Single-step expansion of adipose-derived stem cells with platelet lysate in SCINUS Cell expansion system

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INTRODUCTION

Adipose-derived stem cells (ASCs) can be isolated from fat tissue obtained after e.g. abdominoplasty. Advantages of fat tissue over bone marrow as a source for stem cells include easier accessibility and availability of larger volumes. Cost-effective production of cellular therapies requires efficient culture platforms that address major cost drivers: labor costs, clean room requirements and consumable expenditure. At the same time, process automation can increase quality and reliability of the cell product. Here we present the culture of over 500 million ASCs starting directly from a stromal vascular fraction in medium supplemented with human platelet lysate (hPL) using our SCINUS Cell Expansion system (figure 1). Human PL was used as an alternative to FBS as it contains no animal derived products and it is a rich source for varying growth factors.

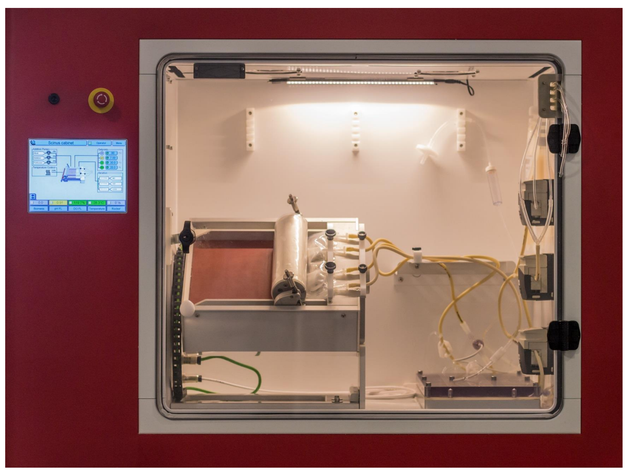


Figure 1 The SCINUS Cell Expansion system, a closed bioreactor for cell therapy production

MATERIALS AND METHODS

Isolation of ASCs

The stromal vascular fraction (SVF), containing the ASC population, was obtained after enzymatic digestion of 125 grams of abdominal fat (Figure 2). The SVF was used to inoculate the SCINUS.

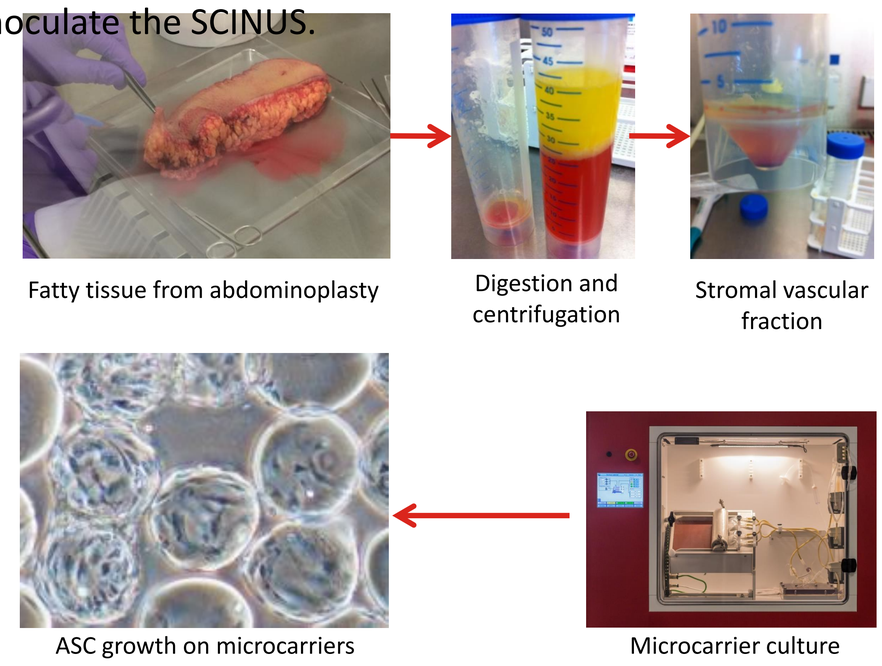


Figure 2 Overview ASC isolation from abdominal fat tissue by enzymatic digestion and subsequent culture

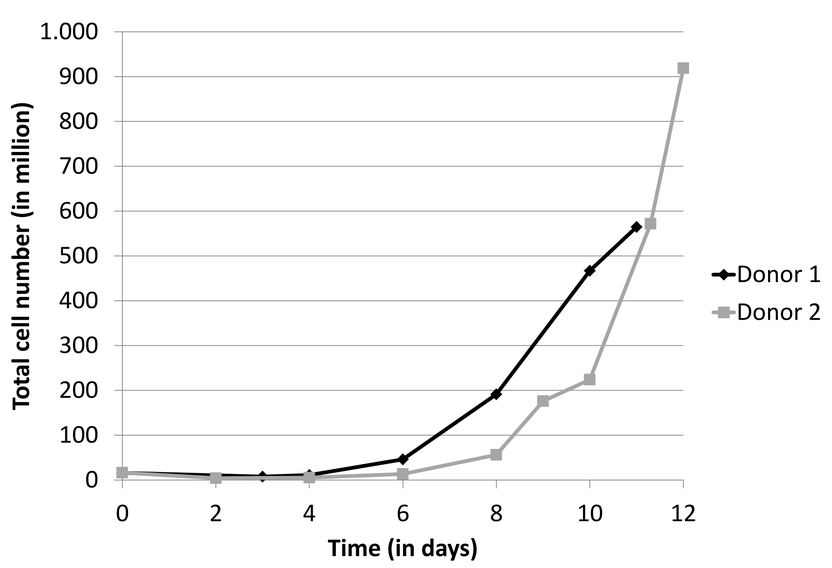
Culture in SCINUS

The SVF was seeded directly into the SCINUS which contained 5 g/L denatured collagen dissolvable microcarriers (DC microcarrier by Corning) in a max volume of 130 mL culture medium supplemented with 5% hPL (provided by prof. dr. K. Schallmoser). During culture in the SCINUS the pH and pO_2 were kept at 7.4 and 17% respectively. An agitation routine maintained a homogenous environment. During 11 days of culture the volume of the culture bag was increased twice, to 430 mL and 1350 mL respectively, by the unique expanding feature of the SCINUS while keeping the DMC-LC concentration at 5 g/L. The culture medium was refreshed based on glucose and lactate measurements. Every 1-3 days samples were taken to estimate cell count, cell morphology and cell distribution. Population doubling time (PDT) was calculated for both donors. At the end of the culture the harvested cell population was characterized using flow cytometry.

RESULTS

Succesful ASC culture in SCINUS

In two separate experiments the SVF from two donors was used to inoculate the SCINUS. The volume of the culture bag was expanded on days 2-3 and on day 6 to its maximal volume when a cell concentration of >30.000 cells/mL was reached. Donor 1 reached a final cell number of 565 million cells after 11 days of culture (figure 3). Donor 2 reached a final total of 919 million cells after 12 days of culture. PDT in the SCINUS was found to be similar for both donors. Interestingly, the PDT of both monolayers was considerably higher compared to the SCINUS. During the whole culture only 2.5 L culture medium was used.



	PDT (h)
Donor 1 Scinus	31.0
Donor 1 monolayer	35.1
Donor 2 Scinus	31.7
Donor 2 monolayer	34.9

Figure 3 The total number of cells in the SCINUS was determined throughout the culture. Cell proliferation was similar in both experiments, both reaching a minimum of 500 million cells at the end of the culture. PDT (in log-phase for microcarrier culture and P1 for monolayer culture) was calculated for both experiments

Excellent distribution and proliferation on DC microcarriers

Visual inspection showed good cell attachment and homogeneous distribution among the dissolvable microcarrier (figure 4). Bead-to-bead transfer was observed in both experiments. After 9 days of culture, only a few empty microcarriers could be observed. At the end of the culture period, almost all microcarriers were completely covered with ASCs, and only small microcarrier aggregates had formed.

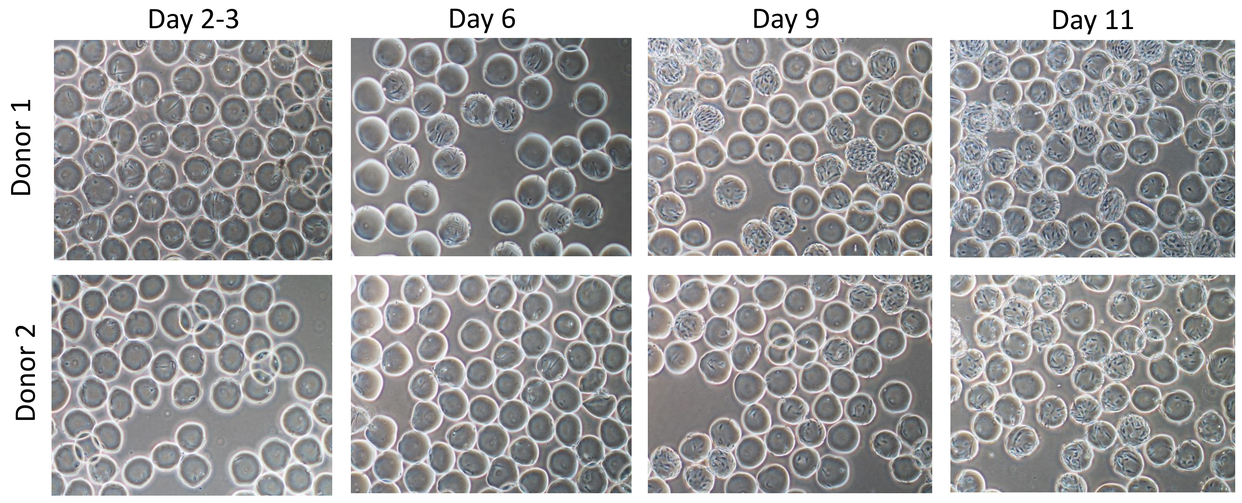


Figure 4 Overview of visual inspection during ASC culture in the Scinus

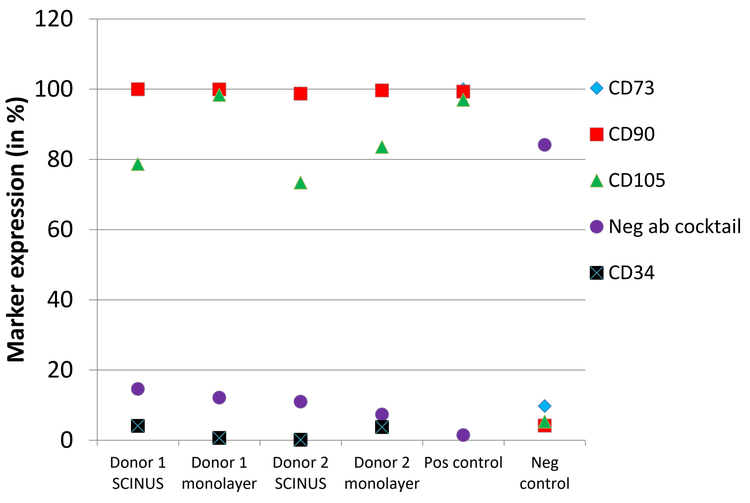


Figure 5 Overview of flow cytometry analysis of ASCs cultured in the Scinus and monolayer.

Cell characterization using flow cytometry

Flow cytometry was used to characterize the cells cultured in the SCINUS (Figure 5). The results showed deviating CD105 expression on the harvested cells. Previous experiments have shown that our harvesting procedure affects the CD105 receptor, thereby lowering the CD105 expression.

CONCLUSION AND DISCUSSION

ASCs were successfully cultured directly from the SVF and a total cell number well over 500 million cells could easily be obtained using the SCINUS Cell Expansion system using culture medium supplemented with human platelet lysate. Cells cultured in the SCINUS had a considerably lower population doubling time compared to the monolayer.

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