Overview ASC isolation from abdominal fat tissue by enzymatic digestion and subsequent culture

Culture in Scinus Cell Expansion system

Twenty million ASCs (P2) were seeded onto dissolvable MCs in the Scinus Cell Expansion system.
During cell culture the pH and DO settings were 7.3 and 17 % pO_2 respectively. During seeding the Scinus Cell Expansion system rocks 5 minutes every hour. After seeding, the Scinus Cell Expansion system was set on continuous rocking with a 1h pause every 8h. During the whole culture the number of ASCs was determined by cell count and vitality was monitored using visual inspection.

RESULTS

Dissolvable microcarriers

For these experiments a newly developed microcarrier by Corning was used. This microcarrier consists of calcium/crosslinked polygalaturonic acid (PGA). To harvest the ASCs from the microcarriers, TrypLE is used to round up the cells. Next, the microcarriers are dissolved using a solution containing EDTA and pectinase. Our results showed that an incubation time of 15 minutes with the harvest solution resulted in complete digestion of the microcarriers, after which a single cell suspension was obtained.

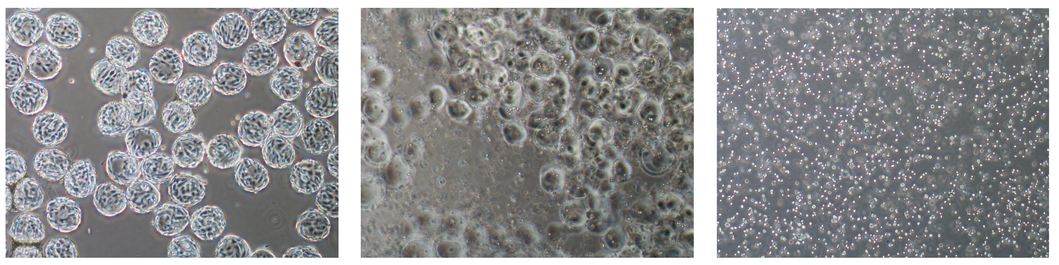


Figure 3 Confluent microcarriers (A) were incubated with TrypLE of ensure accessibility of the harvest solution to the microcarrier. The microcarriers were dissolved using a solution containing EDTA and pectinase (B), resulting in a single cell suspension (C)

ASC culture on microcarriers

Visual inspection showed good cell attachment to the dissolvable microcarrier (see Figure 4). After 7 days of culture, cells were distributed evenly among the microcarriers. At the end of the culture period, almost all microcarriers were completely covered with ASCs, and small microcarrier aggregates had formed. At the end of the culture the total number of ASCs cultured in the Scinus bioreactor was approximately 500 million cells (see Figure 5).

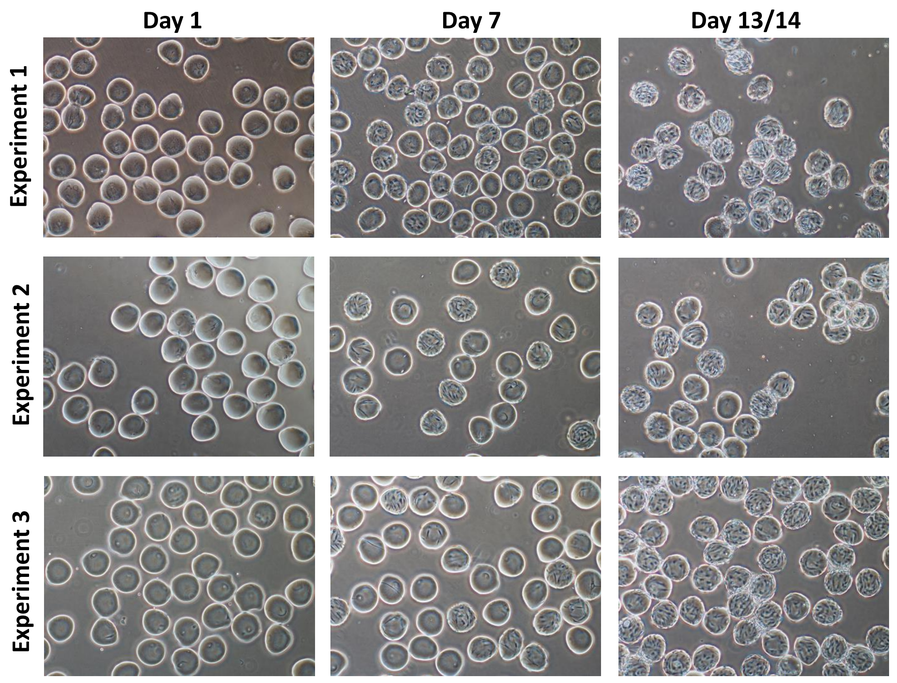
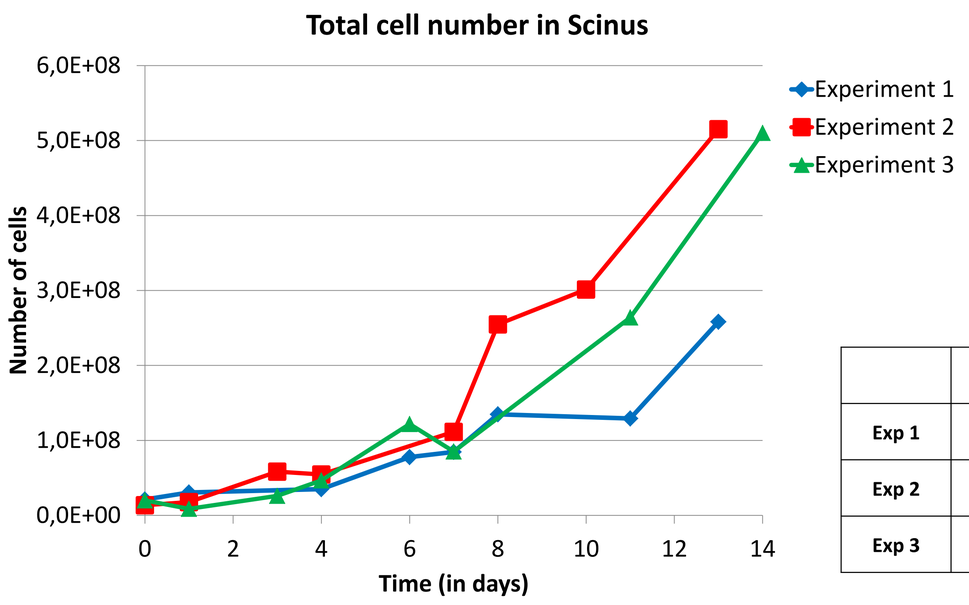


Figure 4. Overview of visual inspection during ASC culture in the Scinus Cell Expansion system



	PDL	PDT (h)
Exp 1	3,6	74,9
Exp 2	5,2	66,7
Exp 3	4,7	69,6

Figure 5 The number of cells inside the Scinus Cell Expansion system was determined throughout the culture. Cell proliferation was similar in the last 2 experiments, both reaching approximately 500 million cells at the end of the culture. PDL and PDT (in log-phase) were calculated for all experiments

CONCLUSION AND DISCUSSION

Our results showed that high ASC concentrations can be quickly reached, and easily and efficiently harvested using the Scinus Cell Expansion bioreactor.

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