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# Automated isolation and expansion of human adipose tissue-derived stem cells for a seamless translation into clinical trials

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## Abstract

***Objective:*** **Adipose tissue-derived stem cells (ASCs) are currently being investigated for a number of uses in regenerative medicine. Certain clinical approaches would require controlled and reproducible methods for producing a high number of cells. Here we describe such a workflow.**

***Materials and Methods:*** **Lipoaspirate from 3 different human donors was processed in the operating theatre to stromal vascular fraction (SVF) using an automated device before isolation and expansion of ASCs was done using flasks or an automated hollow fiber bioreactor. Cell growth and characteristics were measured, and expanded ASCs were injected into diabetic mice.**

***Results:*** **The cells of the SVF went through 3.53±0.90 doublings over 10.7±1.1 days in the bioreactor, similar to results from parallel cultures of SVF in flasks (n=3 $p$=0.34). The resulting cellular population was identified as ASCs by plastic adherence, tri-lineage differentiation capability, and surface marker expression. ASCs were then expanded in a second bioreactor, yielding $4.94x10^8$±$1.61x10^8$ ASCs after 6 days of incubation. Cell doubling count in the bioreactor was similar to ASCs expanded in flasks (n=3 $p$=0.25). Levels of soluble factors released by ASCs were not significantly different between cells grown in a bioreactor or flasks. Levels of these factors were also similar through passaging of the cells. Injection of bioreactor expanded ASCs into diabetic mice resulted in a significantly lower fasting glucose and improved glucose tolerance test ($p$<0.05).**

***Conclusions:*** **Our workflow allows the automated isolation and expansion of large number of ASCs which can be used for *in vitro* or *in vivo* experiments. Our workflow could also be used for future clinical trials.**

## Introduction

Adipose tissue-derived stem cells (ASCs) are cells found in adipose tissue, and have been shown to have properties of self-renewal and differentiation to several different cell types[1,2]. Similarly to mesenchymal stem cells (MSCs) from other sources, ASCs are defined by their plastic adherence, positive and negative expression of surface markers, and differentiation ability[3,4]. However, compared to MSCs from bone marrow, ASCs are found in greater quantities for equal amount of tissue, and ASCs can be harvested using safe and well-established procedures of liposuction[5]. The lipoaspirate

obtained by liposuction can be processed to a cell rich fraction called stromal vascular fraction (SVF), from which plastic adherent ASCs can be isolated by washing out non-adherent cells[3].

ASCs have been demonstrated to have wound healing, anti-inflammatory, angiogenic, and immunomodulatory properties, and are therefore being investigated in a multitude of settings such as liver cirrhosis, fistulas, cardiovascular disease, limb ischemia, graft *vs.* host disease, and multiple sclerosis[6,7]. MSCs from various sources are also being investigated for the use in diabetes[8]. We have previously shown how human ASCs improve human islet viability and function and protect mice against chemically induced diabetes[9], and a recent clinical study has shown that bone marrow derived MSCs can to potentially delay disease progress in type 1 diabetes (T1D)[10]. Although therapeutic doses used in clinical studies vary, the number of cells is usually high, with tens of millions of cells used for local injections[11], and 1-5 million cells/kg for intravenous administration[10,12]. To transform the promises of cell-based therapies into reality, controlled and reproducible manufacturing methods must be developed. Manufacturing of ASCs for clinical use requires the production of large batches with consistent product quality at acceptable cost, while adhering to the principles of good manufacturing principles (GMP) and meeting local regulatory requirements. SVF can be isolated from lipoaspirate using an automated system in the operating theatre[13], and an automated cell culture bioreactor with large surface area provided by synthetic hollow fibers has been demonstrated to expand embryonic stem cells[14], bone marrow MSCs[15,16], unprocessed bone marrow[17], and periosteum derived stem cells [18]. Expansion in this bioreactor has been shown to be faster and less labor intensive than flask-based cell culture[19]. Genetic stability has been shown to be maintained with no malignant transformation of cells after transplantation into mice, and no micronucleus formation or chromosomal aberrations[16].

Here we demonstrate a workflow for produce clinical grade ASCs in large quantities. We found that SVF obtained by an automated system can be seeded directly into a hollow fiber bioreactor for automated isolation and expansion of ASCs. We describe growth kinetics and yield of such a workflow and compare this to cells grown in flasks. We describe the soluble factors released form our cells both before and after cryopreservation, and finally we demonstrate biological activity of our ASCs in a mouse diabetes model.

## Materials and Methods

### *Experimental design*

Lipoaspirate was obtained in the operating theater under sterile conditions, and SVF was produced using an automated device. A closed syringe with the SVF (1-5 mL) was then transferred to our cleanroom facility and seeded into both a bioreactor and tissue culture flasks. Expansion of SVF in the bioreactor or flasks yielded P0 ASCs. These cells were then expanded to P1 in a second bioreactor or new flasks. The P1 cells produced in the second expansion were aliquoted into various fractions and stored in liquid nitrogen for use in experiments (Fig. 1).

### *Tissue harvest and Isolation of SVF*

120-150 ml lipoaspirate was obtained from the flank or thigh of 2 females and 1 male healthy donors aged 61.3 ±8.0 years (range 53-69) undergoing elective plastic surgical operations at the Department for Plastic Surgery, Oslo University Hospital Radiumhospitalet. All donors signed informed consent and the use of ASCs was approved by the Regional Committee for Medical and Health Research Ethics (2014/838). The stromal vascular fraction (SVF) was isolated using the automated Celution® system (Celution 800/CRS, Cytori Therapeutics Inc., USA) according to the manufactures instructions. Briefly the adipose tissue was washed with Ringer's solution (Braun, GER), enzymatically digested by a proprietary enzyme solution (Celase®) during constant agitation, then washed again with Ringer solution and concentrated to a volume of 5 ml by centrifugation[13]. The syringe with SVF (1-5 mL) was transported (by courier) directly to the GMP cleanroom within Department of Cellular Therapy at Oslo University Hospital. Upon arrival at our facility, and within 60 minutes after isolation, the SVF was washed once with human serum albumin 5% (HSA) (Albunorm, Octapharma, UK) followed by centrifugation at 400 g for 5 minutes. Mononuclear cells were counted using a hemocytometer (Kova, USA) and viability was estimated by dye exclusion test using

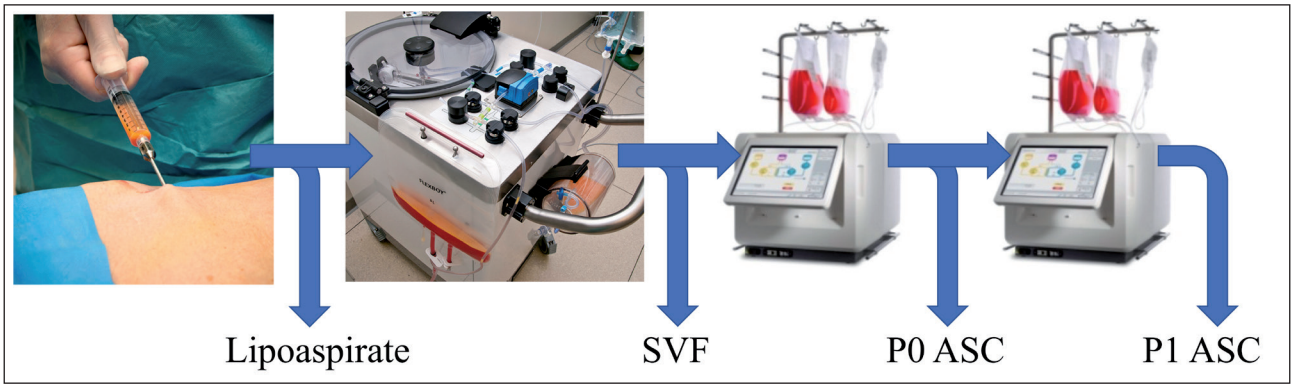


**Figure 1.** Workflow for isolating and expanding ASCs. Lipoaspirate is processed to SVF using the Celution® system. SVF is then loaded into the first Quantum® Cell Expansion bioreactor, yielding P0 ASCs after the first run, and P1 ASCs after the second run.

Trypan Blue Solution 0.4% (Gibco, Thermo Fisher Scientific, USA). ASCs count and viability were determined using Nucleocounter NC-200 (Chemo-Metec, Denmark).

### *Incubation and expansion of cells in the Quantum System*

Two equal Quantum ® Cell Expansion Systems (Terumo BCT, Japan), for the purposes of this paper referred to as bioreactors, were used for experiments. One day before seeding the cells, single use cell expansion sets were loaded into the Quantum system according to the manufactures instructions. All bags and lines were connected to the system using sterile welding connector device (Terumo, Japan). Lines and hollow fibers were first primed with phosphate-buffered saline (PBS) (Hyclone, GE Heathcare, USA), and the lumens of the hollow fibers were coated with 5 mg fibronectin (Sigma Aldrich AS, USA) for 24 h. After this, PBS and fibronectin were washed out of the system and replaced by complete media; MEM alpha medium (Gibco, Thermo Fisher Scientific, USA) containing 5% human plate lysate (HPL) (Cook Medical, USA) and 5 ug/ml Gentamicin (Braun, Denmark). SVF or ASCs were then loaded into the bioreactor or T175 flasks (Nunc, Thermo Scientific, USA) in parallel in equal density. After loading the bioreactor with SVF, cells were given 48 h to adhere before automated continuous medium change was initiated and non-adherent cells were washed out, while after loading ASCs, continuous media change was initiated after 24 h. The bioreactor replaced its media at a rate of 0.1-0.8 ml/min depending on lactate levels following recommendations from the manufacturer, while medium in the flasks was replaced the first or second day after seeding, and then every third or fourth day. Levels of lactate were measured daily using a Lactate Plus Meter (Nova Biomedical, UK). Confluence in the bioreactor was estimated by lactate production, calculated from an estimated base lactate production of $1.54x10^{-8}$ mmol/day/cell. Flasks were incubated in 37°C, 5% $CO_2$, and the bioreactor system were set to maintain similar conditions by using a gas mixture of 5% $CO_2$, 21% $O_2$ in $N_2$ (AGA AS, Oslo, Norway) at constant supply. At the time of harvest both bioreactor and flasks were first washed using PBS before TrypLE Express (Invitrogen, USA) was added to flasks (10 ml) or the bioreactor (100 ml) and incubated at 37°C, 5% $CO_2$ for 15 minutes. Complete medium was added, and cells were harvested from the bioreactor into a harvesting bag or from the flasks into 50 ml collecting tubes (Corning, USA). Cells were counted and viability was assessed as described above and either re-seeded into the second bioreactor, cryopreserved, or used in experiments.

### *Cryopreservation, thawing, and growth estimation in flasks*

To investigate ASC characteristics after crypreservation, P1 ASCs were suspended in medium containing 10% dimethylsulfoxide (DMSO) and 20% HSA before initially cooled to -80°C at a rate close to -1°C/min using a freezing container (Mr. Frosty, Thermo Fisher Scientific, USA) and then subsequently transferred to a -196°C liquid nitrogen vapour tank for storage. Cells were thawed in room temperature, and DMSO was rapidly diluted

in supplemented ASC medium before centrifugation and use in experiments. For ASC growth estimates P1 cells were directly seeded into T175 flasks (Nunc, Thermo Scientific, USA) at a density of 667 ASCs/cm$^2$ in complete medium and were incubated in standard incubation conditions (37°C, 5% $CO_2$) with one medium change before harvest on day 5. Cells were washed with PBS (Hyclone, GE Heathcare, USA) before TrypLE (Invitrogen, USA) was added and cells were incubated 10 min at 37°C. TrylpLE was neutralized by supplemented medium, and cells were counted as desvcribed. P2 cells were then immediately seeded into new T175 flask at 667 ASCs/cm$^2$ for a second 5-day expansion and cell counting.

### *Analysis of surface markers, differentiation potential, and Colony-forming Unit-fibroblast assay (CFU-F)*

Analysis of surface antigen expression was done by flow cytometry using a BD FACS Canto II (Becton Dickinson, USA) and BD Stemflow Human MSC Analysis Kit (BD biosciences, USA) according to manufactures instructions. Briefly, P0 or P1 ASCs were thawed and 5 x $10^5$ cells/100 µl were incubated with the conjugated monoclonal or isotype matched IgG control antibodies, then analyzed by FACS to measure the levels of positive (CD105 PerCP- Cy™5.5/CD73 APC/CD90 FITC) and negative (CD45/CD34/CD11b/CD19/HLA-DR PE) markers of ASCs[4].

Differentiation capacity was evaluated using StemPro, chondrogenic, osteogenic, and adipogenic differentiation kits (Gibco, Thermo Fisher Scientific, USA) following the manufacturer's instruction. Briefly, P0 or P1 ASCs were thawed and allowed to expand one passage before 1 x $10^4$ cells/cm$^2$ cells were seeded into 12-well tissue culture plates (Costar, Corning, USA) and incubated for 14 days in species specific medium changed every 3-4 days. At the end of the differentiation period, all cultures were fixed with 4% formaldehyde before morphological identification was done by staining with 1% Alcian-Blue solution (Sigma Aldrich AS, USA) to identify chondrocytes, 2% Alizarin Red S solution (Sigma Aldrich AS, USA) to identify osteoblasts, and 0.3% Oil Red O solution (Sigma Aldrich AS, USA) to identify adipose tissue with. CFU-F was performed as described by Gnecchi [20]. Briefly; P0 ASCs were seeded in 10 ml complete Mesencult media (STEMCELL, CAN) in 100mm dishes at 3 different densities; 2000, 1000, or 500 ASC/10 ml. After 11 days, medium was removed, and cells were fixated and stained by adding crystal violet 0.5% solution containing 20% methanol. Separate colonies were counted and compared to the number of cells seeded.

### *Calculations used to estimate cell doubling and doubling time*

Cell doublings and doubling time were calculated using the following formulas:

$$\text{doubling number} = \frac{\ln(\text{cells at harvest}) - \ln(\text{cells seeded})}{\ln(2)}$$

$$\text{doubling time} = \frac{\ln(2)}{\ln(\text{cells at (harvest)/cells seeded)/ time incubated}}$$

These formulas are applicable given that only initial and final cell numbers are known. Therefore, any lag phase or stationary growth will not be accounted for.

### *Bacterial contamination testing*

Bacteriological testing was performed by sampling supernatants from the Quantum system thorough at sterile sampling port at the time of harvest. Anaerobic culture was done in Bactec Plus Anaerobic/F Medium flasks (BD, USA) while aerobic culture was performed in Bactec Peds Plus/F Medium flasks (BD, USA), both supplemented with Bactec FOS-Culture Supplement (BD, USA). Incubation and bacterial detection was performed over 5 days in a Bactec FX system (BD, USA).

*Measurement of soluble factors released by ASCs*

Cell-free conditioned media (CM) was collected from P1 ASCs incubated in flasks or the bioreactor and cryopreserved at -80°C before thawing and analysis by multiplex for 45 different factors using a Human Cytokine/Chemokine/Growth Factor Panel 1 (Product 18 No. EPX45012171-901) (ProcartaPlex; eBioscience, USA) according to the manufacturer´s instructions. Levels of analytes were determined by a Bio-Plex 26 200 dual laser flow-based analysis system using software version 6 (Bio-Rad, USA), and to determine levels of ASC released factors, the results from non-conditioned complete medium was subtracted.

### *In vivo*

The research protocol was approved by the Norwegian National Animal Research Authority (Project License No. FOTS id 7793). The animal experiments were performed in accordance with the European Directive 2010/63/EU and the Guide for the Care and Use of Laboratory Animals, 8th edition (NRC 2011, National Academic Press). The animals were housed no more than five mice per cage, maintained in a 12-h light/dark cycle, and given free access to food and water. The mice were handled by an experienced animal technician at all times, and all efforts were made to minimize suffering. The same technician monitored animal welfare in accordance to standardized requirements for the animal unit at Oslo University Hospital, administered the treatment, and performed the blood sampling. Diabetes was induced in 7 to 12-week-old male Balb/c recombination activating gene 1-deficient (Rag 1-/-) immunodeficient mice (C.129S7(B6)-Rag1tm1Mom/J; stock 003145; Jackson Laboratory, Sacramento, CA, USA) by intravenous streptozotocin (STZ) (Sigma-Aldrich) injections (50 mg/kg body weight) repeated daily for 4 days to induce insulitis and mimic onset of T1D. Mice were randomized into two groups, receiving either 0.2 ml of PBS (PBS, Lonza, Switzerland)(n=7) or $2x10^6$ bioreactor expanded P1 ASCs in 0.2 ml PBS (n=12, 4 form each of the ASC donors). ASCs or PBS were injected one day prior to STZ, the third day of STZ injections, and 6 days after the last STZ injection. Fed blood glucose level was measured twice first week, then once weekly at 9:00 a.m. using a glucometer (AccuCheck, Roche, Germany). Fasting blood glucose level was measured after 4 h of fasting starting at 9:00 a.m. An oral glucose tolerance test (OGTT) on day 45 after the last STZ injection. OGTT was performed by administering oral gavage of 1.5 g/kg D-glucose (Fresenius Kabi, Oslo, Norway) to fasting animals. Blood glucose was measured using a glucometer (AccuCheck, Roche, Germany) prior to glucose administration, and 15, 30, 60, and 120 min after. At the end of the experiment, the mice were anesthetized with an intraperitoneal injection of 0.1 ml/10 g body weight tiletamine and zolazepam 3.32 mg/ml (Zoletil Forte; Virbac, Carros, France), 0.45 mg/ml xylazine (Rompun; Bayer), and 2.65 µg/ml fentanyl (Actavis, Oslo, Norway) before being sacrificed by dislocation of the neck.

### *Statistical Analysis*

Differences between groups were examined for significance with a two-tailed Student's test, and Wilcoxon signed rank test was used in paired analysis. Calculations and graphs were made by the GraphPad Prism statistics software (GraphPad Prism 6.07; GraphPad Software Inc., San Diego, CA, USA). A value of $p<0.05$ was taken to indicate the presence of a significant difference.

## Results

### *Isolating ASCs from SVF in a bioreactor*

The number of mononuclear cells (MNC) in the SVF varied between the donors from 1 $x10^6$ to 7 $x10^6$ MNC/ml, and the bioreactor was seeded with a total number of 0.348 - $1.26x10^7$ MNCs. After 10.7±1.1 days of incubation, 0.202-2.46 $x10^8$ P0 ASCs were harvested from the bioreactor (Table 1). Comparing the cell growth in the bioreactor to the growth in flasks, we counted that seeding 600, 500, or 166 cells/$cm^2$ in flasks or bioreactor yielded 11 714, 6 143, or 962 ASCs/$cm^2$ in the bioreactor, or 17 714, 25 143, or 823 ASCs/$cm^2$ in flasks (Fig. 2A). Analyzing the cell doublings, we found an average of 3.53±0.90 doublings in cell numbers occurred in the cells incubated in the bioreactor, compared to 4.30±1.76 doublings for the cells incubated in flasks (Fig. 2B). There was no statistical significance between these two groups ($p$=0.34). The fraction of viable cells was determined to be >95% for all samples. Adherent cells from the bioreactor were found to fulfill the criteria for ASCs with expression of CD73 (99.9%±0.1), CD90 (99.9%±0.1), and CD105 (94.8%±8.4) while expression of CD34, CD11b, CD19, CD45, HLA-DR was low (2.3%±3.6). Differentiation towards chondrocyte, adipose, and osteoblast lineages was determined by histology (Fig. 2C). Microbiological tests performed on the culture supernatants were all negative.

### *Expanding P0 ASC in a bioreactor*

P0 ASC was seeded at a density of 695±49 ASC/$cm^2$ in the second bioreactor or 696±47 ASC/$cm^2$ in the flasks and cultured for 6.2±0.7 days. After culture, the cell density was estimated to 23 508±7 662 ASC/$cm^2$ in the bioreactor, and 29 365±14 951 in the flasks (Fig. 3A). Total cell yield after culture in the bioreactor was $4.94x10^8$±$1.61x10^8$, while cell yield after culture in flasks was $4.45x10^6$±$3.38x10^6$

**Table 1.** Donor characteristics of SVF and total number of adherent cells harvested from the bioreactor and flasks.

| | | | | | | Bioreactor | | Flasks | |
|---|---|---|---|---|---|---|---|---|---|
| | Sex | Age | SVF (ml) | SVF (MNC/ml) | Incubation (days) | MNC seeded | P0 ASCs harvested | MNC seeded | P0 ASCs harvested |
| Donor 1 | F | 62 | 3 | $7x10^6$ | 10 | $1.26x10^7$ | $2.46x10^8$ | $1.02x10^5$ | $3.10x10^6$ |
| Donor 2 | F | 53 | 5 | $3x10^6$ | 12 | $9.50x10^6$ | $1.29x10^8$ | $8.75x10^4$ | $4.40x10^6$ |
| Donor 3 | M | 69 | 5 | $1x10^6$ | 10 | $3.48x10^6$ | $2.02x10^7$ | $2.90x10^4$ | $1.44x10^5$ |

SVF; Stromal vascular fraction, MNC; Mononuclear cells, P0; passage 0, ASCs; Adipose-derived stem cells.

(Fig. 3B). ASCs went through 5.0±0.48 doublings in the bioreactor group, and 5.2±0.73 doublings in the flasks (Fig. 3C), corresponding to a doubling time of 30.99±2.87 h for the ASCs in the bioreactor, and 28.4±1.48 h for the ASCs incubated in flasks (Fig. 3D). There was no statistical difference in cell doublings ($p$=0.25) or doubling time ($p$=0.36) between the two groups. The fraction of viable cells was determined to be >95% for all samples. Microbiological tests performed on the culture supernatants were all negative.

### *Surface markers, differentiation, growth kinetics, and CFU-F of P1 ASCs isolated and expanded in bioreactor before cryopreserved*

Cryopreserved P1 ASCs previously expanded in a bioreactor were thawed and subjected to flow analysis as described in methods. The cells retained expression of CD73 (100%±0), CD90 (100%±0), and CD105 (95.0%±7.9), while expression of CD34, CD11b, CD19, CD45, HLA-DR remained low (1.2%±1.3). Differentiation into chondrocyte, adipose, and osteoblast lineages was confirmed by positive staining of stimulated cells (Fig. 4A). Next, growth of cells seeded directly after thawing (P1) was compared to cells after one passage (P2). After 5 days of culture, P1 cells increased to 6 817±2 822 ASC/$cm^2$, while P2 cells increased to 36 244±9925 ASC/$cm^2$ (Fig. 4B). This corresponds to 3.26±0.65 doublings for P1 cells compared to 5.73±0.39 doublings for P2 cells (Fig. 4C). Calculating the doubling time for the ASCs, we find a calculated doubling time of 37.85±8.0 h for the recently thawed P1 cells, which would also include a lag phase after thawing, compared to a doubling time of 21.02±1.44 h for the P2 cells (Fig. 4D). Using the P2 doubling time, number of doublings, and incubation time, we estimate the lag-period for the cells to be 51.7±12.5 h. There was no statistically significant difference between groups ($p$=0.25). CFU-F was performed on cryopreserved P1 ASCs and results varied between the donors, with a CFU-F of 68.3±8.0 CFU/$10^3$ ASC from donor 1, 82.0±7.2 for donor 2, or 32.3±10.0 for donor 3 (Fig. 4E).

### *ASC released soluble factors in the CM*

Analyzing the levels of 45 soluble factors released by ASCs in the CM harvested from P0 ASCs incubated in flasks or the bioreactor, we found detectable levels of VEGFa, HGF, LIF, MCP1, IL8, CXCL1, IL6, SCF, SDF1, bNGF, and VEGFd, and levels of these factors were comparable from the two groups (Fig. 5A). We also compare the levels of soluble factors in the CM of previously cryopreserved ASCs expanded through P1 and P2 and find comparable levels of the factors VEGFa, HGF, LIF, MCP1, IL8, CXCL1, IL6, and SDF1 over these two passages (Fig. 5B).

### *Biological effect in vivo*

To measure biological activity, we inject mice with P1 ASCs expanded in the bioreactor (n=4 from each donor) or PBS (n=7) before and after multiple low dose STZ injections, and random blood glucose measurements are shown in Fig. 6A. Fasting blood glucose was measured om day 5, 9, 39, and 45 after the last STZ injection, and fasting glucose on day 45 was significantly lower in mice injected with ASCs (10.9±2.7 mmol/L) compared to mice injected with PBS (15.6±5.8 mmol/L) ($p$=0.038) (Fig. 6B). Following an OGTT on day 45, we showed that blood glucose was significantly lower 15 minutes after glucose injection in mice injected with ASCs (23.4±4.6 mmol/L) compared to mice injected with PBS (28.6±3.6mmol/L) ($p$=0.025) (Fig. 6C).

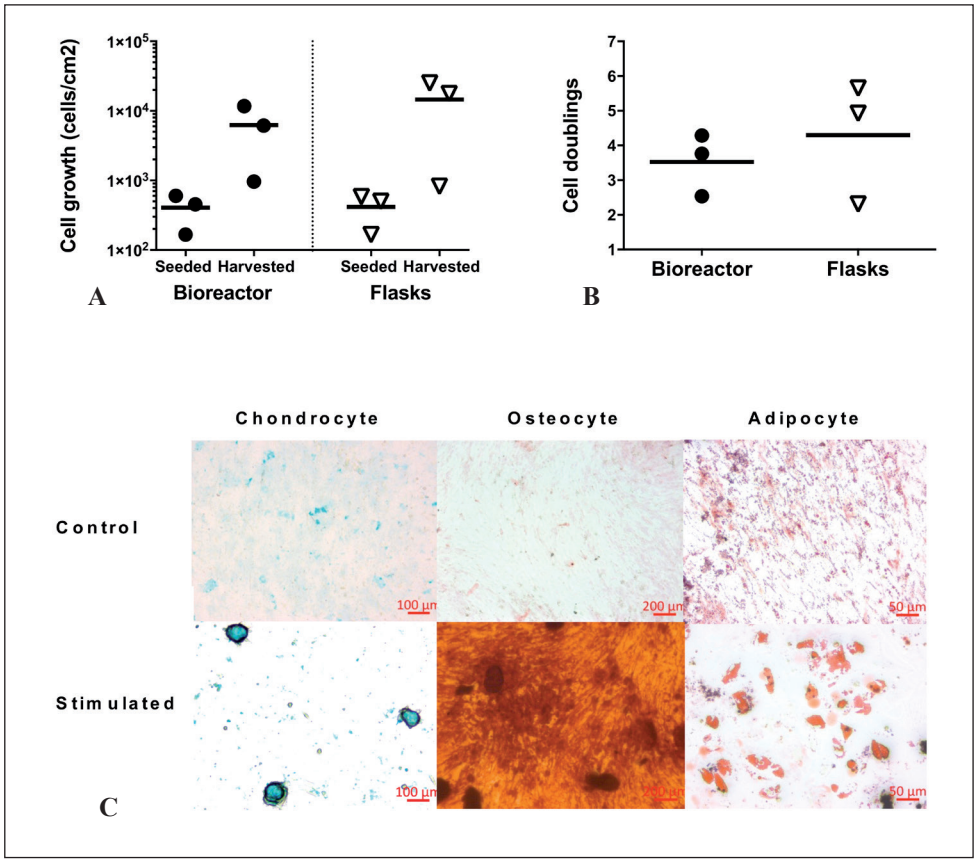


**Figure 2.** Growth characteristics of SVF MNCs from three different donors in a bioreactor of flasks and differentiation capability of harvested adherent cells. Cell growth described as cells/cm$^2$ ***(A)*** or cell doublings ***(B)*** for MNCs. There was no statistical significance in cell doublings between these two groups ($p$=0.34). Adherent cells from the bioreactor were stained with alcian blue *(left panel)*, alizarin red *(middle panel)*, and oil red O *(left panel)*, to identify chondrogenic, osteogenic, and adipogenic differentiaton ***(C)***.

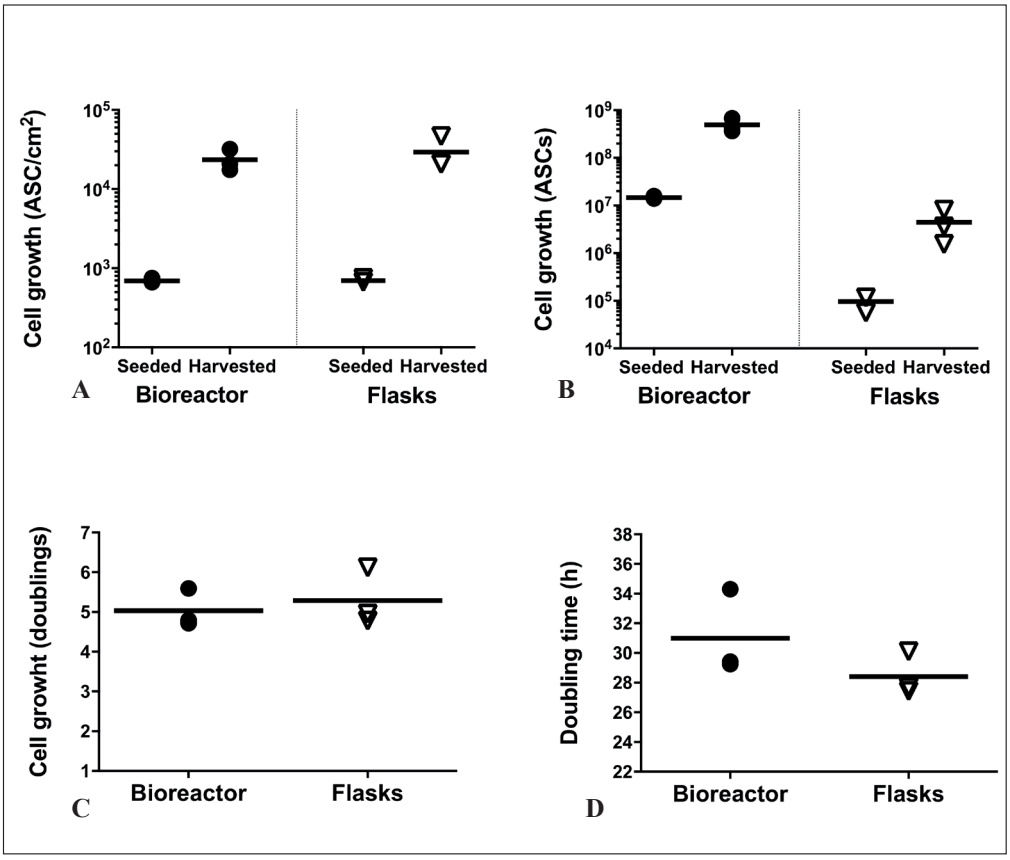


**Figure 3.** Growth characteristics of P0 ASCs from 3 different donors during expansion in a bioreactor or flasks. Cell growth expressed as ASCs/cm$^2$ ***(A)*** or as total number of cells ***(B)***. Calculated cell doublings ***(C)*** and doubling time of cells ***(D)***. There was no statistically significant difference in cell doublings ($p$=0.25) or doubling time ($p$=0.36) between the ASCs expanded in a bioreactor compared to flasks.

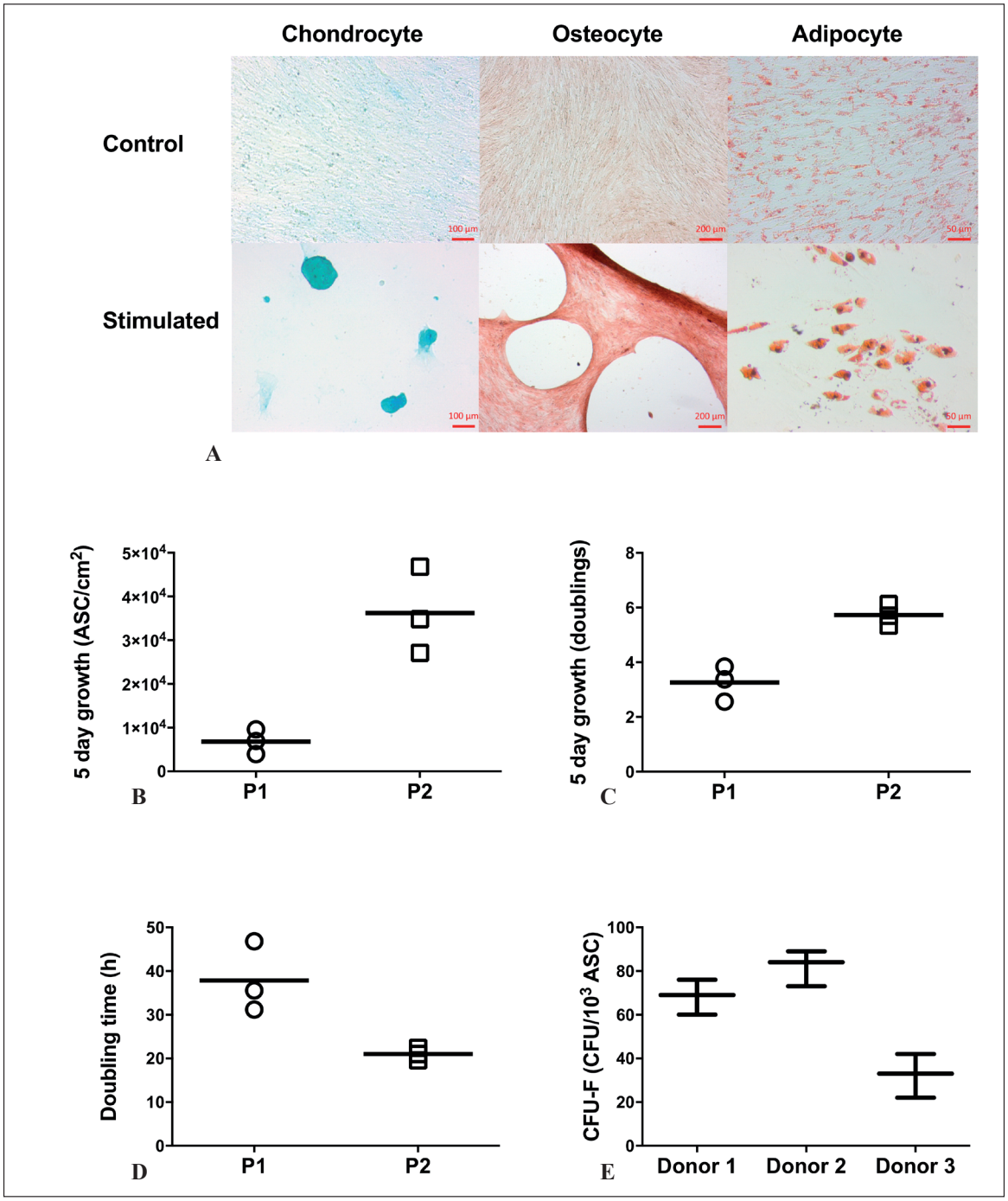


**Figure 4.** Differentiation capability, growth characteristics, and CFU-F results for P1 ASCs isolated and expanded in bioreactors before cryopreservation. Differentiation potential of P1 ASCs ***(A)***. ASC growth over 5 days for cells seeded directly after thawing (P1) or after passaging (P2) shown as ASC/cm$^2$ ***(B)***, number of cell doublings ***(C)***, or calculate doubling time ***(D)***, cells from 3 different donors, no statistically significant difference between groups ($p$=0.25). Colony forming units for cells from each donor after CFU-F assay (n= 3 for each donor, line at mean with whiskers min to max) ***(E)***.

## Discussion

Here we demonstrate a workflow to produce potential clinical grade ASCs in large quantities using automated systems both to obtain SVF and then isolate and expand ASCs. We find that SVF obtained by an automated device can be directly seeded into an automated bioreactor for isolation

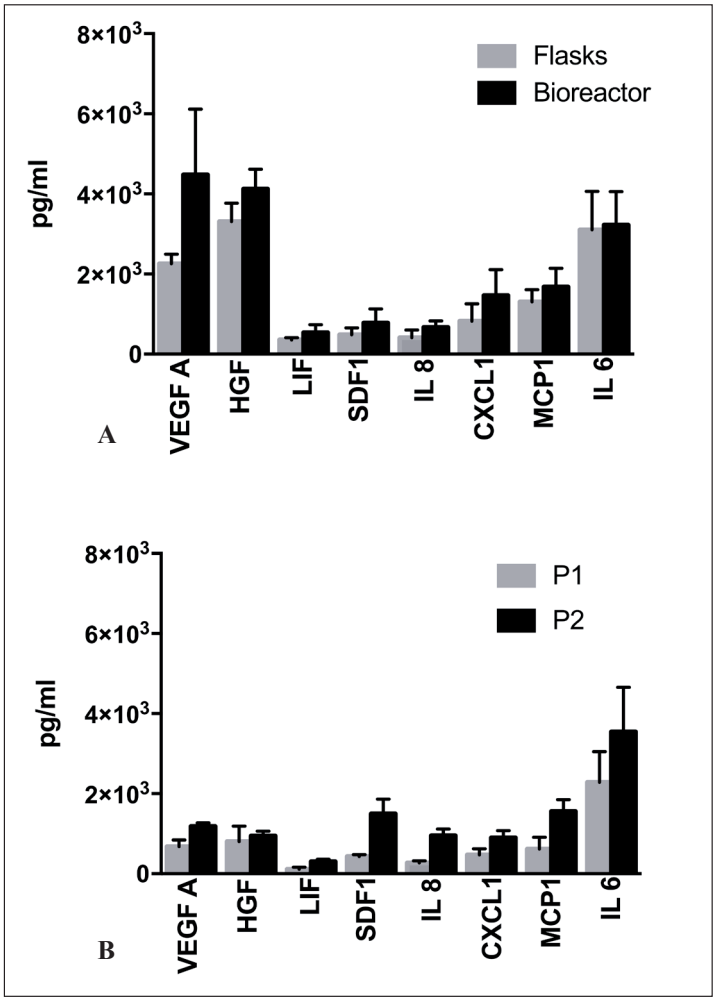


**Figure 5.** ASC released soluble factors in conditioned media (CM). Levels of factors released by P0 ASCs incubated in flask or bioreactor ***(A)***. Levels of factors released by previously cryopreserved P1 and P2 ASCs ***(B)***. Data is shown as mean ±SEM (n=3).

and expansion, yielding cells that meet the criteria for ASCs. We find similar growth kinetics and yield of ASCs in the bioreactor compared to ASCs isolated and expanded in flasks. We describe factor release of ASCs expanded in flasks or a bioreactor, and finally, we demonstrate biological activity of the ASCs in a mouse diabetes model.

This paper show the successful isolation and expansion of ASCs using the Quantum Cell Expansion System, similar to the results reported by Haack-Sorensen et al[21]. However, our setup includes the automatic isolation of SVF in the operating theatre. Our setup also includes two quantum devices in parallel, bypassing the need for a 4-hour coating protocol between passages. Furthermore, our cells were expanded in medium containing 5% HPL, not fetal bovine serum (FBS). We chose to use HPL in order to create an isolation an expansion protocol where the ASCs are not exposed to possible xenogenic antigens in FBS[22]. In contrast to the findings of Haack-Sorensen we did not find evidence of enhanced ASC expansion in the Quantum device, but rather similar rates of expansion compared to flasks. Our findings are similar to what has been reported on expansion of human periosteum derived MSCs in the Quantum device[18]. Reports on expansion of bone marrow derived MSCs vary, with some reporting higher growth[19,23], while others report slower[15,16]. Our results seem to support that ASC growth in a hollow fiber bioreactor is non-inferior compared to flask based culture, despite significantly reduced labor needed to expand cells in such a system[19].

Soluble factor release has been proposed as an ASCs mechanism of action in various disease models[24-26]. When measuring soluble factors released in the media of P0 cells, we find similar levels of factors in both bioreactor and flask groups. P1 ASCs were cryopreserved before further expansion and when we compared factor released between P1 and

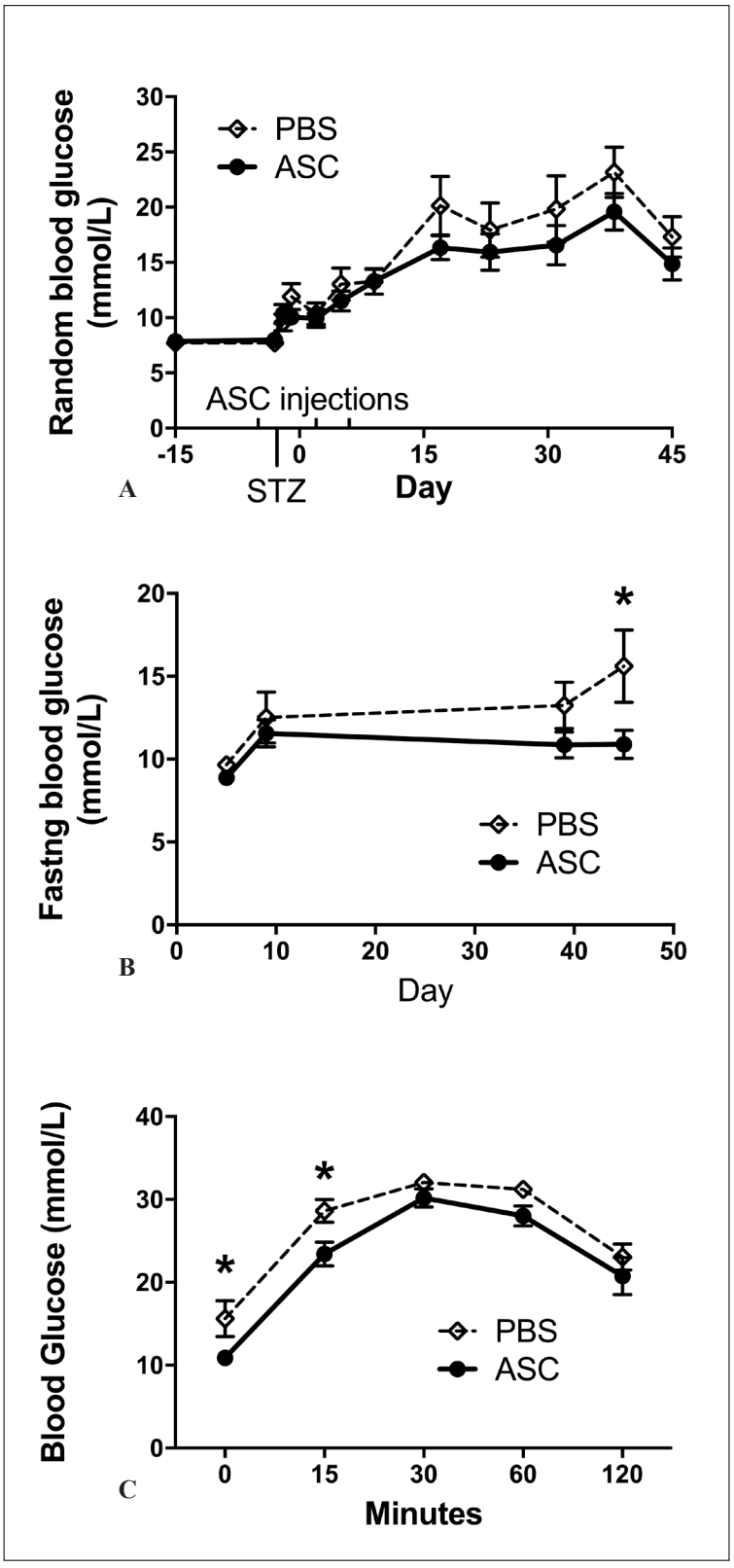


**Figure 6.** ASCs limit STZ damage to mouse pancreas. Mice were given injections of either $2x10^6$ ASCs (n= 4 for each ASC donor (n=3) or PBS (n=7) once prior and twice after administration of low dose STZ (50mg/kg) over 4 days. Random blood glucose was measured every 3-8 days ***(A)***. Fasting glucose was measured on day 5, 9, 39, and 45 after the last STZ injection ***(B)***. Oral glucose tolerance test was performed on day 45 ***(C)***. Data is presented as mean ±SEM. *$p$<0.05 vs. the PBS group.

P2 ASCs we found no difference in factor release between these two early passages. We did however measure lower levels of VEGF-A and HGF in the CM of P1 and P2 ASCs compared to P0 ASCs. This is important as both VEGF-A and HGF have been shown to influence islet function in diabetes models[27,28] and suggest that a low passage number and optimal cryopreservation method can be important ASC function. Donor age has also been shown to negatively influence VEGF and HGF release[29], and donors for this experiment were all older than 50 years. Despite this, we were able to show biological activity of our P1 ASCs in a mouse diabetes model, which to our knowledge is the first report of biological activity by bioreactor expanded ASCs on beta cell injury.

## Conclusions

Here we demonstrate a workflow for isolation and expansion of ASCs using automated systems. We show that this workflow does not alter properties of the ASCs or factors secreted compared to cells cultured in flasks, and we demonstrate biological activity of our expanded cells in a mouse diabetes model. The large number of cells produces allow cellular banking so that ASCs from the same expansion can be used in multiple experiments. This system also could be used in clinical trials where large number of cells expanded in closed and reproducible manners are needed. Finally, our results show a possible altered release of soluble factors after cryopreservation, highlighting the importance of further research into this field.

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