Comparative Study of Adipose-derived Stem Cells from Abdomen and Breast

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Abstract

Background: Abdominal tissue enriched with adipose-derived stem cells (ASCs) is often used in cell-assisted lipotransfer procedures for breast reconstruction. However, as the tissue microenvironment and stem cell niche play important roles in defining the characteristics of the resident cells, it is hypothesized that the stem cell population present in the donor abdominal tissue has dissimilar properties as compared to the cells in the recipient breast tissue, which may ultimately affect the long-term success of the graft.

Methods: ASCs were isolated from breast and abdominal fat tissues and characterized for mesenchymal specific cell surface markers, and their population doubling, colony forming capabilities and proliferative properties were compared. The multi-lineage potential of both cell populations was also investigated.

Results: ASCs from both tissue sites were found to possess similar marker expression and multi-lineage differentiation potential. However, breast fat-derived ASCs (BF_ASCs) were observed to have a higher self-renewal capability and an unstable population doubling as compared to abdominal fat-derived ASCs (AF_ASCs). Gene expression studies revealed that the BF_ASCs were predisposed to the osteogenic lineage and the AF_ASCs to the adipogenic lineage.

Conclusion: Cells derived from both fat tissues possess different characteristics in terms of their growth kinetics and predisposition to the osteo- and adipo-lineages. In particular, ASCs from the abdominal tissue appear to contribute to adipose tissue turnover, whereas ASCs from breast tissue, if used for cell-assisted fat grafting, may potentially be responsible for complications in fat grafting such as oil cysts, calcifications, fat necrosis and tumors.

Keywords

Adipose tissue, adipose-derived stem cells, microenvironment, stem cell differentiation

INTRODUCTION

Autologous fat grafting is a conventional technique that has been used for more than a hundred years. However, it has been reported that in some cases, about 30% to 70% transplant volume is resorbed over time in an unpredictable manner.[1, 2] Therefore, in an attempt to improve long-term graft retention and overall transplant outcome, cell-assisted lipotransfer is currently being carried out.[1-8] This procedure, which consists of enrichment of the lipoaspirate with stromal vascular fraction (SVF) or adipose tissue-derived stem cells (ASCs), has been known to improve graft survival over time and overall aesthetic outcomes.[3, 9] The ASCs could potentially be differentiated into adipocytes, maintain the fat tissue, and also support angiogenesis and accelerate wound healing by secreting various growth factors.[10, 11] However, the risk of complications such as oil cyst formation, fat necrosis, calcifications and tumor inductions[11, 12] from fat grafting procedures to the breast has also been observed. Hence, it is still unclear whether cell-assisted procedures are superior to autologous fat transfer methods in terms of a successful long-term fat graft.

Numerous studies have shown that fat tissue distribution varies considerably among individuals and that the different fat depot sites vary in size, function and potential contribution to various diseases.[13, 14] Whilst a few studies have tried to study the role of donor tissue site in the volume retention of fat grafting,[10, 15-17] these clinical studies involved a number of variables and hence it was difficult to reach a consensus on this subject. Nevertheless, based on earlier studies by others on the role of the stem cell niche,[18, 19] we hypothesize that the ASC populations from different fat depot sites are highly affected by their microenvironment and hence, would have different characteristics that could play a role in the overall outcome of the fat graft.

To test this hypothesis, ASCs were isolated from both donor (i.e. abdomen) and recipient (i.e. breast) tissues, characterized for self-renewal, multipotency and proliferative abilities, and differentiated into osteocytes, adipocytes and chondrocytes. As the cells were cultured in similar conditions *in vitro*, the effect of their native tissue microenvironment on their characteristics and predisposition in terms of lineage differentiation is compared. Overall, a comparison of the ASCs from abdominal tissue and ASCs present in the breast tissue provides insights into the possible role of these cells in cell-assisted fat grafting procedures.

MATERIALS AND METHODS

Isolation and Culture of ASCs

All cell culture reagents were purchased from Gibco, Life Technologies, USA, unless otherwise stated. The waste fat tissue from elective surgery was obtained from the National University Hospital, Singapore (DSRB Ref: 2011/01721) and Tan Tock Seng Hospital, Singapore (DSRB Ref: 2012/00071). Fat tissue samples were either from the breast or the abdomen and the cells isolated from these tissues were termed as BF_ASCs and AF_ASCs respectively. Three different samples were pooled for each of the types and used for this study in order to overcome any biological variations. The donors were all women and the mean and the median of their ages were 42.16 and 45 respectively. All donors had BMI <27 and did not have a history of chronic viral, metabolic, autoimmune, ischemic, or other systemic diseases. The procedure for isolation of ASCs from the two tissues was carried out according to previously established protocols.[20] The tissue samples from the breast were in the form of fat grafts and those from the abdomen were in the form of lipoaspirates. The procedure for isolation of ASCs from the two tissues was similar with an addition of a mincing step at the beginning for the

breast fat graft. Briefly, the fat tissue samples were washed with 1X phosphate buffer saline (PBS) and in the case of tissue grafts, minced using sterile scissors and scalpel blade. The minced graft tissue or the lipoaspirate was then washed with 1X PBS, sieved, weighed and digested with 0.075% collagenase type I in 1X PBS for 30 mins in a shaking water bath at 37°C. The digestion reaction was quenched with an equal volume of Dulbecco's modified Eagle's media (DMEM) supplemented with 10% fetal bovine serum (FBS) and centrifuged at 180 g for 10 mins, after which the supernatant was discarded and the pellet was subjected to RBC lysis (0.15M NH_4Cl, 10mM $KHCO_3$, 0.1mM EDTA, and sterile distilled H_2O) for 5 mins. After quenching the lysis buffer with an equal volume of DMEM, the mixture was filtered through a 100 µm cell strainer and centrifuged at 180 g for 10 mins. The supernatant was then discarded and the resulting stromal vascular fraction (SVF) pellet was plated in a 75 cm^2 tissue culture flask (Nunc, USA). The ASCs are the plastic adherent cells, which were then cultured *in vitro* and passaged on 80% confluence. Both BF_ASCs and AF_ASCs were cultured in tissue culture flasks in 5% CO_2 environment at 37ºC with media changes every other day. The complete proliferation media constituted DMEM/ Ham's F12 (1:1) media supplemented with 10% FBS (Hyclone, USA), 1% Penicillin-Streptomycin, 10mM MEM-Non Essential Amino Acids and 200mM L-Glutamine. The morphology of the ASCs isolated from the two fat tissue sites was observed during the course of *in vitro* cell culture using phase contrast microscopy (Primo Vert, Carl Zeiss, Germany). Imaging was carried out using the Zeiss camera and Axio vision software (Carl Zeiss, Germany).

Cell Surface Marker Expression Analysis

The expression of surface markers present on the ASCs was analyzed by flow cytometry following previously established protocols.[21] Cells were stained for CD73-PE, CD105-PE, CD45-PECy5, CD44-PE and CD29-PE for 1 h at 4ºC and a BD FACSCalibur™ Flow Cytometer (BD Biosciences, USA) was used. Data analysis was carried out using the CELLQuest Software.

Colony Forming Properties

Cells were seeded in wells of a 6-well plate at a seeding density of 10 cells/cm^2 and cultured in the proliferation media up to 14 days with media changes every two days. After 14 days, the cells were fixed with 4% paraformaldehyde and stained with crystal violet and the colonies were measured and counted for both the cell populations. All colonies with a diameter more than 2 mm were scored.[22]

Population Doubling Analysis

Population doubling characteristics of the BF_ASCs and AF_ASCs were analysed from passage 2 to 8 following previously established protocols.[23] Briefly, the cells were trypsinized, counted and plated at a density of 3000 cells/cm^2 at each passage. The cells were cultured to ~80% confluence and again trypsinized, counted and re-plated at the same density in fresh plates. The initial and final cell numbers were recorded at each passage and also the number of days that the cells were in culture (n=5). Cell doubling at each passage was calculated using equation (1), whilst cumulative cell doubling was plotted against the passage number, to study the cell doubling kinetics over passages 2 to 8. Cell doubling time for each passage was calculated using equation (2) and plotted against passage number.

$$\text{Cell Doubling} = \frac{\ln(\text{final cell number} - \text{initial cell number})}{\ln(2)} \quad \text{..... (1)}$$

$$\text{Cell Doubling Time} = \frac{\text{Cell culture time in days}}{\text{Cell doubling}} \quad \text{..... (2)}$$

Cell Proliferation

Proliferation of the cells at passage 2 was measured using the alamarBlue® assay (Life Technologies) following previously established protocols[24] on days 0, 3, 7 and 10 under culture conditions of proliferation, adipogenic and osteogenic differentiation. Briefly, the cells cultured in well plates were incubated with serum free media containing 10% alamarBlue® reagent in 5% CO_2 environment at 37ºC. After 2 h, the reagent containing media was transferred to a 96-well plate and fluorescence was measured at 560/590nm using a plate reader (Molecular Devices, USA). Fresh media was added and the cells were incubated at 37ºC and 5% CO_2 until the next time point.

Differentiation of ASCs

The differentiation potential of the ASCs isolated from the two tissue sites was investigated by exposing the cells to media conditions that constitute differentiation supplements for osteogenic, adipogenic and chondrogenic differentiation.[24] The osteo-differentiated and adipo-differentiated cells were stained with Alizarin Red (Sigma) and Oil Red O (Sigma) for calcium deposits and lipid globules respectively using previously established protocols.[25] After staining, the cells were then washed with distilled water and air dried before visualizing under the light

microscope (Primo Vert, Carl Zeiss). The pellet cultures of chondro-differentiated cells were paraffin embedded and 8 μm thick sections were used for Alcian blue staining.

Gene Expression

RNA from the ASCs was isolated using the RNA-Solv (Omega bio-tek) and RNeasy mini kit (Qiagen) according to manufacturer's instructions. The expression levels of osteocalcin and peroxisome proliferator-activated receptor gamma (PPARγ) were measured and normalized against the expression of housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH) using the primer sequences shown in Table 2.

Statistics

All statistical analyses were carried out using the Mann-Whitney U test for the colony forming assay, population doubling and cell proliferation studies. Kruskal Wallis, non-parametric one way ANOVA analysis was carried out for the gene expression studies. The differences were considered statistically significant when $p<0.05$.

RESULTS

Establishing the Identity of ASCs

The morphology of the ASCs derived from the breast (BF_ASCs) and the abdominal (AF_ASCs) fat tissues were visualized under phase contrast microscopy on day 3 of cell culture as seen in Figure 1. Cells from both types of fat tissue were observed to have the typical mesenchymal stem cell morphology, which consists of a small cell body with few thin and long cell processes. During proliferation, the cells maintained this morphology *in vitro* (Supplemental Digital Content 1). Phenotypic profiling for the ASCs showed that the cells from both tissue sites were CD73+, CD105+, CD44+, CD29+ and CD45- (Table 1 and Supplemental Digital Content

2). Overall, both the cell populations were identified to be ASCs and no significant difference between the two in terms of their cell surface marker expression was observed. In terms of colony forming abilities it was found that 60.2% of the BF_ASCs formed colonies, but a smaller percentage (i.e. 40.0%) was observed for the AF_ASCs (Figure 2b). Hence, BF_ASCs have a significantly higher self-renewal ability compared with AF_ASCs. Overall, the cells isolated from both sites were capable of forming colonies by self renewal and hence along with morphology and cell surface marker profiling were therefore identified as ASCs.[26]

Growth Kinetics of ASCs

Results from the population doubling studies revealed that the cumulative cell doubling numbers over passages 2 to 8 were significantly higher ($p<0.05$) for the AF_ASCs, with a maximum of 144 cell doublings as compared to 22 for the BF_ASCs at passage 8 (Figure 3a). The cell doubling time plotted against the passage number showed that the AF_ASCs have similar doubling times per passage (Figure 3b). For BF_ASCs, the doubling time per passage was found to be significantly longer ($p<0.05$) before passage 3 but subsequently shortened after passage 4 (Figure 3b). Hence, the AF_ASCs appeared to have a more stable growth profile *in vitro*, as compared to the BF_ASCs. Also, within a fixed period of time, higher AF_ASC cell numbers could be achieved as opposed to the BF_ASCs, which took time to adjust to the culture environment, as seen from the longer doubling times at early passages.

Differentiation of ASCs

Upon differentiation, it was observed that, the proliferation rate of the cells grown in differentiation media was slower than those grown in proliferation media (Figure 4a), which is expected, since stem cells tend to lose their proliferative ability upon differentiation.[27] Under

osteogenic conditions (Figure 4b), the cell numbers of both cell populations were found to be similar over 10 days of culture. However, under adipogenic media conditions (Figure 4c), cell numbers of AF_ASCs were found to be significantly higher (p<0.05) than the BF_ASCs. For both AF_ASCs and BF_ASCs, the differentiated osteoblasts produced calcium that was positively stained with Alizarin Red (Figure 5(i)), whereas the adipocytes accumulated oil droplets in the cells that were visualized by staining with Oil Red O (Figure 5(ii)), and the cells differentiated into chondrocytes in a pellet culture showed positive Alcian Blue staining for glycosaminoglycans (Figure 5(iii)). Overall, these qualitative staining results confirmed the multipotent behavior of both cell populations.

The expression levels of osteocalcin, a marker for osteogenesis[28], and PPARγ, a marker for adipogenesis[29] was measured by real-time PCR (Figure 6). Overall, the expression levels of osteocalcin were found to be significantly higher (p<0.05) in osteo-differentiated BF_ASCs when compared to osteo-differentiated AF_ASCs. No significant difference in gene expression was observed for adipo-differentiated BF_ASCs and AF_ASCs (Figure 6a). On the other hand, PPARγ expression was significantly higher (p<0.05) in both the adipo-differentiated BF_ASCs and AF_ASCs as compared to their osteo-differentiated counterparts (Figure 6b). However, an increase in PPARγ in osteo-differentiated AF_ASCs was observed and that was not the case with BF_ASCs (Figure 6b).

DISCUSSION

In this study, the identity of the ASCs isolated from the two fat tissue sites was established by defining their morphology, cell surface marker expression and self-renewal by colony

forming abilities. As seen from Figure 1 and Supplemental Digital Content 1, the ASCs from both sites had a the typical fibroblast-like morphology of mesenchymal stem cells[30] and were found to be CD73+, CD105+, CD44+, CD29+ and CD45- (Table 1 and Supplemental Digital Content 2). Although both BF_ASCs and AF_ASCs were capable of colony formation, the BF_ASCs had a significantly higher (p<0.05) colony forming potential as compared to the AF_ASCs, suggesting that the ASCs from breast fat tissue have a greater potential for self-renewal *in vitro*. Hence, the ASCs from breast fat (BF_ASCs) have a higher chance of maintaining and increasing their stem cell population, as more cells remained in the undifferentiated state, as compared to the AF_ASCs.

On the other hand, population doubling analysis of the ASCs from the two different fat tissue sites revealed that the AF_ASCs had a more consistent population doubling time as compared to the BF_ASCs for passages 2 to 8. This meant that the AF_ASCs had a more stable growth rate as compared to the BF_ASCs, which grew faster with every population doubling. The pathways involved in cellular fate decisions of stem cells have been shown to be affected by microenvironmental factors that can result in a continuously proliferating cell population, which is often cancerous.[31] In terms of growth stability, it appears that the ASCs from the abdominal fat, which is the most common site of harvesting donor tissue for breast augmentation, is an ideal choice in terms of safety and efficacy. However, further analysis is required in order to elucidate the effect of the presence of remnant cancerous cells on the overall properties of the implanted AF_ASCs in patients with history of breast cancer.[32] It has previously been shown that whilst ASCs support tumor growth and metastasis,[33] under the influence of signals from tumor cells, the proliferation of ASCs could also be further sustained and adipogenesis of ASCs could be

inhibited.[34] Hence, it is this reciprocal relationship between ASCs and cancer cells that appears to drive tumor progression.[35, 36].

In cell-assisted fat grafting, freshly isolated ASCs are directly implanted along with the lipoaspirate. Hence, only cells harvested and re-injected in the operating theatre are truly clinically translatable.[37, 38] Whilst, other researchers are developing in vitro suspension culture systems for ASCs in attempts to simulate the actual clinical scenario,[39] for this study, low passsage cells were used in order to simulate the in vivo conditions. For this study, we observed that at passage 2, both BF_ASCs and AF_ASCs showed similar proliferation profiles. However, their population doubling time varies with prolonged *in vitro* passage. Thus, even though the BF_ASCs and AF_ASCs show characteristic MSC traits, they belonged to different microenvironments, which probably played a role in influencing their long-term growth and function. In fact, the behavior of ASCs has previously been shown to be highly dependent on their inherent properties as well as the cues provided by their native microenvironment, which work together to dictate the fate of the ASCs.[40] Overall, the irregular cell divisions and growth kinetics of the BF_ASCs may contribute to complications that can occur post fat grafting and affect the long- term viability of the graft. This is in concurrence with previous studies that demonstrated the possibility of the BF_ASCs playing a larger role in problems such as breast tumor progression as compared to the bone marrow-derived MSCs.[41] Here, our findings suggest that AF_ASCs should be used for cell-assisted lipotransfer procedures instead of BF_ASCs, since in addition to stable growth kinetics; it is the AF_ASCs that contribute to the neo-formation of adipocytes and thus play a pivotal role in fat tissue turnover upon fat grafting.[42, 43]

Upon culturing in differentiation media conditions *in vitro*, both BF_ASCs and AF_ASCs were able to differentiate into the osteogenic, adipogenic and chondrogenic lineages when given

the necessary external cues.[44] Also, the proliferation of both cell populations was overall decreased upon differentiation. The AF_ASCs however showed higher cell numbers than BF_ASCs in adipogenic media, which suggests that these cells are still in the early stages of adipogenesis, as cell expansion has been shown to cease during the late stages via a CCAAT/enhancer-binding proteins-mediated pathway.[45]

Further analysis of the osteo-differentiated cells showed that the expression levels of osteocalcin was increased in both BF_ASCs and AF_ASCs as compared to undifferentiated cells, with the expression levels in BF_ASCs being significantly higher ($p<0.05$) than the AF_ASCs. However, no significant difference in osteocalcin expression levels was observed for cells grown in adipogenic media. Osteocalcin is a protein secreted by osteoblasts upon bone formation, and reflects terminal differentiation of ASCs into the osteogenic lineage.[46] From the current study, it appears that the BF_ASCs are more susceptible to osteogenic differentiation, when subjected to appropriate biochemical cues (i.e. media supplements), since a higher number of terminally differentiated cells led to higher osteocalcin expression level as compared to the AF_ASCs.

On the other hand, the expression levels of PPAR, a major regulator of adipogenesis of preadipocytes into mature adipocytes was found to be significantly higher ($p<0.05$) in both BF_ASCs and AF_ASCs upon adipo-differentiation, with no significant difference observed between the two cell populations. Interestingly, the expression level of PPARγ was significantly higher, in osteo-differentiated AF_ASCs than BF_ASCs, suggesting that AF_ASCs are predisposed to the adipogenic lineage, regardless of microenvironment. Hence, it is conceivable that the AF_ASCs are more likely to differentiate into adipocytes rather than osteoblasts during fat grafting, as seen from the increased accumulation of lipid droplets in adipo-differentiated

AF_ASCs (Figure 5(ii)). Our observation that the AF_ASCs expressed high amounts of PPARγ even in the presence of osteo-differentiation factors suggests that AF_ASCs may have epigenetic cues that take precedence over new microenvironmental factors. The importance of the donor microenvironment was also highlighted in animal studies, where the new adipocytes in the fat graft were found to be derived from differentiation of donor ASCs and not the recipient tissue itself.[15]

Our current results suggest that the AF_ASCs most likely contribute to adipogenesis and tissue turnover, whereas the BF_ASCs are more responsive to microenvironment cues. Indeed, earlier studies have shown that BF_ASCs play an important role in breast cancer, due to their close proximity to the mammary tissue, which leads to an exchange of factors and cytokines between the two that is responsible for tumour invasion into the surrounding healthy breast tissue.[41, 47] Taken together, ASCs that are derived from inherently similar types of fat tissue, but from different locations in the body may display similar traits, but possess varying differentiation predisposition and behavior as a result of their native microenvironment. Although the exact mechanisms have yet to be discovered, such findings may later be exploited for developing alternative treatment options for breast cancer.

CONCLUSION

We found from this study that the ASCs from commonly used donor abdominal fat tissues and recipient breast fat tissues both exhibited characteristic MSC morphology, cell surface marker expression and differentiation potential. However, BF_ASCs have a higher self-renewal capability and an unstable population doubling profile as compared to the AF_ASCs. Overall, this study showed that the cells derived from the donor and recipient fat tissues have different

characteristics in terms of their growth kinetics and predisposition to the osteo- and adipo-lineages. Hence, other factors in the tissue microenvironment that play a role in defining the behavior of the cells need to be fully deciphered in order to provide safe and effective procedures for ASC-enriched autologous fat grafting. Overall, findings from this study showed that compared to BF_ASCs present locally in the recipient tissue, ASCs from the abdomen would be capable of providing a better regenerative potential, due to their tendency to differentiate into adipocytes and contribute to tissue turnover upon grafting. Together with their stable growth kinetics, the AF_ASCs make an ideal source of cells for enriching autologous fat tissue.

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FIGURES

FIGURE 1. Cell morphology of (a) BF_ASCs and (b) AF_ASCs in proliferation media conditions on days 3 of *in vitro* culture, as visualized under the phase contrast microscope. Typical fibroblast-like morphology consisting of a small cell body with few thin and long processes was observed for both types of cells. *(Scale bar = 50 μm)*

FIGURE 2. Colony forming units (CFUs) of BF_ASCs and AF_ASCs, (a) scored after 14 days of culture at a seeding density of 10 cells/cm^2 and (b) representative images of a colony plate of (i) BF_ASCs and (ii) AF_ASCs stained with crystal violet on day 14 of culture. *(Scale bar = 15 mm) (Inset: higher magnification images of a single colony (Scale bar = 500 μm)* (*$p<0.05$)

FIGURE 3. Population doubling studies for BF_ASCs and AF_ASCs shown as (a) Cumulative cell doubling numbers and (b) Cell doubling time for different passages. *(*$p<0.05$)*

FIGURE 4. Cell proliferation profiles of BF_ASCs and AF_ASCs when cultured in (a) proliferation, (b) adipo-and (c) osteo-media conditions. *(*$p<0.05$)*

FIGURE 5. Differentiation of (a) BF_ASCs and (b) AF_ASCs into (i) osteo-, (ii) adipo-, and (iii) chondro-lineages as shown by Alizarin Red, Oil Red O and Alcian Blue stainings respectively.

Black arrows = presence of mineralization, yellow arrows = presence of lipid droplets and blue arrows = presence of glycosaminoglycans. *(Scale bar= 100 μm)*

FIGURE 6. Gene expression levels for (a) Osteocalcin and (b) PPARγ upon osteo- and adipo-differentiation of BF_ASCs and AF_ASCs. *(*p<0.05)*

TABLES

TABLE 1. Cell surface marker expression for (a) BF_ASCs and (b) AF_ASCs. Both cell populations were found to be CD73+, CD105+, CD45-, CD44+ and CD29+.

TABLE 2. Forward and reverse primer sequences for genes GAPDH, Osteocalcin and PPARγ.

LIST OF SUPPLEMENTAL DIGITAL CONTENT

Supplemental Digital Content 1.tif

Supplemental Digital Content 2.tif

Supplemental Digital Content Legends

SUPPLEMENTAL DIGITAL CONTENT 1: Cell morphology of (a) BF_ASCs and (b) AF_ASCs in proliferation media conditions on days (i) 0, (ii) 3, (iii) 5 and (iv) 7 of *in vitro* culture, as visualized under the phase contrast microscope. Typical fibroblast-like morphology consisting of a small cell body with few thin and long processes was observed for both types of cells at all time points. *(Scale bar = 50 μm)*

SUPPLEMENTAL DIGITAL CONTENT 2: Flow cytometric histograms for (a) BF_ASCs and (b) AF_ASCs showing the expression of mesenchymal stem cell-specific cell surface markers. Both cell populations were found to be CD73+, CD105+, CD45-, CD44+ and CD29+.

Figure

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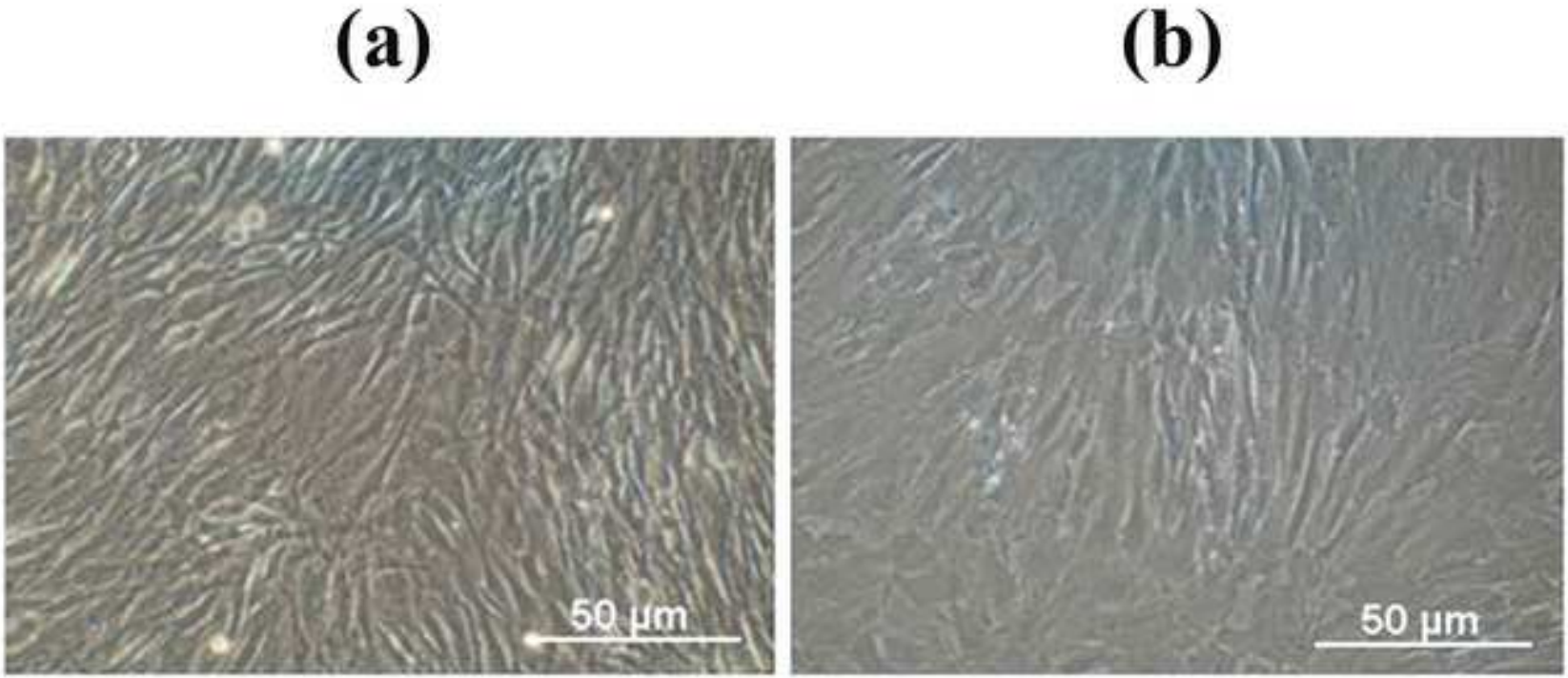

Figure 2

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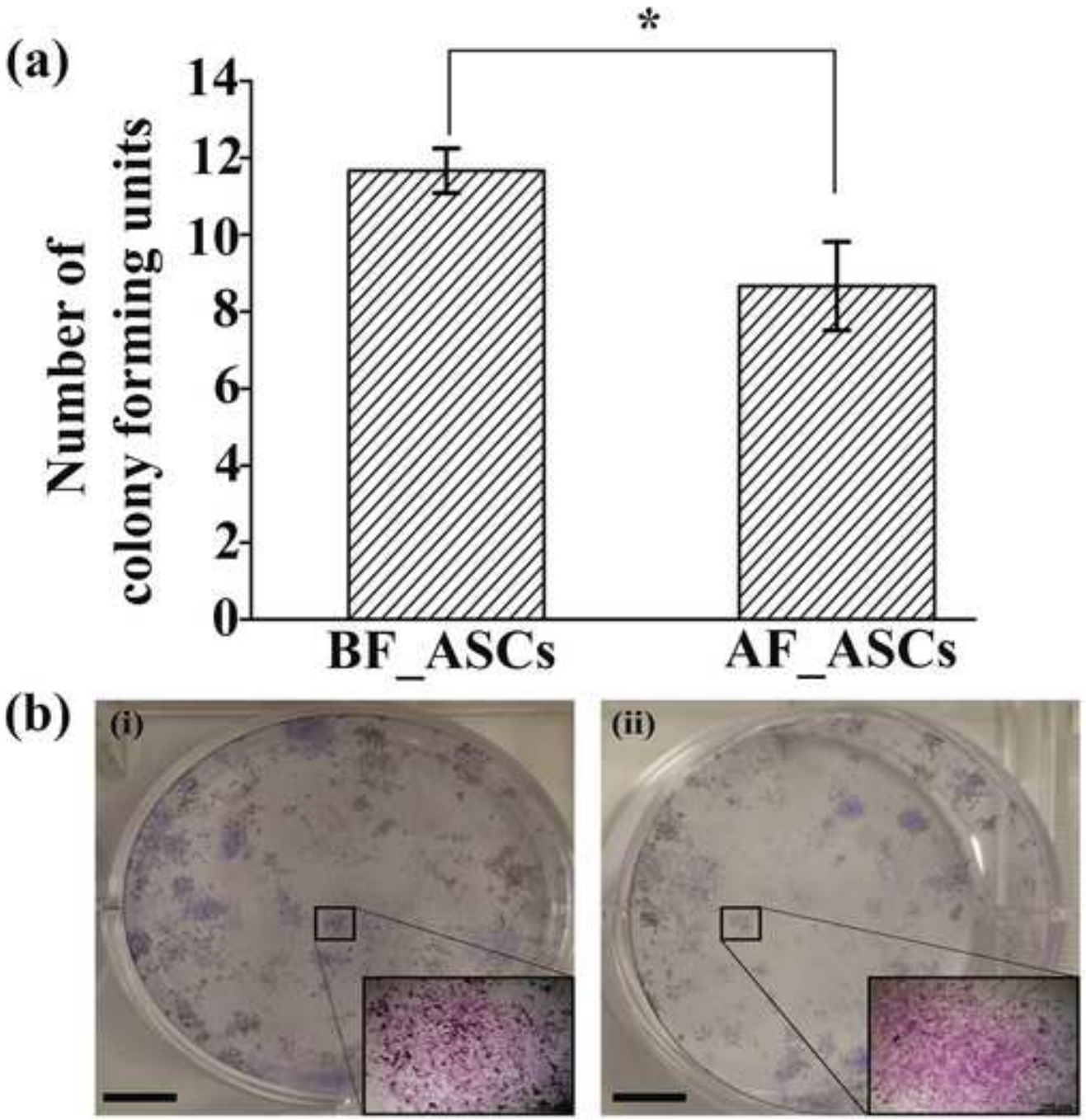

Figure 3

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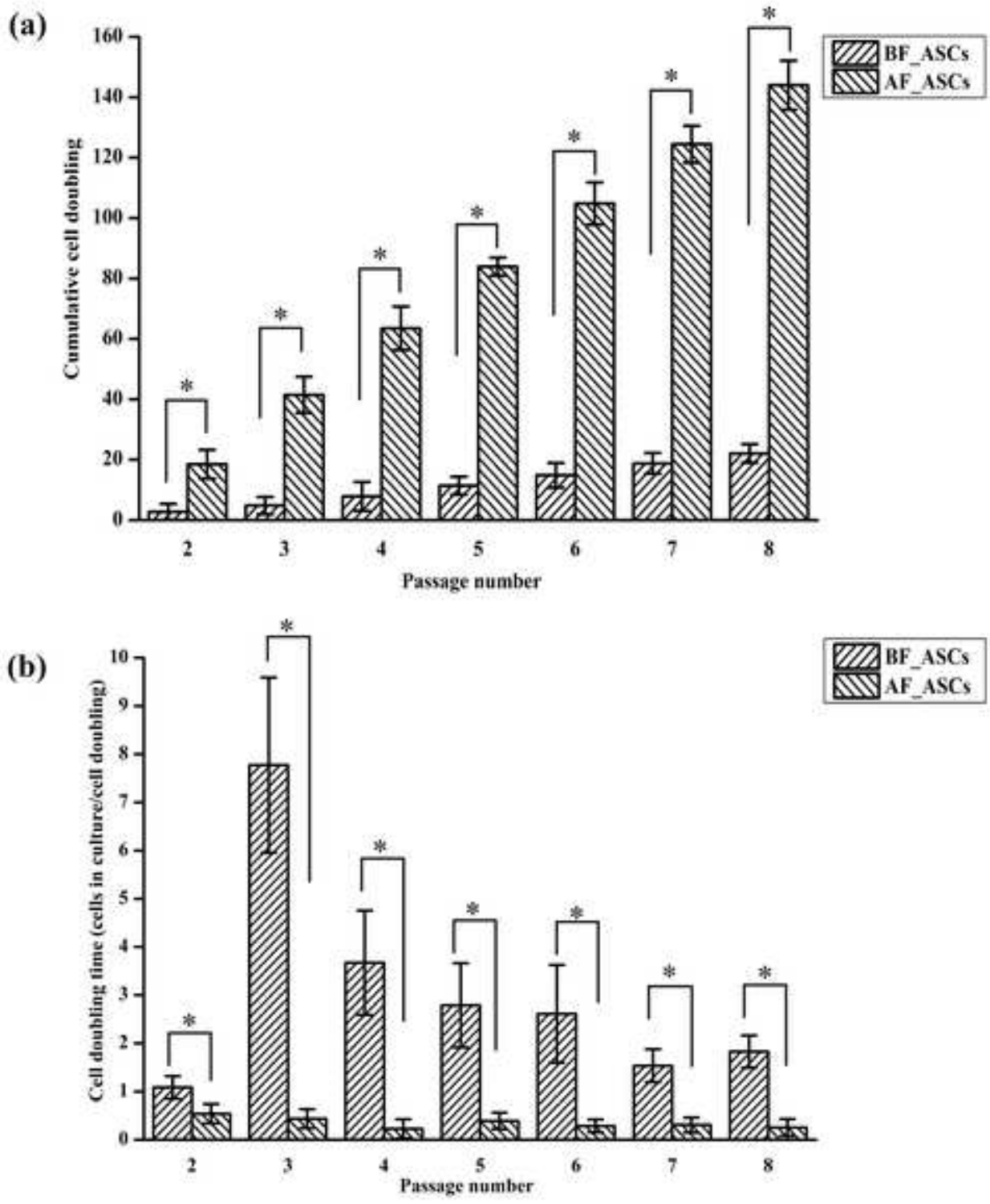

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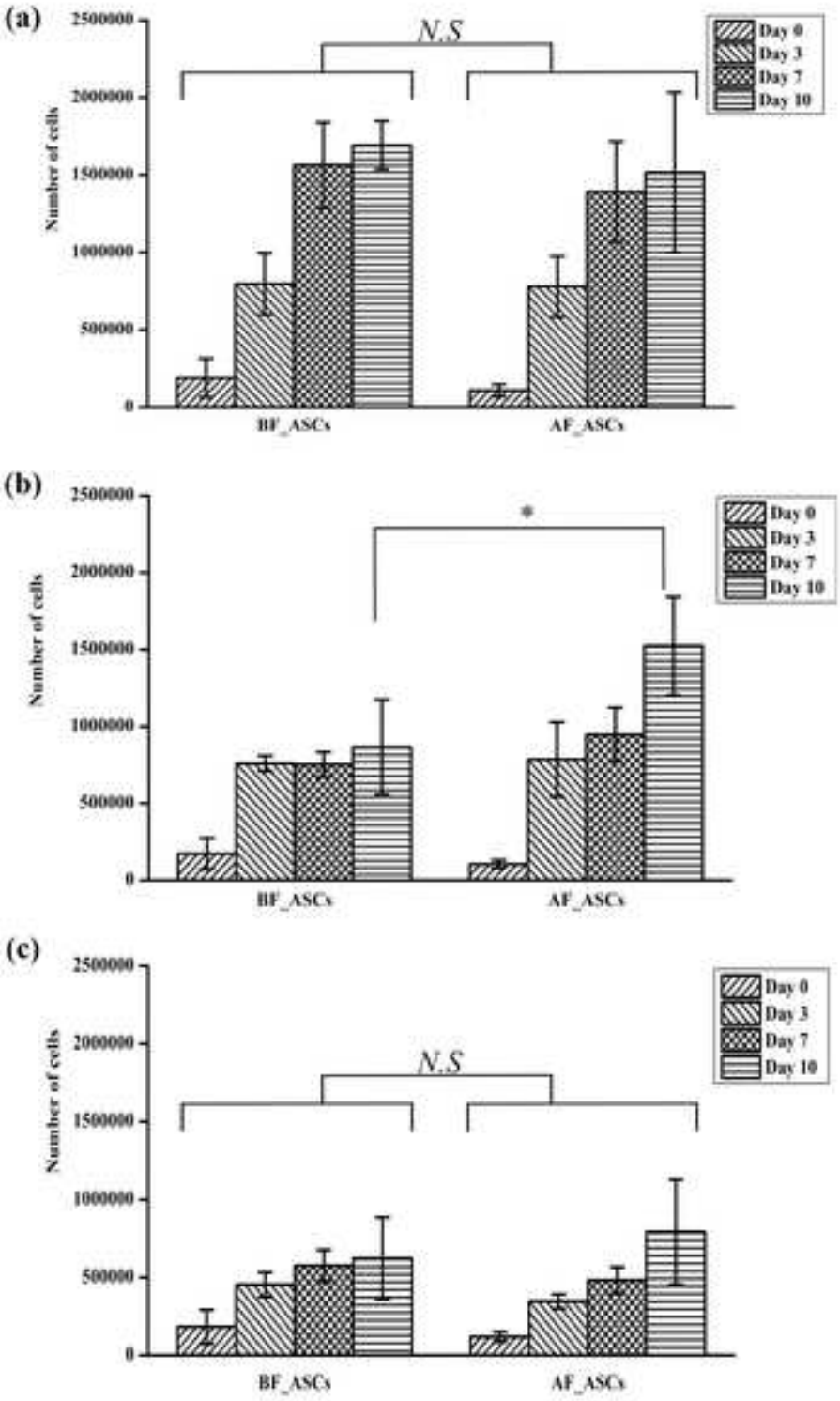

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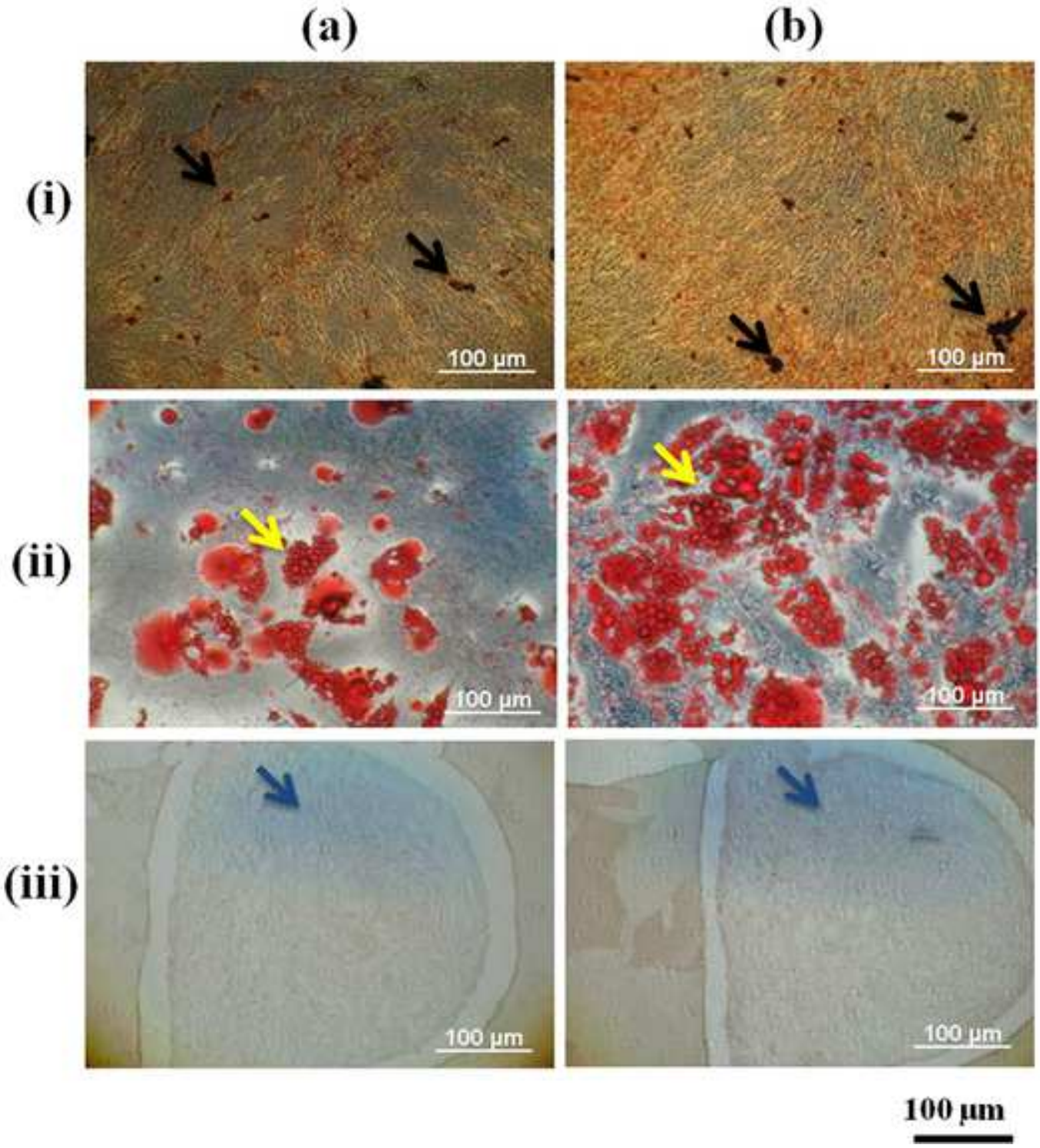

Figure 6

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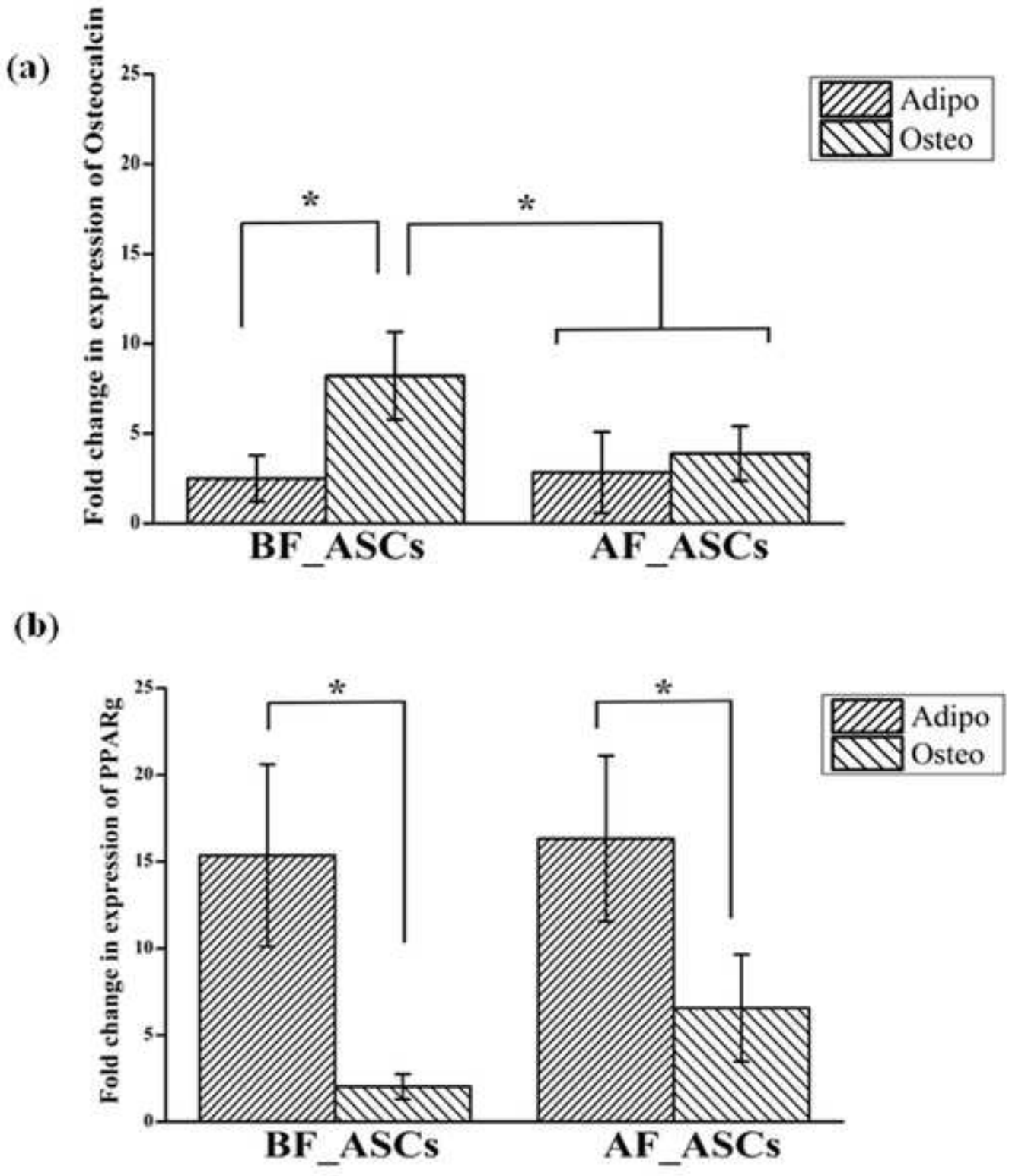

Table 1

	BF_ASCs	AF_ASCs
CD73	98.80±1.24	96.18±3.62
CD105	96.27±3.27	95.16±2.00
CD45	01.29±0.85	00.60±0.11
CD44	99.52±0.52	96.91±1.80
CD29	99.47±0.99	98.00±1.42

Table 2

Gene	Forward Primer	Reverse Primer
Osteocalcin	TCTGACAAAGCCTTCATGTCC	AAATAGTGATACCGTAGATGCG
PPARγ	CTCCTATTGACCCAGAAAGC	GTAGAGCTGAGTCTTCTCAG
Glyceraldehyde 3-phosphate dehydrogenase (GAPDH)	TGGCATATCTCTTATTAAGGGGG	ACTTCTCATCAGCCACCTCG

Supplemental Digital Content Figure 1

Supplemental Digital Content Figure 2