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Yield and proliferation rate of adipose-derived stromal cells as a function of age, body mass index and harvest site—increasing the yield by use of adherent and supernatant fractions?

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Abstract

Background aims. Adipose-derived stem cells are easily accessed and have a relatively high density compared with other mesenchymal stromal cells. Isolation protocols of adipose-derived stem cells (ASC) rely on the cell's ability to adhere to tissue culture plastic overnight. It was evaluated whether the floating ASC fractions are also of interest for cell-based therapies. In addition, the impact of age, body mass index (BMI) and harvest site was assessed. *Methods.* The surface protein profile with the use of flow cytometry, the cell yield and the doubling time of passages 4, 5 and 6 of ASC from 30 donors were determined. Adherent and supernatant fractions were compared. The impact of age, BMI and harvest site on cell yield and doubling times was determined. *Results.* Both adherent and supernatant fractions showed high mean fluorescence intensities for CD13, CD29, CD44, CD73, CD90 and CD105 and comparatively low mean fluorescence intensities for CD11b, CD62L, intracellular adhesion molecule-1 and CD34. Doubling times of adherent and supernatant fractions did not differ significantly. Whereas the old age group had a significantly lower cell yield compared with the middle aged group, BMI and harvest site had no impact on cell yield. Finally, doubling times for passages 4, 5 and 6 were not influenced by the age and BMI of the donors, nor the tissue-harvesting site. *Conclusions.* The floating ASC fraction is an equivalent second cell source just like the adherent ASC fraction. Donor age, BMI and harvest site do not influence cell yield and proliferation rate.

Key Words: *adipose tissue, cell yield, mesenchymal stromal cells, proliferation rate*

Introduction

Mesenchymal stromal cells (MSCs) have been recognized for their potential in regenerative medicine for some time (1). MSCs have been isolated from different adult sources including bone marrow (2), peripheral blood (3), umbilical cord blood (4), synovial membrane (5), dental pulp (6), amniotic fluid (7), multiple human organs such as brain, skin, heart, kidney and liver (8) and finally, adipose tissue (9,10). Isolation is based on protocols that primarily rely on MSC ability to adhere to tissue culture plastic (11).

Because lipoaspirates have a relatively high frequency of stem cells (1–5% of the isolated cells) in contrast to other stem cell sources such as bone marrow (with only 0.001–0.1%), adipose-derived stem cells (ASCs) are becoming more attractive within the stem cell community because the need for *in vitro* propagation is potentially reduced by high cell yields. In addition, ASCs are easily accessible and widely available. The yield of isolated ASCs is reported to depend on the site of adipose tissue harvesting (12,13), with the lower abdomen and the back being mostly enriched (14,15). ASCs are easily isolated, with basically a digestion step, followed by centrifugation and seeding (16). In addition, deviations from the standard isolation protocol by addition of a simple initial washing step after 60 min result in a homogenous ASC population (17). Moreover, there is minimal morbidity on harvesting fat tissue. Finally, in contrast to embryonic stem cells, ethical and legal permissions are easily accessible (18).

Consequently, ASCs are used in plastic surgery and tissue engineering of organ grafts (19,20,21).

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Table I. Summary of sex, age, body mass, height, BMI, harvest site and harvest technique of thirty donors.

Sex (male = 1)	Age, y	Mass, kg	Height, cm	BMI	Harvest site	Harvest technique
0	25	58	168	20.9	Hip	Solid
0	41	84	168	30.2	Subabdominal	Solid
0	45	71	167	25.5	Subabdominal	Solid
0	44	79	165	29	Subabdominal	Solid
0	65	76	149	34.2	Subabdominal	Solid
1	63	105.6	170	36.5	Subabdominal	Solid
0	38	70	168	24.8	Abdominal	Liposuction
0	28	67	163	25.2	Subabdominal	Solid
0	53	103	180	31.8	Subabdominal	Solid
0	40	65	163	24.5	Subabdominal	Solid
0	36	53	150	23.6	Subabdominal	Solid
0	48	66	160	25.8	Back	Solid
1	47	135	172	45.6	Back	Solid
0	41	58	165	21.3	Abdominal	Solid
0	37	65	163	24.5	Abdominal	Liposuction
0	67	75.5	168	26.8	Abdominal	Liposuction
0	29	64	163	24.1	Abdominal	Liposuction
1	39	111.3	173	37.2	Subabdominal	Solid
0	74	90	158	36.1	Subabdominal	Solid
0	30	62	160	24.2	Abdominal	Liposuction
0	27	67	170	23.2	Abdominal	Liposuction
0	40	138	170	47.8	Subabdominal	Solid
0	44	60	165	22	Abdominal	Liposuction
0	38	74.5	156	30.6	Abdominal	Solid
0	40	115	173	38.4	Abdominal	Liposuction
0	37	85	163	32	Abdominal	Solid
0	54	56	152	24.2	Abdominal	Solid
0	35	80	172	27	Abdominal	Solid
0	27	58	170	20.1	Arm	Solid
1	56	86	160	33.6	Abdominal	Solid

Solid, lipectomy, biopsy of solid fat.

In this respect, having as high yields as possible of isolated ASCs is of great interest. In our previous isolations of ASCs, we observed a relatively high number of initially nonadherent cells. Therefore, we investigated the surface protein profile with the use of flow cytometry, the cell yield and the growth rate of ASCs that do not adhere in the first 24 h and compared their behavior to that of the adherent ASCs. If their behavior was similar or even the same—thus the hypothesis—the supernatant ASC fraction would increase the overall stem cell yield, being an advantage in cell-based therapies. Moreover, with a pool of 30 donors showing a wide range of age and body mass index (BMI), the impact of age, BMI and harvest site/harvest technique on the cell yield and proliferation capacity of ASCs was investigated.

Methods

Lipoaspirates

Thirty human adipose tissue samples (100−600 g) were obtained from lipectomies and liposuctions (healthy patients, no diabetic donors). Twenty-six donors were female and four donors were male. Age, mass, height, BMI and adipose tissue source (harvest site and technique) are summarized in Table I.

All ASCs were isolated from fat tissue, with the consent of the patients according to Swiss (KEK-ZH: StV 7-2009) and international ethical guidelines (ClinicalTrials.gov; Identifier: NCT01218945). The extraction procedure was according to Zuk *et al.* (16). Cells were cultured in Dulbecco modified Eagle's medium (DMEM) medium (Biowest, Teco Medical, Sissach, Switzerland), complemented by 10% of fetal bovine serum (FBS) (Biowest, Teco) and 50 μg mL^{-1} gentamycin. Cells that adhered to the plastic dishes during the first 24 h were called the "adherent fraction." The floating cells were collected and reseeded. No apoptotic cells with typical cell morphology changes were observed with the use of a light microscope (Leica DM 6000 B). The fraction of cells that adhered in the following 24 h was called the "supernatant fraction." Passaging of ASCs was performed with 2.5% trypsin/0.23 mmol/L ethylenediamine tetra-acetic acid (EDTA). Cell counting was done with Scepter (Scepter 2.0 Handheld Automated Cell Counter, Merck Millipore, Switzerland) and the Neubauer chamber ($n = 4$).

Characterization of ASCs by flow cytometry

ASC phenotyping was characterized according to established procedures (21,22). For flow cytometric analyses (FACS), ASCs were harvested with the use of trypsin-EDTA digestion and resuspended in phosphate-buffered saline (2×10^6 mL^{-1}). Aliquots of ASC (1×10^5) were stained for 30 min at room temperature (RT) with the respective monoclonal antibody solution (1:25), subsequently fixed with paraformaldehyde (0.1% final) for 30 min at RT and fluorescence measured by FACS. Primary antibodies labeled with phycoerythrin (PE) were CD11b-PE (R0841, Dako, Denmark), CD13-PE (Becton-Dickinson), CD62-L-PE (12-0629-73, eBioscience, UK), CD34-PE (BioLegend, 303106) and intracellular adhesion molecule (ICAM)-1−PE (sc-107 PE, Santa Cruz); antibodies labeled with allophycocyanin (APC) were CD73-APC (BioLegend, 344005) and CD90-APC (R&D Systems FMC002); unlabeled were CD29 (sc-59828, Santa Cruz), CD44 (sc-59758, Santa Cruz) and CD105 (sc-18838 Santa Cruz). Unlabeled antibodies were detected through the use of a respective secondary antibody (goat anti-mouse immunoglobulin G−PE, Santa Cruz, USA). In this case, ASCs were washed once in phosphate-buffered saline after primary staining and were then incubated with the PE-labeled secondary antibody (1:25) for 30 min at RT. PE fluorescence of individual cells was measured with a FACSCalibur flow cytometer (Becton Dickinson AG, Basel, Switzerland), gating on physical parameters to exclude cell debris. A minimum of 10,000 events per gate were counted per sample. Results are reported as percentage of positive cells.

Differentiation capacity

Lineage-specific differentiation of ASC toward the osteoblast, the endothelial cell, the adipogenic and the chondrogenic cell lineage were achieved through the use of cell culture media supplementation according to Zuk *et al.* (16). Briefly, as osteogenic medium α-MEM was used (22571, Gibco, Switzerland) supplemented by 10% FBS, 1% 10 mmol/L β-glycerophosphate (50020, Fluka, Switzerland), 100 nmol/L dexamethasone (D2915, Sigma, Switzerland) and 50 µmol/L ascorbic-2-phosphate (A8960, Sigma, Switzerland). The chondrogenic medium consisted of α-MEM supplemented with 1% FBS, 50 U/mL penicillin, 50 µg/mL streptomycin and 2 mmol/L L-glutamine (Gibco), 1 mmol/L sodium pyruvate (BIOTECH GmbH, Switzerland), 0.15 mmol/L L-ascorbic acid-2-phosphate (Sigma-Aldrich, Switzerland), 0.1 µmol/L dexamethasone and 10 ng/mL transforming growth factor-β1 (300-023, RELIATech, GmbH, Switzerland). For endothelial cell differentiation, we used a ready-to-use medium from Promo Cell: Growth Medium 2 (C-22011), containing 20% FBS, 5 ng/mL epidermal growth factor (recombinant human), 10 ng /mL basic fibroblast growth factor (recombinant human), 20 ng/mL insulin-like growth factor (Long R3 IGF-1); 0.5 ng/mL vascular endothelial growth factor 165 (recombinant human), 1 µg/mL ascorbic acid; 22.5 µg/mL heparin and 0.2 µg/mL hydrocortisone. For adipogenic differentiation, DMEM was used with insulin transferrin selenium 1:100 (Gibco), 1 µmol/L dexamethasone (RELIATech, GmbH, Switzerland), 1 mmol/L isobutyl-methlyxanthine (Sigma-Aldrich, Switzerland), 3.5 mg/dm^3 glucose (Merck, Switzerland), 200 µmol/L indomethacin (Fluka, Switzerland) and 10% FBS (Biowest, Teco Medical). Van Kossa staining was used to semiquantitatively evaluate osteogenic differentiation capacity (Supplementary Figure 1A,A'), CD31 immunohistochemical staining to observe the endothelial cell differentiation (Supplementary Figure 1B,B'), Alcian blue staining to evaluate the ability of the ASCs to differentiate toward chondrocytes (Supplementary Figure 1C,C') and oil red O staining to verify adipogenic differentiation (Supplementary Figure 1D,D').

Growth kinetics

Determination of doubling times (DT) was performed by seeding ASCs from each donor in duplicates of 1.5×10^5 cells per well into six-well plates starting from passage 3 (P3). After 3 d, cultures were split and again duplicates of 1.5×10^5 cells were seeded into six-well plates (P4) and cultivated for 6 d. The same procedure was used for P5 (8 d of cultivation) and P6 (8 d of cultivation). Different cultivation times for P4, P5 and P6 resulted from the fact that cultures were passaged just before confluence. Before splitting, cell counting with Scepter and the Neubauer chamber of united duplicates was performed ($n = 4$) and factor of cell population increase was calculated by dividing the total cell number by 3×10^5 ($2 \times 1.5 \times 10^5$ cells). DTs were calculated for P4, P5 and P6 according to:

$$\text{Doubling time} = \text{Number of days} \times 24\ \text{h} / \left(\frac{\log \text{factor of population increase}}{\log 2} \right)$$

Statistical analysis

The data were analysed with StatView 5.0.1 software. One-way statistical analysis of variance was conducted to test the significance of differences between

different groups of age, BMI and harvest site. Pairwise comparison probabilities (P) were calculated by means of the Fisher protected least-squares difference *post hoc* test to test for differences between the groups. P values <0.05 were considered significant. Values are expressed as mean ± standard deviation.

Results

Surface protein profile of adherent and supernatant fractions

A typical example of FACS analysis is shown in Figure 1. Whereas the mean fluorescence intensities (MFI) of all extracted fat samples were high in CD13, CD29, CD44 and CD105, especially at low passages (P1–P2), the MFI were comparatively low for CD11b, CD62L, ICAM-1 and CD34. Adherent and supernatant fractions showed the same pattern. Generally, the supernatant ASC fraction had slightly higher MFI for CD13, CD29, CD44 and CD105 compared with the adherent ASC fraction at the same time point and for the same passage.

DTs of adherent versus supernatant fractions

The percentage of supernatant ASCs after first seeding was 30–50%; therefore up to a maximum of half of the extracted ASCs were found to stay in the supernatant (floating) cell culture medium and not attach to the plastic dish in the first 24 h. These cells were decanted, separated and seeded again (called supernatant fraction). Approximately 60% of the supernatant cells adhered after another 24 h of cultivation. Thus, using both the adherent and the supernatant fraction of ASCs increases the total stem cell yield from approximately 50% (only adherent fraction) to approximately 80% (adherent and supernatant fractions).

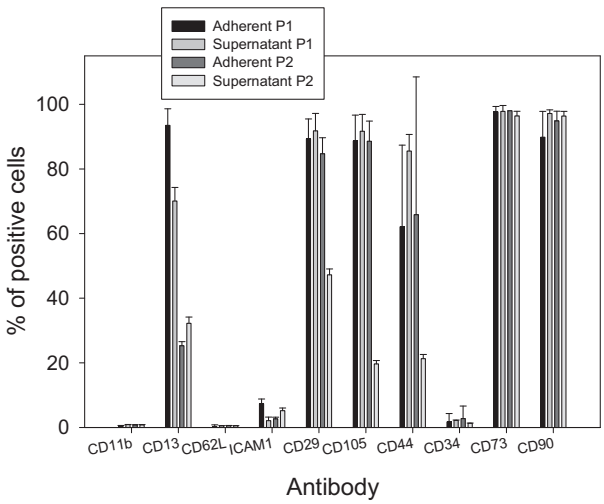

Figure 1. Percentage of positive cells of flow cytometry analysis (FACS) of three representative ASC cell lines of adherent cell fraction (P1 and P2) and supernatant cell fraction (P1 and P2), respectively. Whereas CD13, CD29, CD44, CD105, CD73 and CD90 were strongly expressed, for both adherent and supernatant ASC fractions, the percentage of positive cells was comparatively low in CD11b, CD62L, ICAM1 and CD34 (values are mean ± standard deviation, $n = 3$).

Averages of DTs for adherent and supernatant ASCs for passages 4, 5 and 6 (P4, P5 and P6) ranged from 74 h to 103 h (Figure 2). When DTs of P4 and P5 were compared for supernatant and adherent fractions, no significant differences were found. Only one significant difference was observed for P4 and P5 among adherent ASCs; however, because the days in passage for P4 and P5 were different (6 d and 8 d, respectively), no direct comparison is possible.

Cell yield as a function of age, BMI and harvest site/harvest technique

The cell yield was compared for three age groups: young donors (25–37 y), middle-aged donors (38–44 y) and old donors (45–74 y) (Figure 3A). The old age group had a significantly lower ASC cell yield compared with the middle-aged group. Moreover, cell yield was compared for three groups having different BMI values (group 1: BMI = 20–25, group 2: BMI = 25–30 and group 3: BMI = 30–48) (Figure 3B). Groups were formed according to Spalding *et al.* (23), in which they define lean subjects with BMI <25 and obese subjects with BMI >30. No significant differences were found between the three BMI groups. Additionally, three groups for harvest site/harvest technique were made, with abdominal ($n = 6$), subabdominal ($n = 12$) and liposuction ($n = 8$). Results are shown in Figure 3C. No significant differences were found between these three

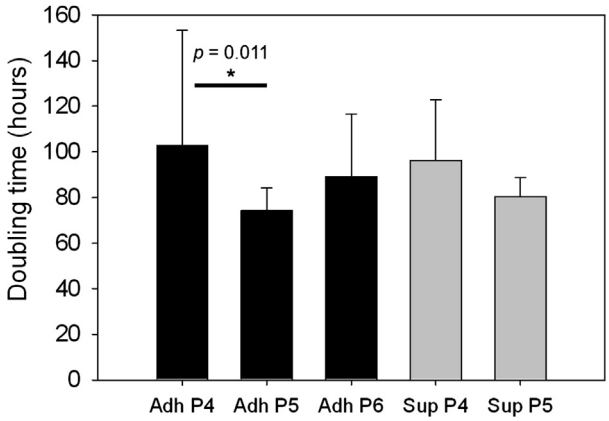

Figure 2. Doubling times for adherent ASC fractions (black) and supernatant ASC fractions (gray). Adh, adherent; Sup, supernatant; P4, P5 and P6, passages 4, 5 and 6. Number of donors involved in each group: $n = 16$ (Adh P4), $n = 18$ (Adh P5), $n = 17$ (Adh P6), $n = 5$ (Sup P4) and $n = 5$ (Sup P5). Error bars indicate standard deviations of means.

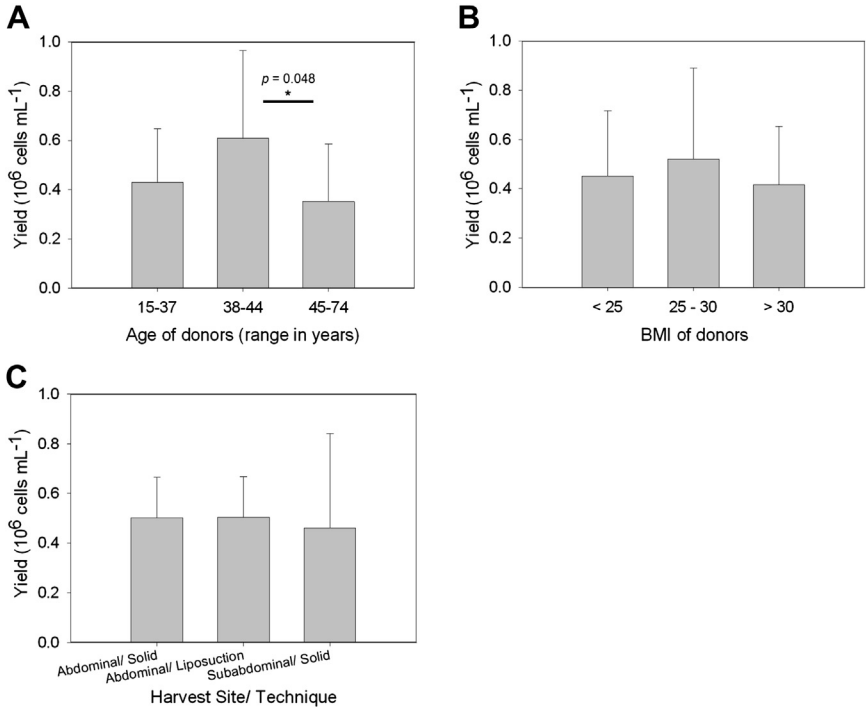

Figure 3. Total cell yield (adherent and supernatant) as a function of age (A) with $n = 10$ donors for each group, BMI (B) with $n = 10$ donors for each group and harvest site (C) with $n = 6$ donors for abdominal, $n = 8$ donors for liposuction and $n = 12$ donors for subabdominal. Ranges of age and BMI are given for the three groups. Error bars indicate standard deviations of means.

harvest sites. Finally, the influence of sex on the cell yield was not statistically different (data not shown). However, with 26 female and four male donors, the effect of sex on cell yield could not be ascertained.

DT as a function of age, BMI and harvest site/harvest technique

DTs were determined for three groups of age (age groups as defined above) and for three passages: P4, P5 and P6 (Table II). Because the growth rate for P4 was determined with cultures that were 6 d in culture, these growth rates are not directly comparable with the ones of P5 and P6 (both being 8 d in culture). Obviously, P4 had higher DTs than P5 and P6, with, on average, 100 h, whereas P5 and P6 had 80 hours on average. Whereas culturing for 6 d results in a cutoff of the log phase, culturing for 8 d includes the whole log phase (24). Consequently, DTs are smaller for growth kinetics, including the whole log phase. Comparison of the three different age groups gave no significant differences, except for P5, in which the old age group had significantly higher DTs compared with the middle-age group. This trend was also found for P4 and P6, both showing higher DTs for old age groups compared with middle-age groups.

Regarding the BMI groups, lean donors were defined as having a BMI <25, followed by a middle group of BMI 25–30 and an obese group with BMI >30 according to Spalding *et al.* (23) (Table III). No significant differences between DTs within the same passage were observed. However, there was a trend for all three passages; the higher the BMI value, the higher the average DTs.

Table II. Mean cell DT and standard deviations for three age groups of 25–37 y, 38–44 y and 45–74 y and passage length (d).

Passage	25–37 y DT (h) mean	38–44 y DT (h) mean	45–74 y DT (h) mean	Days in passage
P4	91 ± 42	100 ± 40	111 ± 62	6
P5	74 ± 11	66 ± 7*	79 ± 8*	8
P6	89 ± 29	75 ± 16	96 ± 31	8

Whatever the donor's age, there was no statistically significant difference in DT within the same passage, with the exception of group 38–44 years and 45–74 years in P5 ($P = 0.0381$), marked with an asterisk.

Table III. Mean cell DT and standard deviations for three BMI groups of <25 (lean), 25–30 and >30 (obese) and passage length (d).

Passage	<25 DT (h) mean	25–30 DT (h) mean	>30 DT (h) mean	Days in passage
P4	92 ± 42	100 ± 28	114 ± 73	6
P5	69 ± 8	76 ± 9	77 ± 11	8
P6	80 ± 27	85 ± 17	101 ± 35	8

Whatever the donor's BMI, there was no statistically significant difference in DT within the same passage.

Table IV. Mean cell DT and standard deviations for three combinations of harvest sites/harvest techniques: abdominal/solid fat, abdominal/liposuction and subabdominal/solid fat and passage length (d).

Site Technique Passage	Abdominal solid fat DT (h) mean	Abdominal liposuction DT (h) mean	Subabdominal solid fat DT (h) mean	Days in passage
P4	77 ± 8	78 ± 19	127 ± 62	6
P5	68 ± 6	74 ± 4	78 ± 12	8
P6	82 ± 12	74 ± 17	99 ± 33	8

Whatever the harvest site of the fat sample, there was no statistically significant difference in DT within the same passage.

Finally, no significant differences between DTs within the same passage were found for three different harvest sites/harvest techniques (Table IV). As a trend and found for or all three passages, however, subabdominal fat source showed higher DTs compared with abdominal and liposuction sources—the latter two behaving very similarly in this respect.

Discussion

Many studies dealing with ASCs focus on the fraction of adipose tissue–derived stem cells that attach to plastic dishes being a widespread criterion and way of selection for mesenchymal stromal cells (11,15,25). Because in some cases a considerable amount of up to 30% of ASCs is reported not to adhere to the plastic dishes in the first run (9), we collected these cells in the supernatant cell culture medium and reseeded them. Their characteristics such as surface markers and growth rate of different passages were compared with the adherent fraction of the same donor. The main outcome was that the supernatant fraction adhered after reseeding and was thus a very useful second cell source and a considerable additional amount of ASCs that would have been discarded following widely established protocols (25). Moreover, the supernatant stem cell fraction had at least a same or even more expressed surface protein profile compared with the adherent fraction, with positive and negative markers standing in accordance with the findings of Tapp *et al.* (26,27). Finally, DTs of adherent and supernatant ASCs did not differ significantly. From these results, we suggest that the supernatant fraction of ASCs should also be used for cell-based therapies, just as the adherent fraction is used.

Most all of the studies dealing with the isolation of ASCs only include the fraction that adheres to the plastic surface, exemplified by the isolation of ASCs from rats, rabbits and pigs (28), from mice (29) or from human lipoaspirates (30–32). Small modifications in the isolation protocol are reported by Griesche *et al.* (17), in which a washing step after 60 min led to less heterogeneity in the such-isolated ASC population; however, only initially adherent ASCs were used as in the standard protocol. To our knowledge, the use of other fractions than the first adherent fraction is, to date, not reported as a regular procedure in stem cell–based therapies including *in vitro* expansion of ASCs. The reason that a considerable amount of isolated ASCs does not adhere in the first run remains to be elucidated. It can be speculated that the initially nonadherent ASC fraction has a different maturation or activation state with different proteins on the surface that help to adhere to plastic. Another reason may be that factors secreted from already-adherent ASCs prevent supernatant ASCs from adhering in the first run.

In addition, we compared yields and DTs of ASCs for donors of different age, BMI and harvest site. In our study, an average cell yield of $0.46 \pm 0.29 \times 10^6$ mL^{-1} was found for the whole set of 30 donors, with eight undergoing liposuction (cell yield, $0.50 \pm 0.16 \times 10^6$ mL^{-1}) and the rest delivering solid lipoaspirates. Generally, cell yields per milliliter of solid lipoaspirates have been reported to be always in the same range of $0.308 \pm 0.140 \times 10^6$ mL^{-1} (33), $0.375 \pm 0.147 \times 10^6$ mL^{-1} (34), 0.38×10^6 mL^{-1} (35), $0.404 \pm 0.206 \times 10^6$ mL^{-1} (28) and lower for liposuction samples such as $0.1475 \pm 0.032 \times 10^6$ mL^{-1}, $0.26 \pm 0.12 \times 10^6$ mL^{-1} or $0.287 \pm 0.034 \times 0.38 \times 10^6$ mL^{-1} (13,36,37). In contrast to the reported significantly lower yields for liposuction samples compared with solid fat sources, we did not find any statistically significant different cell yields for the three harvest sites considered, namely abdominal, liposuction and subabdominal.

In contrast, reports dealing with the influence of age, BMI or sex on yields and proliferation rates vary tremendously in their outcomes. For example, Yu *et al.* (34) claim to have found a positive correlation between cell yield and donor age (linear correlation coefficient $r = 0.30$) as well as BMI ($r = 0.26$). Other studies deny such a correlation and claim that BMI and age of donors (37) do not influence the cell yield (28,35,38). Our results indicate that the yield of ASCs might be influenced by the age of the donor because we found a significantly higher ASC cell yield of donors aged 38–44 years compared with older donors ages >45 years. In contrast, de Girolamo *et al.* (39) report higher cellular ASC yields for female donors >45 years of age ($0.61 \pm 0.40 \times 10^6$ mL^{-1}) compared with female donors <35 years of age ($0.27 \pm 0.13 \times 10^6$ mL^{-1}). It appears to be a controversial issue that highly depends on the coincidental composition of the donor population and the number of donors involved in the corresponding studies. Such comparative studies focussing on the impact of age, BMI or sex include at maximum 125

donors (38), usually less such as 22 donors (12), 15 donors (24), 12 donors (15) or only 10 subjects (13). Thus, representative results are difficult to obtain. The same is true for the influence of BMI on the ASC cell yield. In the study presented here, no significant influence of BMI on cell yield was determined. Other studies report a positive correlation of ASC yield and BMI (34) or a negative correlation (28). Finally, the question of whether male or female donors have higher ASC cell yield was difficult to answer. In the present study, we found no significant difference; however, the number of donors was imbalanced in the two subsets consisting of 26 female donors and only four male donors.

Regarding DTs, the average DTs found for P4 corresponded well to values of 4.4 ± 2.3 days with time in passage of 6.6 ± 2.0 d reported by Mitchell *et al.* (9). The impact of age, BMI or harvest site/technique on growth kinetics was found to be minor within the same passage; only one exception occurred for P5, in which the old age group had significantly higher DT compared with the middle-age group. This stands in accordance with the finding of Zhu *et al.* (24), in which a positive correlation of DT and donor age is reported ($r = 0.62$). Neither BMI nor sex was found to have any significant impact on DT of P4, P5 and P6, which stands in accordance with the findings of Mojallal *et al.* (35). In a comparison of DTs for ASC coming from different tissue-harvesting sites/techniques (abdominal, liposuction and subabdominal), no impact on DT of P4, P5 and P6 was found, standing in accordance with the findings of Jurgens *et al.* (12), who report similar growth kinetics for ASC from abdomen and hip/thigh.

Conclusions

Comparison of adherent and supernatant fractions of ASCs resulted in similar surface protein profiles and not significantly different growth kinetics, proposing inclusion of the supernatant (floating) stem cell fraction in cytotherapy besides the routinely used adherent stem cell fraction. Moreover, the impact of age, BMI and harvest site on the cell yield and the growth kinetics was found to be minor, with no statistically significant differences. These findings should encourage surgeons to use ASCs for cytotherapy regardless of the age and BMI of the donor, nor the collection harvest site and collection technique of the adipose tissue.

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Supplementary data

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