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Biological Properties of Adipose-Derived Stem Cells (ADSCs) and Bone Marrow Stem Cells (BMSCs)

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1. Abstract

1.1. Background: Osseointegration is also a clinal problem especially in patients with systemic diseases. Mesenchymal stem cells provide a new technology to solve this problem. Among them, ADSCs and BMSCs are the most utilized. But which one is better remains a mystery.

1.2. Results: In our research, cell sheet of ADSCs and BMSCs all could enhance the osseointegration. BMSCs improved the osteogenesis rather than ADSCs. while ADSCs improve the proliferation and migration than BMSCs. They all could improve the gene expression of osteogenesis and BMSCs do it better.

1.3. Conclusions: ADSCs and BMSCs all can improve the osseointegration and could be a therapeutic method in implantology.

2. Background

Mesenchymal stem cells (MSCs) are kinds of cells with self- renewal and multi-directional differentiation ability [1, 2]. They could differentiate into osteoblasts, adipocytes, myocytes, tenocytes, and chondrocytes and so no [3-6]. The International Society for Cell Therapy (ISCT) proposed that mesenchymal stem cells must have plasticity, must express surface markers CD73, CD90 and CD105 (≥90%), and do not express hematopoietic markers CD14, CD34 , CD45, CD19, and HLA- DR (≤2%), and are capable of multi-lineage differentiation [7]. Bone marrow mesenchymal stem cells (BMSCs) are extensively used into clinical of bone tissue engineering because of its easily osteogenesis [8, 9]. However, collecting MSCs has limitations due to the possible pain and complications associated with the bone marrow aspiration procedure, and the limited number of MSCs obtained as there are only relatively small numbers (0.001-0.01%) bone marrow cells are bone marrow mesenchymal stem cells [10]. Meanwhile, BMSCs have an early signs of senescence during expansion [11]. Some efforts were made to seek for an alternative stromal stem cell. ADSCS (Adipose-derived stem cells) is a pluripotent stem cell isolated from adipose tissue. It was usually used because of its easily achieved and no ethic [12]. However, which one have an osteogenic efficiency needs more attention.

However, most of the methods for applying and modifying mesenchymal stem cells are based on obtaining allogeneic stem cells, and their clinical applications are limited [13, 14]. In contrast, autologous mesenchymal stem cells have the advantages of clinical availability, long-term survival, and transplantation tolerance, and have broader application prospects. BMSCs and ADSCs have their own characteristics [15].

There has not been much discussion on the comparison of autologous BMSCs and autologous ADSCs [16]. How to choose the most suitable autologous stem cells in tissue regeneration in patients with bone defect has also become a research hotspot. In this study, by comparing the basic biological properties of ASCs and BMSCs, we elucidate their mechanism of action and provide guidance for future research.

3. Methods

3.1. Cell Isolation and Culture

The adipose tissue of groin was taken, cut into pieces then digested with 0.1% collagenase I (Gibco, USA) in an incubator for 1 hour. The solution was centrifuged at 1000rpm, 5min after filtered through 200 mesh screens. The cell pellet was resuspended into a α-MEM complete medium (10% serum [Gibco, USA], 1% Penicillin–Streptomycin Solution [HyClone, USA], 89% basic medium [HyClone, USA])).

The bilateral lower limb bones were obtained in the process of adipose tissues. PBS was used to rinse the marrow cavity repeatedly until it became clear. The suspension was collected, centrifuged at a 1000 r/min for 5 min and resuspension. Cells were cultured in a α-MEM complete medium as mention above.

3.2. Identification of Surface Markers of ADSCs and BMSCs

ADSCs and BMSCs were washed with 1×PBS for 3 times, digested with trypsin and centrifuged at 1000 r/min for 5mins. The cell suspension was split into 1.5 ml centrifuge tubes controlling with 5×10^5–1×10^6 in each tube. Antibodies (CD29, CD90, CD34 and CD45 [CST, USA]) were added into each tube, and the expression level on the cell surface was detected by flow cytometry [BD, USA].

3.3. Cell Proliferation Assay and Migration Assay

5000/well cells were plated into 4 96-well plates and incubated into incubator. 10ul cck-8 solution (corning, USA) were added into each plate then incubated in an incubator for 30 mins. Absorbance value of 450nm was detected after 4 days of operation.

1×10^5/well cells were plated into 6-well plate and incubated into an incubator. A scratch was made with the tip and photographs were taken again after six hours of incubation in the incubator.

3.4. Osteogenetic and Adipogenic Differentiation Induction

$2.5x10^5$/well of ADSCs and BMSCs were plated into 6-well plates with lipogenic induction medium (1 μM dexamethasone, 200 μM Indometacin, 0.5 mM Isobutylmethylxanthine, 10 μg/ml Insulin [MP, USA]). Samples were fixed with polyformaldehyde and stained with oil red O solution (Sigma, USA) after 10 days. The staining results were observed under the microscope. ADSCs and BMSCs were plated at the same density with osteogenic induction medium (10 mM β-glycerophosphate, 50 mg/mL ascorbic acid, and 10−7M dexamethasone [MP, USA]). cells were fixed and then stained with BCIP/NBT dye (CWBIO, China), alizarin red dye (Solaibio, China) for ALP production and ECM mineralization respectively for 7 and 21 days.

3.5. Real-time Quantitative Polymerase Chain Reaction (qPCR)

Total RNA was extracted using Trizol reagent, quantified and reversed into complementary DNA (cDNA) as described. TB Green® Premix Ex Taq™ II kit (Takara, Japan) was used to detect the expression of genes of osteogenesis with GAPDH as the endogenous reference, using the $2^{-\triangle\triangle}$ CT value. The primers sequences are listed in Table 1.

Table 1: Primer sequencing

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
GAPDH	GTACGACTCACTATAGGGA	AGGTGACACTATAGAATA
ALP	CCTTGTAGCCAGGCCCATTG	GGACCATTCCCACGTCTTCAC
Runx2	CCATAACGGTCTTCACAAATCCT	TCTGTCGGTTCTTGGGTTC

4. Results

4.1. Sheet of ADSCs and BMSCs enhanced the osteointegration of SD rats

Results of micro-CT showed that ADSCs sheet improved the osteointegration and BMSCs sheet enhanced the osteointegration compared to normal rats (Figure 1A). Indexes of BV/TV, Tb.Th and TbN were in accordance to the micro-CT results (Figure 1B).

4.2. Sheet of ADSCs and BMSCs improved the cellular morphology of SD rats

Results of HE showed that ADSCs and BMSCs sheet improved the cellular morphology compared with the normal rats (Figure 2A-2C).

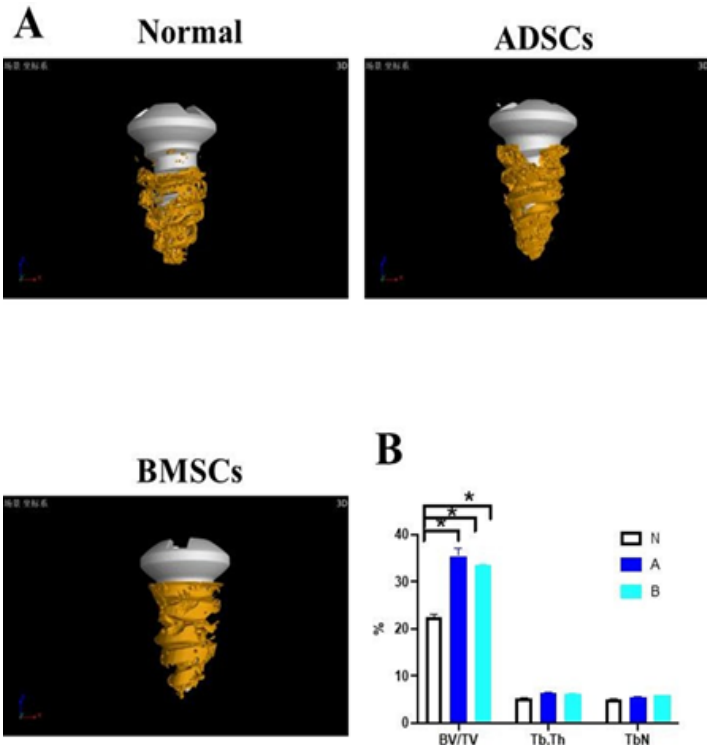

Figure 1: ADSCs and BMSCs promoted the osteointegration
Results of micro-CT showed that ADSCs sheet and BMSCs sheet enhanced the osteointegration of SD rats rather than normal sheet (Fig A), indexes of micro-CT showed that groups of ADSCs sheet and BMSCs sheet upgraded comparing the normal group (Fig B). Normal: implantation site without cell sheet; ADSCs: implantation site with ADSCs cell sheet; BMSCs: implantation site with BMSCs cell sheet.

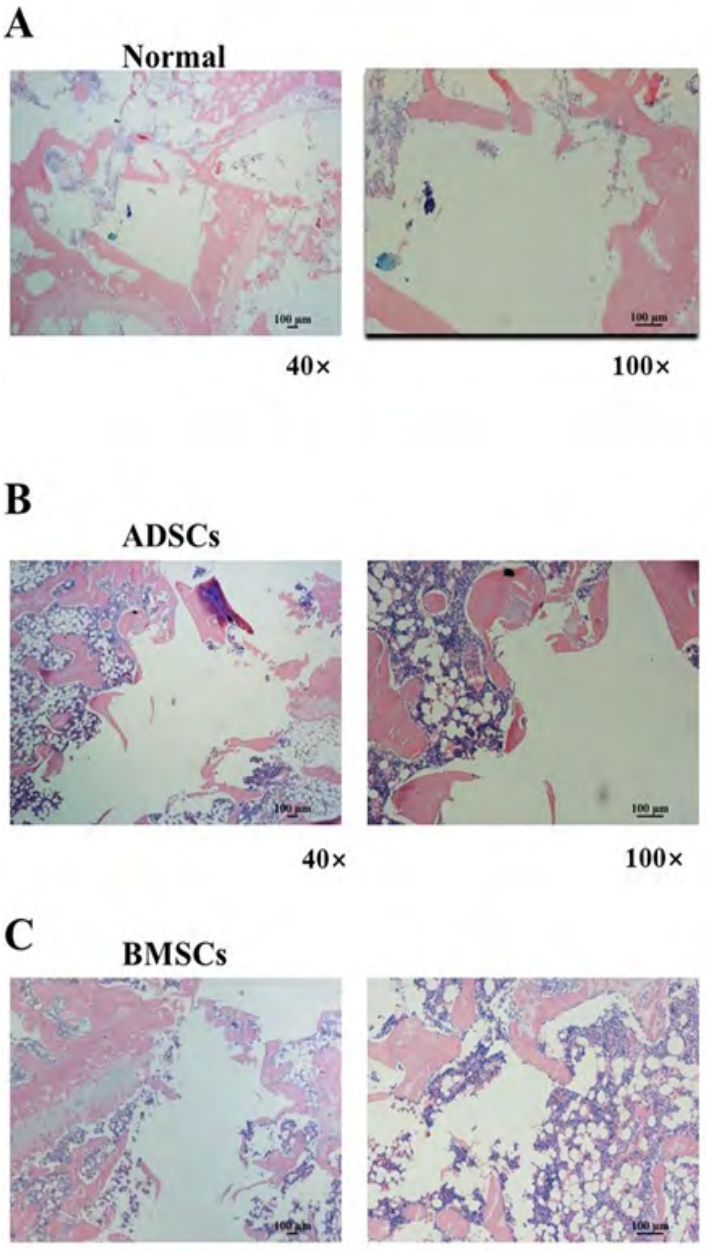

Figure 2: ADSCs and BMSCs improved the cellular state
Results of HE stanning showed that ADSCs and BMSCs sheet improved the cellular state (Fig A- C). Normal: implantation site without cell sheet; ADSCs: implantation site with ADSCs cell sheet; BMSCs: implantation site with BMSCs cell sheet.

4.3. Sheet of ADSCs and BMSCs induced the cartilage formation of SD rats

As showed in Figure 3A-3C, ADSCs and BMSCs sheet induced the cartilage formation compared with the normal rats.

4.4. Identification of ADSCs and BMSCs

For detecting the ADSCs and BMSCs, result of flow cytometry showed that 96.5 percentage of CD29 and 3.8% percentage of CD34. (Figure 4A) ADSCs and BMSCs all in good condition under microscope (Figure 4B-4C).

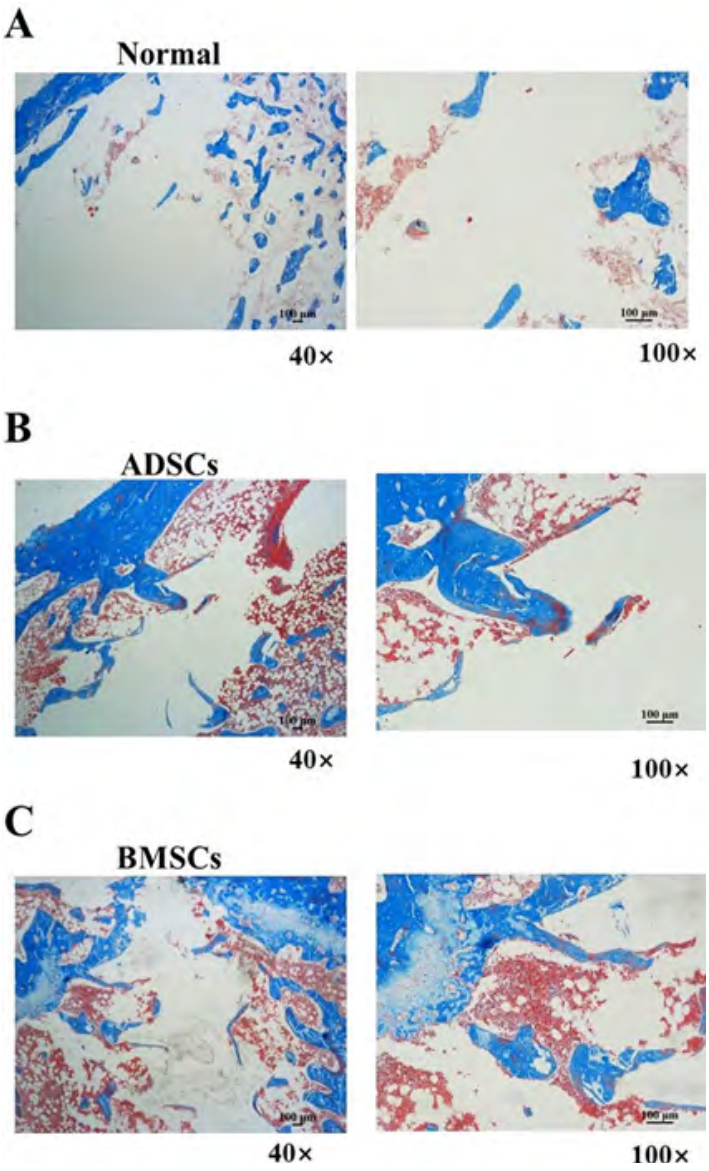

Figure 3: ADSCs and BMSCs induced the new bone formation.
Results of MASSON stanning showed that ADSCs and BMSCs sheet improved the new bone formation between the implant and bone (Fig A-C). Normal: implantation site without cell sheet; ADSCs: implantation site with ADSCs cell sheet; BMSCs: implantation site with BMSCs cell sheet.

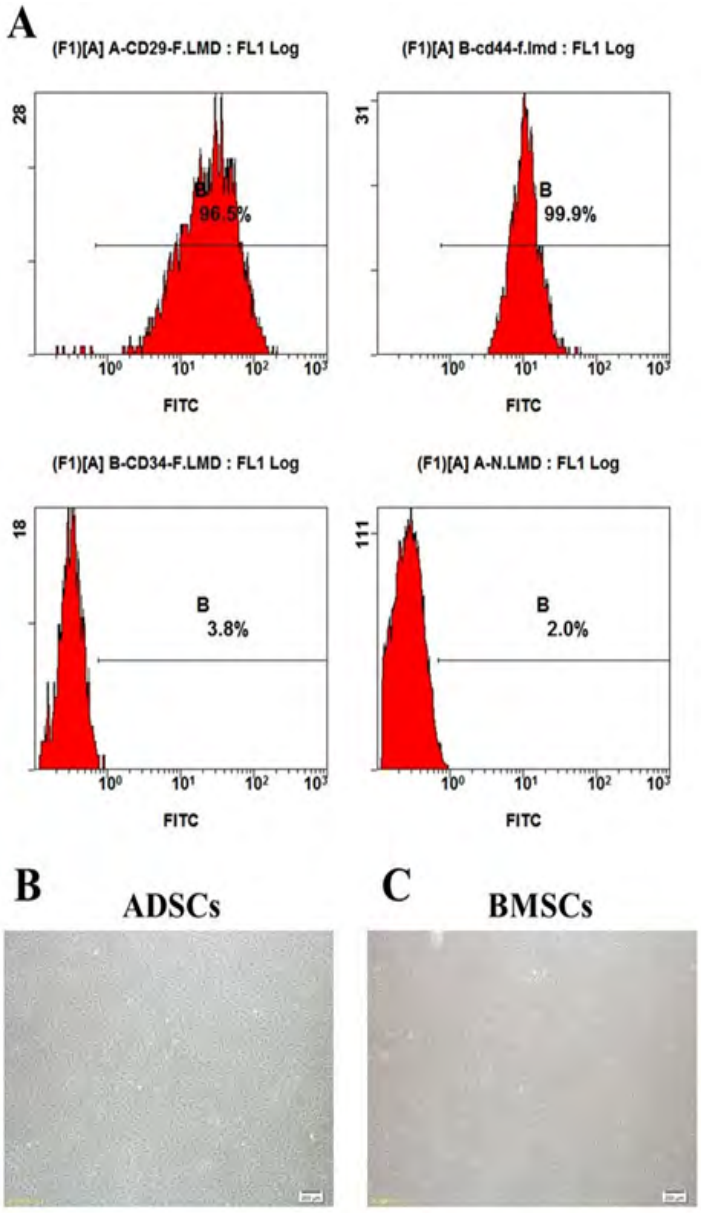

Figure 4: ADSCs and BMSCs had a similar surface marker.
Results of flow cytometry showed that ADSCs and BMSCs are all stem cells and had the similar surface markers such as CD29, CD44 (Fig A). A scheme of ADSCs (Fig B) and BMSCs (Fig C). ADSCs: implantation site with ADSCs cell sheet; BMSCs: implantation site with BMSCs cell sheet.

4.5. The proliferation and migration of ADSCs were higher than BMSCs

CCK-8 results showed that proliferation of ADSCs were higher than BMSCs (Figure 5A). Scratch assay showed migration of ADSCs were higher than BMSCs (Figure 5B).

4.6. The osteogenesis of ADSCs and BMSCs

As shown in Figure 6B, blue purple with BCIP/ NBT dye were stained after osteogenic induction for 7 days. ALP expression in BMSCs was higher than that in ASCs. (Figure 6A) Results of semi- quantitation indicated that BMSCs presented better early osteogenesis (Figure 6B).

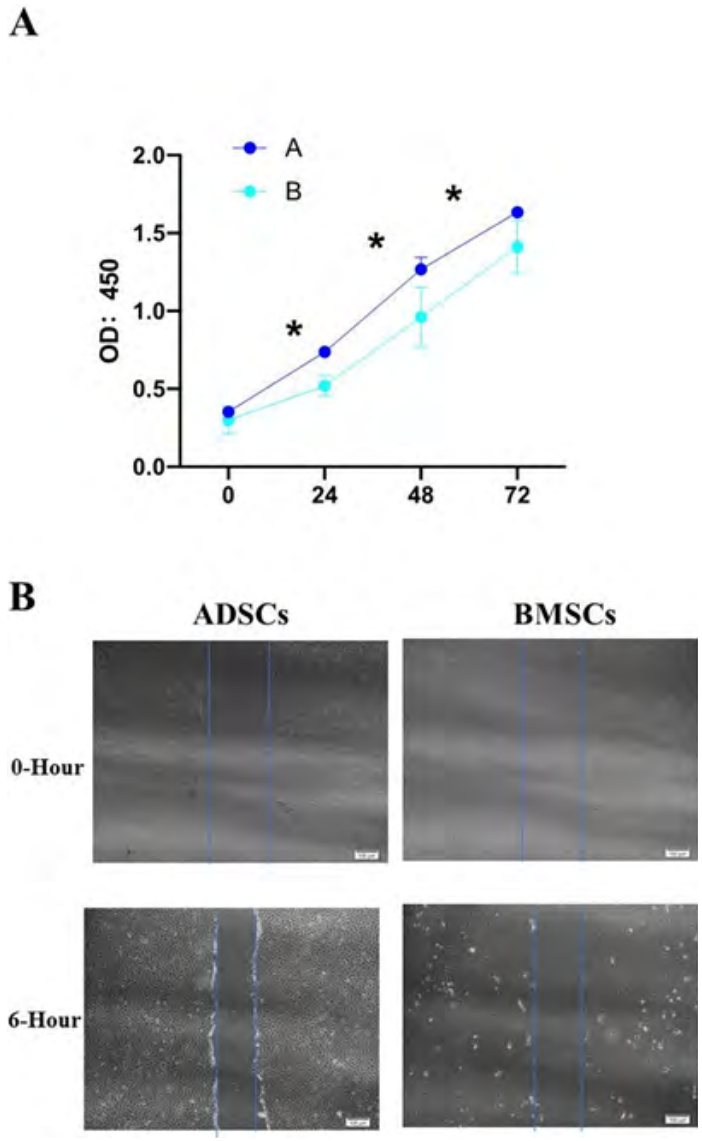

Figure 5: ADSCs improved the proliferation and migration compared the BMSCs. Results of cck-8 showed that ADSCs improved the proliferation (Fig 5A) and scratch assays enhanced the migration (Fig 5B). ADSCs: implantation site with ADSCs cell sheet; BMSCs: implantation site with BMSCs cell sheet. *:p＜0.05.

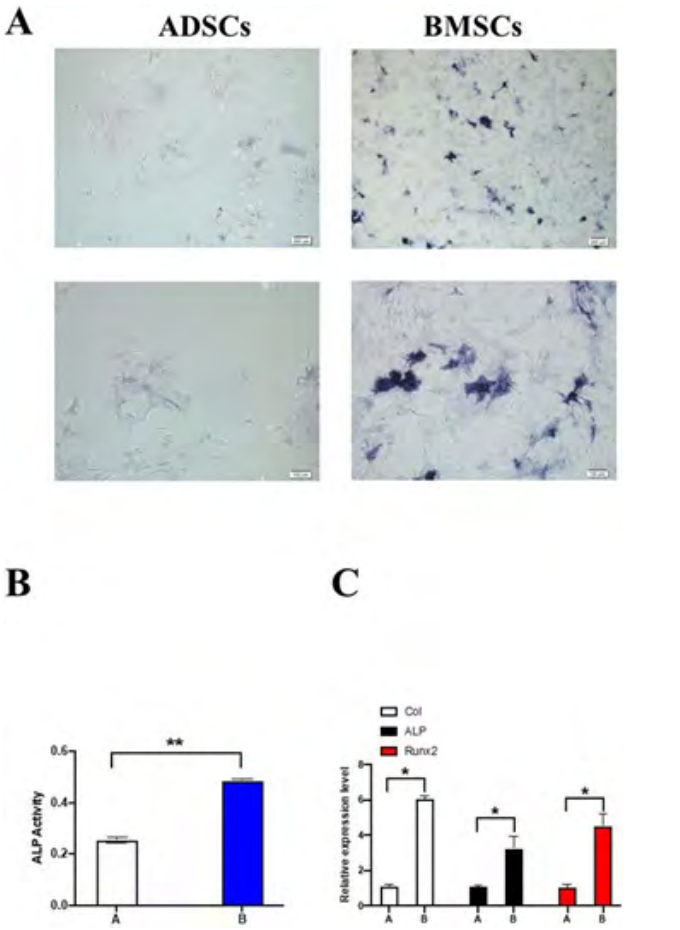

Figure 6: BMSCs induced the osteogenesis rather than ADSCs. Results of ALP stanning showed that BMSCs induced the osteogenesis compared the ADSCS (Fig 6A-6B); qPCR showed that BMSCs and ADSCs improved the expression of ALP, Runx2 and COL1 (Figure 6C). ADSCs: implantation site with ADSCs cell sheet; BMSCs: implantation site with BMSCs cell sheet. *:p＜0.05, **:p＜ 0.01.

4.7. Expression of osteogenic-genes and protein

The expression of mRNA of ALP and Runx2 in BMSCs group were higher than in ADCSs.

5. Discussion

Bone defect is a major clinal problem in oral implantology [17]. In recent years, stem cell has been a promising outcome to improve the osteointegration [18, 19]. Because of long time transplant survival, autologous stem cells become more prevalent [20]. ADSCs and BMSCs are most commonly utilized and achieve widely clinical outcomes among them [21, 22]. However, none effort on the comparison of ADSCs and BMSCs and choose the better one for tissue regeneration.

We extracted the ADSCs and BMSCs from the same rat and compare them. But, if we abide by the request of autologous stem cell that needs early extraction, intermediate detection and later implantation, the difficulty to activate and the death risk of models

are sophisticated. So, we extracted ADSCs and BMSCs from allogeneic rats to clarify their multi-lineage differentiation potential. Variety of methods were employed to explore cell viability and differentiation potential. Among them, cell proliferation is closely related to the therapeutic effect of mesenchymal stem cells on wound closure. The results showed that both cells had self-renewal ability and maintained good proliferation activity within a certain passage. To some extent, ADSCs had a better proliferation potential., which was consistent with a study by Dmitrieva et al with its better adapt to different environment and easily expansion [23]. Cell-sheet could improve the therapeutic effect including stem cells, and it is a technology to concentrate cells, retain intercellular proteins, and ion channels. Yu et al. found that the composite implants BMSCs sheets with implants could meaningfully improve bone formation [21].

The best way to evaluate multidirectional differentiation of stem cell either into osteoblast or adipocytes. ALP and alizarin red stanning were to evaluate early and late osteogenesis of ADSCs and BMSCs. Our results showed that BMSCs had a more noteworthy osteogenesis. In vivo, there has no differences in osseointegration. Although its proliferative ability was poor in early experiments, its excellent osteogenic ability bridged the gap, which may be related to its more pronounced mineralization ability to the matrix. ADSCs is easy availability and cause little pain for patients. Explicitly, improving proliferation could be considered as a relevant strategy to activate the stem cells [24]. Whether BMSCs or ADSCs can be applied directly or modified to increase proliferation or osteogenesis. To be honest, our study only discussed the proliferation and osteogenesis, we should explore its chondrogenesis or wound healing. However, the available data suggested that the specific and dominant nature of MSCs should be considered in the selection and development of MSC-related therapies for optimal matching and improved clinical outcomes

6. Conclusions

Compared with ASCs, BMSCs have better osteogenesis in improving osseointegration. In contrast, ADSCs have more prominent proliferative and osteogenic abilities and have advantages in other aspects as well. This requires us to select or modify stem cells according to the actual situation and their respective characteristics to attain the best therapeutic effect.

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