Current evidence on potential of adipose derived stem cells to enhance bone regeneration and future projection

Quang Le, Vedavathi Madhu, Joseph M Hart, Charles R Farber, Eli R Zunder, Abhijit S Dighe, Quanjun Cui

ORCID number: Quang Le 0000-0002-5124-2398; Vedavathi Madhu 0000-0002-8508-7891; Joseph M Hart 0000-0002-0410-5112; Charles R Farber 0000-0002-6748-4711; Eli R Zunder 0000-0002-0356-1685; Abhijit S Dighe 0000-0003-4234-2618; Quanjun Cui 0000-0003-4285-4488.

Author contributions: Dighe AS and Cui Q contributed conception and designed the research; Le Q, Madhu V and Dighe AS contributed literature search, preparation of the first draft, tables and figures; Le Q, Hart JM and Cui Q contributed clinical trials database search and review; Dighe AS, Farber CR, Zunder ER and Cui Q contributed preparation of seminal draft after reviewing the first draft; all authors wrote, read and approved the final manuscript.

Conflict-of-interest statement: The authors declare no conflict of interest for this article.

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works

Abstract
Injuries to the postnatal skeleton are naturally repaired through successive steps involving specific cell types in a process collectively termed “bone regeneration”. Although complex, bone regeneration occurs through a series of well-orchestrated stages wherein endogenous bone stem cells play a central role. In most situations, bone regeneration is successful; however, there are instances when it fails and creates non-healing injuries or fracture nonunion requiring surgical or therapeutic interventions. Transplantation of adult or mesenchymal stem cells (MSCs) defined by the International Society for Cell and Gene Therapy (ISCT) as CD105+CD90+CD73+CD45-CD34-CD14orCD11b-CD79αorCD19-HLA-DR- is being investigated as an attractive therapy for bone regeneration throughout the world. MSCs isolated from adipose tissue, adipose-derived stem cells (ADSCs), are gaining increasing attention since this is the most abundant source of adult stem cells and the isolation process for ADSCs is straightforward. Currently, there is not a single Food and Drug Administration (FDA) approved ADSCs product for bone regeneration. Although the safety of ADSCs is established from their usage in numerous clinical trials, the bone-forming potential of ADSCs and MSCs, in general, is highly controversial. Growing evidence suggests that the ISCT defined phenotype may not represent bona fide osteoprogenitors. Transplantation of both ADSCs and the CD105- sub-population of ADSCs has been reported to induce
INTRODUCTION

Of the 7.9 million fractures sustained each year in the United States, 5% to 20% result in non-union or delayed healings[1,2]. Since these fractures do not heal naturally, they require therapeutic interventions. Transplantation of multipotent stem cells, reported present in practically all postnatal tissues, is an attractive therapeutic option. Mesenchymal stem cells (MSCs) isolated from bone marrow [bone marrow-derived MSCs (BMMSCs)] are thought to be true, gold-standard osteoprogenitors[3].

To streamline investigations on MSCs, the International Society for Cell and Gene Therapy (ISCT) defined MSCs in 2006 as cells satisfying the following three criteria: Plastic adherent, CD105+CD90+CD73+CD45-CD34-CD14orCD11b-CD79aorCD19-HLA-DR-, and possessing the ability to differentiate into osteoblasts, adipocytes, and chondroblasts in vitro[4-6]. This remains the current definition of adult stem cells or MSCs. This school of thought suggests that MSCs exist in all adult tissues and can give rise to osteoblasts, chondrocytes, marrow stromal cells, and adipocytes. Accordingly, the U.S. Food and Drug Administration (FDA) revealed that 73% of Investigational New Drug applications for stem cell-based products rely on CD105 expression as the “positive” marker for adult stem cells.

A concerted effort involving the scientific community, clinicians, industries, and regulatory bodies to redefine ADSCs using powerful selection markers and strategies to modulate signaling pathways of ADSCs will speed up the therapeutic use of ADSCs for bone regeneration.

Key Words: Mesenchymal stem cells; Adipose-derived stem cells; Endogenous stem cells; Skeletal stem cells; Bone regeneration

©The Author(s) 2021. Published by Baishideng Publishing Group Inc. All rights reserved.

Citation: Le Q, Madhu V, Hart JM, Farber CR, Zunder ER, Dighe AS, Cui Q. Current evidence on potential of adipose derived stem cells to enhance bone regeneration and future projection. World J Stem Cells 2021; 13(9): 1248-1277
URL: https://www.wjgnet.com/1948-0210/full/v13/i9/1248.htm
DOI: https://dx.doi.org/10.4252/wjsc.v13.i9.1248
REGULATORY ASPECTS OF STEM CELLS THERAPY

Although there is general agreement in the scientific community that stem cell therapy holds great promise for bone repair and regenerative medicine applications, there is not much agreement on the definition of adult stem cells. Moreover, several leading experts in the field warn that the existing stem cell-based products are manufactured without vigorous testing and are not backed up by strong scientific evidence. An article titled “Clear up this stem-cell mess” published recently in *Nature* states that the confusion about MSCs is making it easier for industries to sell unproven treatments [26]. In agreement with this observation, another article in *Cell Stem Cell* comments that clinical trials using MSCs have been conducted for more than a generation, but the outcomes have fallen short of expectations[27].

A thorough understanding of the FDA guidelines is necessary for orthopedic surgeons to decide whether the stem cell-based products that they are using or being asked to use by industries are authenticated by the regulatory bodies. It is also necessary to clarify that the FDA guidelines do not establish legally enforceable responsibilities, but they describe FDA’s current thinking and therefore should be viewed only as recommendations unless specific regulatory or statutory requirements are cited. This puts a greater responsibility on clinicians and scientists to make sure that the general public is aware of the effectiveness of stem cell therapy, and more importantly, the patients receiving stem cell therapy are aware of the risk to benefit ratio.

The current guidance issued by FDA is available under the docket number FDA-2017-D-6146 (https://www.fda.gov/media/109176/download). Adult stem cell-based products are regulated by the Center for Biologics Evaluation and Research, similar to human cells, tissues, and cellular- and tissue-based products (HCT/Ps). These regulations are provided by the FDA to HCT/P manufacturers, healthcare providers, and FDA staff, under Title 21 of the Code of Federal Regulations (CFR) Part 1271. These regulations explain the types of HCT/Ps that do not require premarket approval; and the registration, manufacturing, and reporting steps that must be taken to prevent the introduction, transmission, and spread of communicable disease by these HCT/Ps: (1) The product is minimally manipulated; (2) It is intended for homologous use and this is reflected by the labeling, advertising, and the manufacturer’s objective intent; (3) The manufacture of the HCT/P does not involve the combination of the cells or tissues with another article (except for water, crystalloids, or a sterilizing, preserving, or storage agent, provided that these agents are safe); (4) The product is not dependent upon the metabolic activity of living cells for its primary function; and (5) If the product is dependent upon the metabolic activity of living cells or has a systemic effect then it must be only for autologous use.
cells isolated from the person transplanted back into the same person) or allogeneic use in a first-degree or second-degree blood relative or for reproductive use.

In a cautionary observation, Skovrlj et al.\[28\] reported that all five commercially available cellular bone matrices for spine fusion, Osteocel Plus (NuVasive, San Diego, CA, United States), Trinity Evolution (Orthofix, Lewisville, TX, United States), Cellentra Viable Cell Bone Matrix (Biomet, Warsaw, IN, United States), AlloStem (AlloSource, Centennial, CO, United States), and Ovation (Osiris Therapeutics, Columbia, MD, United States), contain live, allogeneic MSCs but claim to meet the FDA criteria under Section 361, 21 CFR Part 1271, and have not undergone FDA premarket review. All of these products are composed of MSCs derived from freshly procured cadaveric bone marrow, cadaveric adipose tissue, or chorion layer of the placenta.

It is important to take notice of the fact that there is no stem cells-based product currently approved by the FDA that can be used for bone tissue engineering purposes or for the treatment of bone diseases. The list of all cell and gene therapy products approved by the FDA can be found on FDA’s website: https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/approved-cellular-and-gene-therapy-products. Thus, detailed investigation on bone-forming potential of stem cells in vitro and in vivo followed by non-industry sponsored clinical studies evaluating the efficacy of stem cells are required. Since ADSCs can be isolated in a non-invasive procedure, in abundant numbers, for autologous use, they offer a promising option for stem cell-based bone repair therapies.

THE CLINICAL TRIALS USING ADSCS

To elucidate the possible clinical benefits of using ADSCs, many clinical trials have been initiated. The clinical trials that will be used in our analysis were acquired from Clinicaltrials.gov in December 2020 using the combination of keywords “Orthopedic Disorder (Condition) - Adipose Stem Cells (Other terms)” and “Bone (Condition) - Adipose-Derived Stem Cells (Other terms).” The first combination of keywords returned 74 registered trials while the second combination returned 17 trials. Following content review, we eliminated any overlapping trials, trials that did not directly involve orthopedic conditions, trials that did not include human patients, and trials that did not explicitly state the use of ADSCs. This resulted in 70 trials being included in this analysis. We found only four trials that addressed bone healing or bone regeneration using ADSCs: NCT02140528, NCT04340284, NCT03678467, and NCT03678467. While NCT03678467 is an ongoing trial, the results of the other three are not published. We, therefore, searched PUBMED using the keywords “adipose stem cells”, “orthopedic”, and “clinical” with the filter “Clinical Study” and found 10 relevant articles[29-38]. The general distribution of the 80 included clinical trials can be seen in Figure 1. The outcomes of clinical trials on bone regeneration are summarized in Table 1.

From the number of trials, it is clear that there is tremendous interest in ADSCs as a therapeutic tool for a variety of orthopedic disorders. The earliest trials were started in 2008. However, the number of initiated trials has been on an upward trend since this time. Moreover, only 37% of trials indicated as completed (total = 29). We will be seeing a large number of trials ending in 2021 (total = 17), which will have important implications for the field. The majority of the trials are in Phase 1 or 2, evaluating the safety and initial efficacy of treatment with ADSC. Only 6 trials (8%) are in phase 3 and one is in phase 4. Of the 29 completed trials, 19 corresponding publications could be found on PubMed using the National Clinical Trial registration number. Of these, we will review in detail 10 publications directly investigating bone regeneration using ADSCs.

SAFETY OF ADSCS ESTABLISHED IN CLINICAL TRIALS

In 2013, Pak et al.\[29\] published the outcomes of long term follow up of 91 patients undergoing injections of autologous ADSCs with platelet-rich plasma in various joints to evaluate the safety of this treatment modality. Participants were observed for an average of 16 mo. During this time, magnetic resonance imaging (MRI) evaluation showed no evidence of neoplasm. Common adverse events included swelling of injected joints, tenosynovitis, and tendonitis, all of which were either successfully
| Bone defect treated                                                                 | Study duration and length of follow up | n  | Intervention                                                                                           | ADSCs source                                      | ADSCs number | Outcome                                                                 | Ref.                                     |
|-----------------------------------------------------------------------------------|---------------------------------------|----|--------------------------------------------------------------------------------------------------------|-------------------------------------------------|--------------|----------------------------------------------------------------------|------------------------------------------|
| Avascular necrosis of hip, osteoarthritis of hip/knee/ankle, spinal disc herniation | 2009-2012, 30 mo                      | 91 | Intraarticular injection of SVF with PRP                                                              | Autologous SVF from abdominal tumescent liposuction | 10 mL of SVF | No evidence of neoplasm, no serious adverse events, common adverse events (swelling of injected joints, tenosynovitis, and tendonitis) were either successfully managed or self-limited, established safety of ADSCs | Pak et al[29]                            |
| Upper arm fracture in elderly patients (62-84 yr)                                  | 2012-2014, 6 mo                       | 8  | SVF seeded porous silicated-hydroxyapatite microgranules with fibrin hydrogel implant                  | Autologous SVF from abdominal tumescent liposuction | 800 microliters of SVF | Evidence of osteogenesis at graft site; circumstantial evidence for direct contribution of SVF cells to fracture healing | Saxer et al[30]                          |
| Large cranial defect                                                               | 2008-2010, 12 mo                      | 4  | ADSCs-seeded β-tricalcium phosphate implant                                                          | Autologous ADSC from abdominal subcutaneous liposuction | 15 × 10⁶ cells | Noted equivalence between newly generated tissue and native bone     | Thesleff et al[31]                      |
| Large cranial defect                                                               | 2008-2016, approximately 7 yr         | 5  | ADSCs-seeded β-tricalcium phosphate implant                                                          | Autologous ADSC from abdominal subcutaneous liposuction | 15 × 10⁶ cells | This study was long term follow up of Thesleff et al[31]; unsatisfactory long-term outcome with significant resorption | Thesleff et al[32]                      |
| Cranio-maxillofacial hard-tissue defects                                            | 2012-2014, up to 52 mo               | 13 | ADSCs-seeded bioactive glass or β-tricalcium phosphate scaffolds, at times with recombinant hBMP-2 | Autologous ADSC from anterior abdominal wall liposuction | Up to 160 × 10⁶ cells | Majority of patients achieved satisfactory clinical and radiographic results; three experienced significant resorptions of the ADSCs graft | Sándor et al[33]                         |
| Long bone nonunion from bone tumor resection or pseudoarthrothesis                 | 2012-2014, 39 mo                      | 6  | ADSCs seeded decellularized bone matrix                                                              | Subcutaneous autologous ADSCs                      | Up to 200 × 10⁶ cells | 50% of the patients achieved bone regeneration and union              | Dufrane et al[34]                       |
| Maxillary sinus floor elevation                                                    | 2009-2015, 36 mo                      | 10 | SVF seeded β- tricalcium phosphate implant                                                            | Autologous SVF from abdominal tumescent lipo-aspiration | 20 × 10⁶ cells | Experimental group exhibited significantly more bone healing compared to control | Prins et al[35]                         |
| Alveolar cleft osteoplasty                                                          | 2015-2016, 6 mo                       | 10 | Lateral ramus cortical bone plate with ADSCs-mounted natural bovine bone mineral                      | Autologous ADSCs from buccal fat pad              | 1.0 × 10⁶      | No significant different in bone regeneration found between experimental group and controls | Khojasteh et al[36]                     |
| Mandibular fracture                                                                | 2010-2015, 12 wk                      | 20 | Direct application of ADSCs                                                                         | Autologous ADSCs                                  | Unreported    | Significantly more osteogenesis in ADSCs-treated group compared to control | Castillo-Cardiel et al[37]              |
| Nonunion following subtalar arthrodesis                                             | 2010-2016, 24 mo                      | 140| ADSC-seeded partially demineralized bone matrix                                                     | Allograft ADSCs                                   | Unreported    | Inferior bone union rate in ADSCs treated group compared to autograft; equivalent clinical evaluations | Myerson et al[38]                       |

ADSCs: Adipose-derived stem cells; SVF: Stromal Vascular Fraction; PRP: Platelet-rich plasma.

managed or self-limited. Evaluation of pain using VAS suggested that most patients experienced a significant reduction in pain three months post-operation.
Figure 1 The clinical trials utilizing adipose-derived stem cells. The data were retrieved from clinical trials databases Clinicaltrials.gov and PUBMED. A: Distribution of adipose-derived stem cells (ADSCs) clinical trials’ start and end dates. Clinical trials on bone regeneration with both identified start and end date are represented in blue. All others are represented in orange; B: Distribution of ADSCs clinical trials’ status; C: Distribution of ADSCs clinical trials’ phase; D: Distribution of ADSCs clinical trials’ targeted condition.

OUTCOMES OF CLINICAL TRIALS USING ADSCS FOR BONE REGENERATION

Saxer et al[30] published in 2016 the results of a study investigating the safety and feasibility of a stromal vascular fraction (SVF) (construct in the treatment of proximal humeral fractures in elderly patients. The construct was made from unexpanded and undifferentiated human SVF derived from abdominal adipose tissue seeded onto a silicated-hydroxyapatite and fibrin hydrogel scaffold. The construct was tested first on male nude rats' 6 mm critical-sized femoral defects. Compared to cell-free control, the SVF-seeded construct was associated with significantly better mineralization and bone volume. Histological staining also confirmed de novo osteogenesis and angiogenesis in SVF-treated rats. The construct was subsequently tested on 8 patients aged 62-84 with displaced, low-energy, proximal humerus fractures who were followed prospectively for up to 12 mo post-surgery. The study confirmed that it was feasible for liposuction, SVF isolation, graft manufacturing, and implantation to all be completed intraoperatively. The implant was deposited into the void space created following open reduction and internal fixation. Over the follow-up period, the authors reported no adverse reaction that could be linked to the graft. Pain evaluation via VAS showed no lasting donor site pain and generally diminished operation site pain. Within one year of the surgery, five out of eight patients had their plates removed, which provided the opportunity for biopsy. The other three patients achieved subjective therapeutic goals and declined plate removal. Histological and micro-CT analysis confirmed osteogenesis at the graft site, either directly connected to or separated from the pre-existing bone. Bone ossicles were also found in scaffold pores. The authors considered these findings as circumstantial evidence for the direct contribution of SVF cells to fracture healing.

In 2011, Thesleff et al[31] presented the results of treatment of 4 patients with critical-size calvarial defects that underwent cranioplasty using grafts of ADSCs seeded on beta-tricalcium phosphate granules. ADSCs were obtained autologously from participants' subcutaneous abdominal fat, isolated, and expanded over three weeks. Participants were evaluated over a one-year follow-up period with computed tomography (CT) scans, which showed ossification. Hounsfield Unit measurements with CT scans showed approximate equivalence between normal bone and regenerated tissue. No serious adverse event was reported. In 2017, the same research group released the results of a 6-year follow-up on the same cohort of patients plus
one more[32]. Unfortunately, the authors determined that the long-term outcomes of ADSCs beta-TCP grafts for cranioplasty remained unsatisfactory. Among the five patients who underwent the procedure, four needed revision surgeries at 0.9, 2.0, 2.2, and 7.3 years following the original operation. Indications for re-operation included infect, partial resorption of graft, complete resorption, and meningioma recurrence. The grafts were then either removed and replaced with titanium, strengthened with titanium mesh, or the patient underwent craniotomy in the case of meningioma. Only one patient retained the original graft at the time of publication, but her skull X-ray did show some level of graft resorption.

Sándor et al[33] in 2014 reported a case series involving 13 patients with cranio-maxillofacial bone defects, three of the frontal sinuses, five of the cranial bones, three of the mandibles, and two of the nasal septa. ADSCs from participants were harvested from abdominal subcutaneous fat, expanded, and seeded on either bioactive glass or beta-tricalcium phosphate scaffolds. In the three mandible cases, rhBMP-2 was also added. Follow-up periods ranged between 12 and 52 mo and showed satisfactory clinical and radiographic results for patients with mandibular, frontal sinus, nasal septum defects. Of the participants with cranial defects, two achieved clinically and radiographically satisfactory ossifications, while the other three experienced significant resorptions of the ADSC graft. One of the nasal septum defect patients resumed habitual nasal picking during follow-up and needed graft removal.

Dufrane et al[34] published a study in 2015 describing the treatment of 6 patients with long bone nonunion resulting from either bone malignancy or pseudoarthrosis. These patients were treated using 3D bone grafts produced from subcutaneous ADSCs, incubated in osteogenic media, and delivered using demineralized bone matrix (DBM) without scaffolding. During the follow-up period of 47 mo, no acute adverse events or tumorigenicity were reported, but there were two instances of infection. Three out of six patients achieved bone regeneration and union.

Prins et al[35] published a study in 2016 evaluating the use of autologous SVF, rich in ADSCs, seeded in calcium phosphate ceramics for maxillary sinus floor elevation. SVF was obtained from the participants’ abdominal wall. A total of 10 participants received either bilateral implants, with one side being SVF with ceramics and one side being ceramics control or a unilateral implant of just SVF with ceramics. Follow-up over three years showed no serious adverse event. Follow-up biopsy and micro-CT showed active bone formation in the study arm with statistical differences in bone volume over control, most notably in SVF with β-tricalcium phosphate ceramics group.

In 2017, Khojasteh et al[36] published a phase I clinical trial involving 7 patients with alveolar clefts treated with autogenous bone osteoplasty in combination with buccal fat pad derived ADSCs. Patients were divided into three treatment arms: Anterior iliac crest (AIC) spongy bone with a collagen membrane, lateral ramus cortical bone plate with ADSCs, and AIC spongy bone with ADSCs and collagen membrane. Results indicated bone generation in all three experimental arms, weakest in the AIC only group and strongest in the AIC with ADSCs group. However, the differences were not statistically significant. No serious adverse event was reported.

Castillo-Cardiel et al[37] published in 2017 the results of a single-blind, randomized, clinical trial involving 20 patients with mandibular angle fractures. Participants were separated into two groups, a control group receiving fracture reduction only and a stem cell treatment group receiving fracture reduction with application of ADSCs as well. ADSCs were obtained from abdominal fat 24 h prior to the mandibular procedure. Evaluation of bone regeneration over 12 wk showed statistically significant improvement in ossification in the ADSC group compared to control.

In 2019, Myerson et al[38] published a multicenter, randomized controlled study to compare safety and efficacy of ADSCs in subtalar arthrodesis (bone fusion of the subtalar joint involving ankle bone and heel bone) with classic bone autograft. This study included 140 patients enrolled in two study arms receiving either autologous bone grafts or ADSCs. Autologous bone grafts were obtained from either the iliac crest or the distal tibia. ADSCs were obtained autologously and deposited on partially demineralized cancellous bone. Patients were followed up for over two years using clinical scores such as AOFAS, SF-12, and FFI-R as well as radiographic evaluation for the fusion of the subtalar joint. Imaging showed a lower rate of fusion in the ADSCs group compared to autograft control. Nonetheless, both groups showed equivalent clinical evaluations.

There are three clinical trials registered at Clinicaltrials.gov with no published outcomes. NCT02140528 sought to evaluate the safety and efficacy of the injection of allogeneic ADSCs on the healing of tibial fractures in 40 patients. Patients were separated into two groups receiving either ADSCs injections or placebo. NCT04340284...
is a retrospective report on the outcomes of 11 patients receiving fluoroscopic guided percutaneous injections of SVF to the site of long bone nonunion. Healing was evaluated over 12 mo using SF-12 and radiographic imaging. ADSCs were also considered for Spinal Cord Injury, which was investigated in trial NCT02981576. This study enrolled 14 participants separated into two arms receiving three intrathecal injections of either autologous ADSCs or autologous bone marrow-derived MSCs. Follow-up was done over 12 mo using ASIA impairment score as well as MRI imaging.

Finally, clinical trial NCT03678467 is an open-label trial using an autologous, anatomically shaped bone graft made from patients’ own ADSCs specifically for patients’ mandible injury or deformity. The main goal of the study is to assess the safety of the treatment. Six patients will be followed over 12 mo on the number of adverse events, quality of life, and bone regeneration with CT scans.

In summary, a total of ten different studies conducted on total of 307 patients suggest that the use of ADSCs is safe, but there is limited evidence that ADSCs can significantly enhance bone regeneration.

RESERVATIONS ABOUT USING ADSCS FOR BONE REGENERATION

Considering the abundant availability of ADSCs and ease of their isolation, several investigators have attempted to use ADSCs to enhance bone regeneration. These studies were conducted using conventional preparations of ADSCs satisfying the ISCT definition of adult stem cells or MSCs as CD105+/CD90+CD73+CD45-/CD34-/CD14orCD11b-CD79orCD19-HLA-DR- cells. These studies suggested a limited ability of ADSCs to induce bone formation or to enhance bone repair and raised serious doubts about their therapeutic utility. The outcomes of the investigations are summarized in this section.

Primary ADSCs failed to enhance bone healing, in defects created in rat calvaria and sheep tibia[39,40]. In a canine maxillary alveolar cleft model, autografts induced significantly higher bone formation than ADSCs-seeded on hydroxyapatite/beta-tricalcium phosphate scaffolds[41]. Godoy Zanicotti et al[42] used titanium surface as the scaffold for delivery of ovine ADSCs to repair sheep femur epicondylic defects. Histology and histomorphometry were used to evaluate the implants one month after surgery. Using PKH26 cell-tracking dye, the authors were able to confirm the persistence of ADSCs in the defect area at one month. Unfortunately, based on histomorphometry results, no significant difference in regenerated bone tissue was found among all experimental and control groups.

When human ADSCs (hADSCs) were implanted in immunodeficient animals, they failed to induce any ectopic bone formation in 8 wk[43-46]. Spheroids of human bone marrow-derived MSCs, but not hADSCs, could consistently induce ectopic bone formation in immunodeficient mice[47]. Surprisingly, hADSCs did not survive in the calvarial defects of nude mice after two weeks, although the recipient mice lacked T cells[48]. While the theory of paracrine factors released by hADSCs in these two weeks being sufficient for bone regeneration awaits more investigation, these data raise questions about the usefulness of ADSCs for bone regeneration in healthy (immunocompetent) recipients. Corroborating this notion, ADSCs could not enhance calvarial defect healing in immunocompetent rats[39].

Attempts by other investigators to improve the bone-forming ability of hADSCs, by the addition of BMP-2 have also failed in a femoral defect model in T-cell deficient nude rats[49]. This was proposed to be the consequence of the failure of hADSCs to respond to BMP-2 in vitro[50]. In agreement with this finding Runyan et al[51] found that recombinant human BMP-2 formed more bone than autologous ADSCs and recombinant human BMP-2 in combination in a porcine model of the periosteal envelope. Keibl et al[52] tested a fibrin scaffold embedded with ADSCs and BMP-2 in the treatment of a non-critical size rat femur defect model. At two- and four weeks post-treatment, the authors found no major difference among the groups indicating no effect of BMP-2 on ADSCs potential and ADSCs alone could not induce any bone repair. This questions the ability of ADSCs to induce bone formation and also their ability to respond to BMP-2. Interestingly, this problem could be overcome by overexpressing BMP-2 and BMP-7 both in ADSCs. Qing et al[53] reported that only the BMP-2/BMP-7 transduced ADSCs, but not non-transduced ADSCs, BMP-2 only ADSCs, and BMP-7 only ADSCs, showed complete filling of the defect area in rat femur defects. However, the combination of growth factors present in non-activated platelet-rich plasma (nPRP), such as PDGF, TGF-b, bFGF, and VEGF, did not show any
beneficial effect on ADSCs during rabbit calvarial defect healing\cite{54}. There was little difference between the nPRP-ADSCs group, ADSCs alone, and PRP alone in terms of newly formed bone surface or volume.

Mazzoni \textit{et al}\cite{55} evaluated the osteogenic capacity of ADSCs on a hydroxyapatite-collagen hybrid scaffold in 50 patients undergoing malar augmentation. The authors reported the follow-up over three years which showed implant stability and osteointegration but histological samples from patients revealed osteogenesis and mature bone only in 70% of specimens.

Testing human stem cells in T-cell deficient animal models has been a regular practice but that may not be the ideal way to test the potential of ADSCs. Recent advances in the bone regeneration field suggest that certain T-cell subsets, CD4+CD25+FoxP3+ Treg cells being a prominent one, are required for stem cells to initiate the bone formation process. We believe that this could explain, at least partially, the inability of ADSCs to induce bone formation in T-cell lacking mice and rats.

\textbf{PRECLINICAL STUDIES}

Publications on preclinical studies utilizing ADSCs were obtained from OVID Medline using the search keywords: “ADSCs”, “Stem Cells”, “Animals”, “Mesenchymal Stem Cells”, “Tissue Engineering” and “bone regeneration”, which returned 90 studies. An additional 14 studies were included from past collections by the researchers. After the elimination of studies that either lacked \textit{in vivo} experiments, lacked a focus on ADSCs, or overlapped with other sections, 52 pre-clinical studies, investigating the bone-forming ability of ADSCs using various animal models, are summarized in this section and in Table 2.

\textbf{COMPARISON OF ADSCS WITH SVF AND MSCS}

Kang \textit{et al}\cite{56} compared canine MSCs from adipose tissue, bone marrow, umbilical cord blood, and Wharton’s jelly in terms of their osteogenic potential \textit{in vitro} and \textit{in vivo}. ADSCs showed the highest proliferation capacity at all passages \textit{in vitro}. Measured levels of ALP activity were highest in ADSC and umbilical cord blood-derived MSCs. When stem cells were mixed with $\beta$-TCP and implanted into the canine segmental defects created in the radial diaphysis, comparable bone healing was observed in all stem cells groups which were significantly higher than the scaffold control group as determined by radiographic union, histology analysis, and the ratio between newly formed bone over total defect size.

Toplu \textit{et al}\cite{57} created the bone defects on the bilateral zygomatic arches of 20 rats. On one side, the defect was left for secondary healing and on the other side, SVF was injected into the defect site. After 20 wk, Micro-CT analysis and histology confirmed a significantly larger volume of newly formed bone in the SVF-injected side\cite{57}.

\textbf{GROUP 1: PRE-DIFFERENTIATED ADSCS}

Kim \textit{et al}\cite{58} treated rabbit 20 mm mid-diaphyseal ulna bone defects using SVF on a PLGA scaffold. Animals were treated with scaffold alone, PLGA containing undifferentiated SVF cells, and PLGA with osteogenically induced SVF cells. Since the PLGA-osteogenic SVF group showed significantly higher bone volume, the authors concluded that osteogenic differentiation was necessary for optimal bone regeneration by SVF. Osteogenically induced ADSCs-seeded coral scaffold showed statistically significant more healing of the canine bilateral full-thickness parietal defect model in comparison with control scaffold\cite{59}.

Investigators have also explored pre-differentiating ADSCs into endothelial lineage. Shah \textit{et al}\cite{60} compared osteogenesis induced by ADSCs differentiated into osteogenic lineage with those differentiated into endothelial lineage. Undifferentiated control ADSCs and differentiated ADSCs were used to treat rats’ calvarial defects. The authors were not able to find any statistically significant difference in osteogenesis and angiogenesis among these groups. Sahar \textit{et al}\cite{61} also compared ADSCs differentiated into endothelial lineage with ADSCs differentiated into osteogenic lineage when implanted in a critical size rat calvarial defect model. The results showed that undiffer-
| Animal model                              | Scaffold used                                                                 | ADSCs per implant | Time frame | Defect healing outcomes                                                                 | Ref.         |
|------------------------------------------|-------------------------------------------------------------------------------|-------------------|------------|----------------------------------------------------------------------------------------|--------------|
| -Beagle Dogs; -Unilateral radial segmental defect -10 mm | β-TCP/poly l-lactide-co-glycolide-co-e-caprolactone composite scaffold          | $1 \times 10^6$ canine ADSCs | 20 wk      | 33.90 ± 4.31                                                                           | Kang et al [56] |
| -Wistar albino rats; -Middle zygomatic arch defect -3 mm wide | No scaffold                                                                 | Rat inguinal fat pad derived SVF | 20 wk      | The average new bone growth in the experimental group was 1.1 mm, significantly higher than control | Toplu et al [57] |
| **Group 1: Pre-differentiated ADSCs**    |                                                                                |                   |            |                                                                                         |              |
| -New Zealand white rabbits; -Mid-diaphysis of left ulna; -20 mm long | Porous polylactic glycolic acid scaffold                                      | $1 \times 10^6$ rabbit SVF cells | 8 wk       | Approximately 55%                                                                       | Kim et al [58] |
| -Beagle dogs; -Parietal bone; -20 mm × 20 mm full-thickness defect | Coral scaffold                                                               | $60 \times 10^6$ of canine ADSCs | 24 wk      | 84.19 ± 6.45                                                                            | Cui et al [59] |
| -Lewis rats; -Calvarial defect -8 mm wide | Polylactic scaffold                                                          | $0.1 \times 10^6$ rat ADSCs | 8 wk       | Coculture of endothelial- and osteoblast-induced ADSC showed no significant improvement over undifferentiated cells | Shah et al [60] |
| -Lewis rats; -Calvarial defect -8 mm wide | Poly (D,L-Lactide) scaffold                                                   | $0.1 \times 10^6$ rat ADSCs | 8 wk       | Osteogenic-induced ADSC generated 0.91 ± 0.65 mm$^3$ new bone, significantly higher than endothelial-induced ADSC | Sahar et al [61] |
| **Group 2: FGF, VEGF, PDGF, and ADSCs**  |                                                                                |                   |            |                                                                                         |              |
| -Osterix::mCherry reporter mice; -Closed transverse diaphysis fractures of the right femur | No scaffold                                                                 | $0.3 \times 10^6$ wild-type mouse ADSCs | 35 d       | The experimental group induced significantly larger mineralized surface and bone callus compared to cell-free and non-transduced controls. | Zhang et al [62] |
| -Balt/c nude mice; -Parietal bone defect; -4 mm wide | Whitlockite::reinforced gelatin/heparin cryogels                             | $1 \times 10^6$ human ADSCs | 8 wk       | > 16%                                                                                   | Kim et al [63] |
| -CD1 nude mice; -Parietal bone defect; -4 mm wide | Coral scaffold                                                              | $1.5 \times 10^6$ human ADSCs | 8 wk       | 95.40%                                                                                   | Behr et al [64] |
| -Sprague Dawley rats; -Distal femoral cancellous bone -3.5 mm wide and 5 mm deep defect | Trimodal mesoporous bioactive glass scaffold                                 | $20 \times 10^6$ cell/mL until saturation; rat ADSCs | 8 wk       | 14.25 ± 3.57                                                                            | Du et al [65] |
| -Nu/Nu J mice; -Parietal bone; -4 mm wide | Polycaprolactone - fibrin scaffold containing heparin-conjugated decellularized bone | $0.2 \times 10^6$ human ADSCs | 12 wk      | The experimental group induced a significantly larger new bone volume compared to the control without PDGF | Rindone et al [66] |
| **Group 3: BMP and ADSCs**               |                                                                                |                   |            |                                                                                         |              |
| -Sprague Dawley rats; -Full-thickness parietal bone defect -5 mm wide | Polylactic glycolic acid scaffold                                             | $0.025 \times 10^6$ human ADSCs | 8 wk       | 33.3 ± 29.0                                                                             | Park et al [67] |
| -Chinese white rabbits; -Full-thickness calvarial defects; -8 mm | Fibrin gel matrix                                                           | $3 \times 10^6$ rabbit ADSCs | 12 wk      | Approximately 48                                                                         | Lin et al [68] |
| -Japanese white rabbits; -Segmental radial defect; -15 mm | Nano-hydroxyapatite/recombinant human-like collagen/poly (lactic acid) scaffold | $2 \times 10^6$ cells/mL; rabbit ADSCs | 12 wk      | 97.25 ± 2.06                                                                             | Hao et al [69] |
Le Q et al. Adipose-derived stem cells for bone regeneration

| Group 4: Genetically manipulated ADSCs | Group 5: Engineered scaffolds and ADSCs | Group 6: Manipulation of recipient host and ADSCs |
|--------------------------------------|-----------------------------------------|-----------------------------------------|
| -Taiwan Lee-Sung minipigs; Mid-shaft left femur defect; -30 mm long | -C57BL6/J mice; -Mid femur defect; -2 mm | -Sprague-Dawley rats; -Calvarial defect; -7 mm wide |
| -CD-1 nude mice; -Full-thickness parietal bone defect; -3 mm wide | -Sprague Dawley rats; -Full-thickness femur defect; -4 mm wide | -Sprague-Dawley rats; -Calvarial defect; -5 mm wide |
| -Nude mice; -Parietal bone defect; -4 mm wide | -Balb/c nude mice; -Cranium defect; -4 mm wide | -Athymic nu/nu mice; -Subcutaneous implantation |
| -Nude mice; -Subcutaneous implantation | -Sprague Dawley rats; -Calvarial defect; -8 mm wide and 1 mm thick | -Sprague-Dawley rats; -Calvarial defect; -7 mm wide |
| -CD-1 nude mice; -Right parietal bone defect; -4 mm wide | -Athymic nude rat; -Mandible defect; -5 x 5 mm | -Athymic nu/nu mice; -Subcutaneous implantation |
| -Athymic nude rat; -Mandible defect; -5 x 5 mm | Chitosan/chondroitin sulfate scaffold | -Osteoporotic Sprague-Dawley female rats; -Distal epiphysis left femur defect; -3 mm wide |
| Apatite coated poly (L-lactide-co-glycolide) scaffolds | Poly(lactic-co-glycolic acid) scaffold | Poly(lactic glycolic acid) scaffold |
| 100 x 10^6 cells/animal; minipig ADSCs | 3 x 10^6 cells/mL; ADSC from C57BL/6 mouse | Poly(lactic glycolic acid) scaffold |
| 12 wk | 6 wk | 0.15 x 10^6 human ADSCs |
| Experimental group’s new bone formation showed equivalent density and volume compared to native bone and is significantly better than non-transduced control | 77% | Up to 100% |
| Fan et al[71] | Lin et al[70] | Weimin et al[73] |
| Group 4: Genetically manipulated ADSCs | -C57BL6/J mice; -Mid femur defect; -2 mm | -Sprague-Dawley rats; -Calvarial defect; -7 mm wide |
| -BALB/c nude mice; -Subcutaneous implantation | Strontium-substituted hydroxyapatite poly (γ-benzyl-l-glutamate) scaffold | -Sprague-Dawley rats; -Calvarial defect; -7 mm wide |
| β-tricalcium phosphate scaffold | 5 x 10^6 C57BL6/J mice ADSCs | Alginate microspheres |
| 2 x 10^6 human ADSCs | 8 wk | 0.5 x 10^6 rabbit ADSC |
| Approximately 30% | Approximately 38% | Approximately 41% |
| Wang et al[76] | Gao et al[78] | Man et al[82] |

**Table:**

| Group 6: Manipulation of recipient host and ADSCs | Group 7: Engineered scaffolds and ADSCs | Group 8: Manipulation of recipient host and ADSCs |
|--------------------------------------|-----------------------------------------|-----------------------------------------|
| -Sprague-Dawley rats; -Calvarial defect; -7 mm wide | -C57 black/DBA mice; -Supracondylar right femur defect; -0.9 mm wide | -Osteoporotic Sprague-Dawley female rats; -Distal epiphysis left femur defect; -3 mm wide |
| -C57 black/DBA mice; -Supracondylar right femur defect; -0.9 mm wide | Hydrogel | Gelatin |
| Poly(lactic glycolic acid) scaffold | 1 x 10^6 human ADSCs | 2 x 10^6 rat ADSCs |
| 12 wk | 8 wk | 5 wk |
| Approximately 60% | Approximately 50% | Approximately 23% |
| Wang et al[83] | Deng et al[84] | Li et al[85] |
Group 7: Allogeneic ADSCs

- New Zealand white rabbits; - Ulna defect; - 15 mm
  Demineralized bone matrix
  $60 \times 10^6$ rabbit ADSCs
  12 wk
  Both allogeneic and autologous ADSC seeded construct induced almost complete defect repair while cell-free control remained un repaired
  Gu et al.[66]

- Sprague Dawley rats; - Ulna defect; - 8 mm long
  Demineralized bone matrix
  $60 \times 10^6$ rat ADSCs
  24 wk
  Radiographs and histology confirmed superior bone healing in the experimental group compared to cell-free control
  Wen et al.[67]

- Beagle Dogs; - Parietal bone defect; - 20 x 20 mm
  Coral scaffold
  $60 \times 10^6$ of canine ADSC
  24 wk
  Approximately 70%
  Liu et al.[88]

- Wistar rats; - Left radius defect; - 4 mm long
  Heterogeneous deproteinized bone
  $0.1 \times 10^6$ rat ADSCs
  8 wk
  Radiographs and histology confirmed improved healing in osteoinduced ADSC/scaffold group compared to undifferentiated ADSC, cell-free, and blank controls
  Liu et al.[89]

Group 8: Non-manipulated or unaltered ADSCs

Decellularized matrices

- CD1 nude mice; - Distal femur defect - 3 mm
  Human cancellous bone scaffold
  $0.5 \times 10^6$ human ADSCs
  8 wk
  hADSCs-seeded scaffold induced significantly superior defect healing compared to cell-free scaffold
  Wagner et al.[90]

- C57BL/6 mice; - Calvarial defect; - 4 mm wide
  Extracellular matrix deposited on porcine small intestinal submucosa
  $0.0025 \times 10^6$ of human ADSCs
  4 wk
  21.77 ± 6.99
  Zhang et al.[91]

- Institute of Cancer Research mice; - Full-thickness parietal defect; - 4 mm wide
  Decellularized tendon
  $1.0 \times 10^6$ human ADSCs
  8 wk
  86%
  Ko et al.[92]

- Sprague Dawley rats; - Two-wall periodontal intrabony defect; - 2.6 x 2.0 x 2.0 mm
  Amniotic membrane
  $0.3 \times 10^6$ human ADSCs
  3 wk
  ADSC-seeded scaffold resulted in a significantly smaller defect size than the control
  Wu et al.[93]

Ceramics

- Sheep; - Tibia; - 3.2 cm long defect
  Hydroxyapatite-based particle in a semi-solid milieu
  $56 \times 10^6$ human ADSCs
  12 wk
  The experimental group showed bridging and significantly better healing compared to control
  Ben-David et al.[94]

- New Zealand White rabbits; - Full-thickness proximal medial tibia defect; - 8 mm wide
  Hydroxyapatite
  $0.2 \times 10^6$ rabbit ADSCs
  8 wk
  The new bone area was equivalent between seeded and unseeded scaffold; however, ADSC seeded construct represented preferable histological characteristics
  Arrigoni et al.[95]

- New Zealand White rabbits; - Full-thickness proximal medial tibia; - 8 mm in diameter
  Hydroxyapatite
  $1.5 \times 10^6$ rabbit ADSCs
  8 wk
  ADSC-seeded scaffold exhibited better scaffold resorption than cell-free scaffold and superior histological characteristics compared to all controls
  De Girolamo et al.[96]

- Fisher 344 rats; - Calvarial defect; - 5 mm wide
  Hydroxyapatite
  $0.4 \times 10^5$ rat ADSCs
  8 wk
  16.88 ± 1.52
  Xia et al.[97]

- T and B cell-deficient NOD SCID mice; - Subcutaneous implantation
  Type I collagen (30%) and magnesium-enriched hydroxyapatite
  $1 \times 10^6$ human ADSCs
  8 wk
  hADSC-seeded presented improved osteogenesis and angiogenesis compared to cell-free scaffold control
  Calabrese et al.[98]

- Miniature Pigs; - Mandibular defect - 3 cm x 1 cm x 2 cm
  Tri-calcium phosphate- poly (D,L-lactide-co-glycolide) scaffolds
  $5 \times 10^6$ porcine ADSCs
  12 wk
  34.8 ± 4.80
  Probst et al.[99]
| Bioactive glass          | 0.5 × 10^6 rat ADSCs | 12 wk | ADSC-seeded scaffold group exhibited significantly more bone repair and higher bone density compared to blank control. ADSC construct’s result was equivalent to that of autologous bone graft |
|------------------------|---------------------|-------|-------------------------------------------------------------------------------------------------------------------------------------|
| Bioactive glass        | 0.5 × 10^6 rat ADSCs | 12 wk | The experimental group saw the complete repair of the defect while all controls showed various degrees of incomplete healing; repair in the experimental group is characterized by mature bone and complete scaffold resorption |
| Polycaprolactone scaffold | 0.05 × 10^6 human ADSCs | 8 wk | Both undifferentiated and osteo-induced ADSC-seeded scaffold resulted in preferable histological features and higher expression of osteogenesis and angiogenesis markers |
| Activated platelet-rich plasma | 1.0 × 10^6 human ADSCs | 6 wk | 68.97 ± 0.91                                                                                                                                 |
| Activated platelet-rich plasma | 0.2 × 10^6 rat ADSCs | 8 wk | 95.60                                                                                                                                 |
| Hyaluronic acid-g-chitosan-g-poly (N-isopropylacrylamide) embedded with biphasic calcium phosphate microparticles and PRP | 0.1 × 10^6 rabbit ADSCs | 16 wk | The experimental group induced obvious significant bone formation and defect bridging. Cell-free scaffold control showed negligible defect repair |
| Multi-layered stacking of electrospun polycaprolactone/gelatin membranes | 0.006 × 10^6 rat ADSCs | 12 wk | Up to 90%                                                                                                                                 |
| 1H,1H,2H,2H-per-fluorodecyl acrylate (97%) and glycidyl methacrylate coated paper scaffold | 1.0 × 10^6 cells/paper human ADSCs | 8 wk | 92%                                                                                                                                 |

ADSCs: Adipose-derived stem cells; SVF: Stromal Vascular Fraction; PRP: Platelet-rich plasma.

entiated ADSC or osteogenic ADSC induced a significantly higher amount of bone tissue than endothelial ADSCs group which was equivalent to acellular control.

**GROUP 2: FGF, VEGF, PDGF, AND ADSCS**

Modulation of the bone-forming ability of ADSCs by expressing genes of FGF, VEGF, PDGF or by tethering these proteins to scaffolds has been reported. Zhang et al.[62] created bone defects in mouse femurs and injected control ADSCs or ADSCs transduced with bFGF intramuscularly adjacent to the fracture site. While no significant improvement was observed in the ADSCs group, improved bone healing
was observed in the ADSCs-bFGF group. Interestingly, using GFP-assisted observation, the authors identified that at day 21, only a very small fraction of the originally implanted ADSCs remained in the healing callus. This supported the idea that ADSCs’ role in bone healing is more reliant on its paracrine function rather than direct cell replacement[62].

Kim et al[63] studied VEGF-transduced ADSCs for the treatment of mouse calvarial bone defects. At week 8, microCT and histology revealed that groups with ADSCs exhibited faster bone regeneration. In that, the VEGF overexpression group was found to have significantly more bone healing. hADSCs pre-treated with VEGF also showed beneficial effects. Behr et al[64] implanted hADSCs that were pre-treated with 2 µg VEGF into critical-size calvarial defects of nude mice using coral scaffold. Quantification of defect filling at week 8 by microCT showed that the VEGF-treated ADSCs group yielded significantly better bone regeneration than all other groups including untreated ADSCs. VEGF is likely to have a direct effect on the differentiation of ADSCs and it might also increase the bone healing rates indirectly by improving angiogenesis during bone regeneration. Du et al[65] have reported that the bioactive glass implant pre-vascularized in vitro for 7 d using endothelial cells and then seeded with ADSCs was associated with enhanced angiogenesis and significantly more bone regeneration in rat femur critical size defect compared to acellular scaffold and non-vascularized ADSC-seeded implant.

Similar to FGF and VEGF modulation, PDGF has been reported to improve the osteogenic ability of ADSCs. Rindone et al[66] designed a scaffold in which PDGF-BB was anchored using heparin-conjugation and simple electrostatic force. When implanted in murine calvarial defects, the experimental group containing ADSCs and PDGF-BB showed significantly higher bone formation compared to controls without PDGF-BB.

GROUP 3: BMP

BMP-2 is FDA-approved for the treatment of bone injuries and is currently being widely used to treat bone defects. It is known to govern osteogenic differentiation of stem cells. Naturally, it has been the focus of investigation for improving the bone-forming potential of ADSCs.

Park et al[67] investigated the ability of BMP-2-transduced ADSCs for the treatment of full-thickness parietal bone defects in rats. Similar to control receiving no implant, scaffold only group and scaffold with osteogenically induced ADSCs group showed either no or incomplete filling. However, mice receiving BMP-2-transduced ADSCs showed complete healing at week 8. Lin et al[68] compared bone formation induced by BMSCs and ADSCs in rabbit calvarial defect model. BMSCs and ADSCs were transduced with a BMP4-carrying-adenovirus vector and seeded on a fibrin gel scaffold. Both transduced BMSC and ADSC groups showed a significantly higher amount of newly regenerated bone tissue compared to their respective non-transduced control. No difference was identified between transduced BMSC and transduced ADSC groups. Hao et al[69] investigated the potential of BMP-2 overexpressing ADSCs in a rabbit critical size radial segmental defect. The authors reported that animals treated with transfected ADSCs-seeded scaffolds demonstrated recanalization of the radial medulla, bone contour modeling, and scaffold degradation. No significant defect repair was found in either scaffold only or scaffold with non-transfected ADSCs groups.

Lin et al[70] overexpressed BMP-2 and VEGF genes in ADSCs and the resulting cells were seeded on a PLGA scaffold which was implanted in a minipig massive segmental left femoral defect model. Bone regeneration in the experimental group was observed as early as two weeks post-procedure and progressively increased to complete union at 12 wk. PET evaluation also revealed improved angiogenesis in the experimental group compared to the control.

Strategies that promote BMP signaling in ADSCs have also been used successfully. Fan et al[71] coated PLGA scaffolds with Phenamil and BMP-2 and then seeded the scaffold with ADSCs. Phenamil is a derivative of the diuretic Amiloride, that acts as a powerful stimulator of BMP-2 signaling. The authors hypothesized that using Phenamil would allow optimal osteogenesis while reducing the needed BMP-2 dose to avoid adverse effects. The construct was tested on a mouse calvarial bone defect model. The authors reported that ADSCs-loaded scaffold treated with both Phenamil and BMP-2 induced significantly improved bone regeneration compared to ADSCs-loaded scaffold with BMP-2 alone group as measured by micro-CT. Li et al[72]
transduced ADSCs to upregulate expression of BMP-2 and miR-148b using a Cre/LoxP-based baculovirus hybrid before seeding onto gelatin-coated PLGA scaffold. miR-148b is a miRNA identified for its osteogenic property when acting with BMP-2. When this construct was used to treat critical-sized calvarial bone defects in nude mice, the authors found that at 12 wk post-procedure, the experimental group showed significantly improved bone healing compared to controls with either no transduction, transduction with only miR-148b, or only BMP-2. Weimin et al.[73] expressed LIM mineralization protein 1 (LMP-1) and hypoxia-inducible factor 1 (HIF-1a) genes in ADSCs to promote osteogenesis. LMP-1 was recognized as a positive intracellular regulator of osteogenesis, upstream of BMP-2, while HIF-1a initiated angiogenesis[41]. After lentiviral expression of genes in ADSCs, the resulting cells were seeded onto a PLGA scaffold and tested on the dorsal subcutaneous pockets of nude mice. Based on histological analysis, the authors claimed that there was more neo-osteogenesis found in LMP-1 and HIF-1a expressing ADSCs than found in controls.

Lentiviral delivery of shRNA to inhibit expression of Noggin, an inhibitor of BMP-2 signaling, in ADSCs has been shown to improve their osteogenic potential[74]. The transduced ADSCs induced more rapid and complete healing of the calvaria defect in nude mice in comparison with non-transduced ADSCs. BMP-2 addition and Noggin inhibition together are known to further improve bone healing outcomes. Fan et al.[75] transfected ADSCs with lentivirus silencing the expression of noggin and loaded them onto a chitosan and chondroitin sulfate scaffold, coated with apatite to ensure BMP-2 attachment and controlled release. The construct induced significantly more bone repair in a rat mandibular defect model in comparison with blank scaffold, scaffold with BMP-2, or scaffold with ADSCs (Nog-/-) at 8 wk.

GROUP 4: GENETICALLY MANIPULATED ADSCS

Wang et al.[76] found that Prostaglandin G/H synthase 1 (PTGS1) is expressed in ADSCs in response to TNF-α in inflammatory conditions and that PTGS1 knockout ADSCs showed higher osteogenic potential. When PTGS1 knockout ADSCs were mixed with Synthograft (Bicon), a commercial beta-tricalcium phosphate product, and were transplanted into the dorsal subcutaneous tissue of mice, they induced significantly more bone formation at week 8 compared to control[76]. Xie et al.[77] used lentivirus to transduce ADSCs to upregulate the expression of miR-135, a microRNA recognized for its role in regulating osteogenesis. Transduced ADSCs were implanted in rats’ critical-sized calvarial bone defect model. The authors observed almost complete sealing of defect area when treated with miR-135 transduced ADSCs. All other groups showed from nonexistent to incomplete filling of the defect.

GROUP 5: ENGINEERED SCAFFOLDS

Gao et al.[78] developed a microcarrier from Strontium (Sr)-substituted hydroxyapatite, which was found to release Sr ions, known activators of the Wnt/b-catenin pathway, consistently at the right concentration. When these microcarriers were seeded with ADSCs and implanted into mouse femur nonunion defect, they were found to stimulate significantly more bone formation than control at 8 wk. Doğan et al.[79] incorporated boron into PLGA scaffold (NaB/PLGA) and seeded ADSCs onto it to test this implant in a rat femur defect model. One month after implantation of ADSC-NaB/PLGA, the ADSC-NaB/PLGA group showed the highest Hounsfield units which represented superior bone regeneration compared to all other groups. Shin et al.[80] constructed a system in which siRNA lipidoid nanoparticles, designed to target and silence the osteogenesis inhibitor guanine nucleotide-binding protein alpha-stimulating activity polypeptide (GNAS), were immobilized on PLGA scaffolds, and hADSCs were seeded onto this PLGA scaffold for treating mouse critical-sized calvarial defect. The authors hypothesized that using this system, hADSCs could undergo genetic modification and osteogenic induction after being seeded onto the scaffold, eliminating the need for activation using culture-based protocols. At eight weeks post-procedure, the experimental group showed significantly more bone regeneration in comparison with no treatment control, construct without siRNA, and construct with scrambled siRNA.
Wang *et al* [81] combined collagen scaffold with Resveratrol (RSV), an antioxidant with anti-inflammatory and osteogenic properties, and seeded ADSCs on the construct. The authors reported that hADSCs-seeded collagen-RSV scaffold was the most effective in bone regeneration in a rat calvaria defect model when compared with other groups: collagen scaffold only, collagen scaffold with resveratrol, ADSCs seeded collagen scaffold, and ADSCs seeded collagen scaffold with resveratrol, based on their micro-CT results at 12 wk.

Man *et al* [82] evaluated the effect of PRP on the osteogenic potential of ADSCs encapsulated in alginate microspheres. The microspheres were combined with 5% PRP, 10% PRP or 15% PRP and injected subcutaneously in athymic nude mice. Only groups receiving ADSC-Alginate with 10% and 15% PRP showed mineralization at 1 and 3 mo with the 15% PRP group showing a dose-dependent increase.

**GROUP 6: MANIPULATION OF RECIPIENT HOST**

Wang *et al* [83] used the hADSC-seeded PLGA constructs for the treatment of rat critical-size calvarial defect and also evaluated the impact of locally injected Alendronate (Aln), a bisphosphonate often used for the treatment of osteoporosis. At 12 wk the acellular groups (control and PLGA-Aln) showed limited bone formation while both PLGA-ADSCs and PLGA-ADSC-Aln showed abundant mature neo-osteogenesis. Complete bridging of the defect was observed only in the PLGA-ADSC-Aln group.

Deng *et al* [84] found that exendin-4 enhanced the ability of ADSC to induce bone regeneration in a mouse femur metaphyseal defect model. Exendin-4 is a glucagon-like peptide 1 receptor agonist previously recognized for its role in glycemic control, control of bone resorption, and increased bone mass [84]. After creating the femur metaphyseal defect, the authors planted hydrogels with ADSCs into the defect site followed by daily intraperitoneal exendin-4. This experimental group was compared with wild-type non-defective bone, defect bone without treatment, and defect bone treated with ADSC only. The results of bone regeneration after 8 wk showed that this experimental group exhibited significantly more repair than ADSCs only group as well as the controls.

Li *et al* [85] reported that miR-214 targeted the Wnt pathway to favor adipogenesis in ADSCs isolated from osteoporotic ovariectomized rats and this microRNA was found at a high level in aged osteoporotic patients as well. Implantation of ADSCs genetically manipulated to silence miR-214, but not unaltered ADSCs isolated from osteoporotic rats, could lead to complete healing of critical size femoral metaphyseal defects in ovariectomized rats when delivered using a gelatin scaffold.

**GROUP 7: ALLOGENEIC ADSCS**

The use of allogeneic stem cells is currently prohibited by FDA. However, there is limited data available that allogeneic ADSCs can be as effective as autologous ADSCs in rabbit, canine, and rat bone defect models.

Gu *et al* [86] investigated the osteogenic capacity of ADSCs-seeded DBM to treat critical-sized ulna defects in a rabbit model. Micro-CT was used to compare three experimental groups: Allogenic ADSC-seeded DBM, Autologous ADSC-seeded DBM, and DBM only. The authors reported that both Allogenic and Autologous ADSC groups showed bone formation that bridged the defect gap. DBM alone group, on the other hand, did not show bridging of the defect but only loose fibrous tissue. Wen *et al* [87] also used a DBM as a scaffold for allogeneic rat ADSCs to promote bone healing in rat critical-sized ulnar bone defect model. At 24 wk, superior osteogenesis in defects treated with ADSCs-DBM was recognized grossly and radiographically. ADSCs-DBM treatment was also associated with significantly higher ulnar bone strength than those treated with DBM only. Allogeneic ADSCs were shown to be as effective as autologous ADSCs for the treatment of cranial critical-sized defects in canine models as well [88]. There was no significant systemic immune reaction as measured by the ratio of CD4/CD8 as well as serum levels of IL-2, IL-4, IL-10, IFN-γ, and TGF-β. MicroCT evaluation showed equivalent bone regeneration between allogeneic and autologous groups with both groups inducing significantly better healing than the scaffold-only group. When GFP-positive ADSCs were implanted, they could still be detected in osteocyte lacunae and bone matrix at 24 wk, pointing to their direct role in osteogenesis.
Liu et al[88] found that allogeneic ADSCs require pre-differentiation to be effective. Undifferentiated allogeneic ADSCs failed to induce bone formation. They seeded allogeneic ADSCs on heterogeneous deproteinized bone (HDB) and delivered the construct for the treatment of critical-sized bone defects in a rat radius model[89]. The authors investigated four groups: no implant, HDB implant only, non-induced ADSCs seeded on HDB or osteogenically induced ADSCs on HDB scaffold. It was found that at 8 wk, the group treated with osteogenic ADSCs on HDB showed evident bridging with new bone completely filling the defect area. All other controls, on the other hand, did not improve healing at 8 wk.

**GROUP 8: SCAFFOLD TYPES USED FOR NON-MANIPULATED OR UNALTERED ADSCS**

To this end, many different materials have been experimented with in vitro and in vivo in combination with ADSCs. Commonly used materials include decellularized tissues used as matrix, ceramics, polymers, as well as hybrid materials.

**Decellularized matrices**

Various natural matrices such as human cancellous bone, porcine small intestinal submucosa, bovine tendon, human amniotic membrane, have been used successfully, after their decellularization, for delivery of unaltered ADSCs and this approach has attained reasonable success in enhancing bone regeneration.

Wagner et al[90] investigated the combination of hADSCs and freeze-dried human cancellous bone for treatment of femur critical-sized defect in rats. They optimized the seeding density of ADSCs and found that a cell number of 250000 cells (84600 cells/100mm.) was optimal. At 4 wk, the authors observed a significant elevation of bone regeneration in the ADSCs group compared to unseeded control. Zhang et al[91] explored a scaffold made from the extracellular matrix (ECM) deposited on porcine small intestinal submucosa (SIS). This porcine SIS construct was cultured with osteoblasts to induce deposition of osteogenic ECM, followed by decellularization and ADSCs seeding. The ADSCs-seeded ECM-SIS scaffold induced significantly more healing of mouse critical-sized calvarial defects than SIS only, ECM-SIS, ADSC-SIS groups. Ko et al[92] evaluated decellularized, bovine Achilles and neck tendons as the scaffold for osteogenically induced hADSCs to evaluate bone regeneration in a mouse calvarial bone defect model. The implant was placed in two layers into the calvarial bone defects and its capacity for bone regeneration was evaluated. Results at 4 and 8 wk showed significantly better filling of the defect in the experimental group compared to all controls. Wu et al[93] obtained amniotic membranes (AM) during cesarian delivery, decellularized them, and co-cultured with ADSCs to initiate seeding. This construct was used to treat two-wall periodontal bone defects in rats. CT imaging of the defect 29 d after implantation showed a smaller defect volume in the ADSCs-AM group compared to no treatment control, AM only, and ADSCs only.

**Ceramics**

Hydroxyapatite and β-tricalcium phosphate are the two most widely used matrices in this group and have shown successful outcomes in supporting ADSCs-mediated bone regeneration. ADSCs seeded and grown on hydroxyapatite-based mineral particles could successfully treat full cortex segmental tibial defect in sheep[94]. Following implantation of the ADSCs-seeded particles, statistically higher newly formed bone volume was observed in the treatment group compared to the control. Arrigoni et al[95] compared bone regeneration in a rabbit critical-sized tibial defect model mediated by hydroxyapatite alone and ADSCs-seeded hydroxyapatite implant. The authors reported that the ADSCs-seeded group displayed superior performance. de Girolamo et al[96] also reported somewhat similar findings in the rabbit model when they used autologous ADSCs seeded hydroxyapatite scaffold to treat full-thickness defects in New Zealand rabbit’s proximal tibial epiphysis. At 8 wk, the authors reported that there were no significant differences in defect filling and bone mineral density, but the ADSCs-scaffold group induced the most mature bone that was quite similar to native tissue. The structure of hydroxyapatite is reported to play an important role. Based on in vitro results and data from the healing of the rats’ bilateral calvarial defects, the micro-nano-hybrid structure, which is a hybrid of nanorod and microrod, was found to be the most effective surface topography for the delivery of ADSCs[97]. Calabrese et al[98] evaluated the ectopic bone formation induced by hADSC seeded on a collagen-
hydroxyapatite scaffold through subcutaneous implantation in mice. The scaffolds seeded with ADSCs exhibited faster hydroxyapatite formation and increased vascular generation, both statistically significant in comparison with scaffold control.

Probst et al.[99] examined the efficacy of pig ADSCs with tri-calcium phosphate poly(lactic-co-glycolic) acid scaffold for regeneration of critical-sized mandibular bone defects in minipigs. ADSCs were induced with an osteogenic medium prior to seeding. MicroCT showed a significantly higher ratio of bone volume to total volume in the ADSCs group in comparison with control but even in the test group, the regenerated bone volume was only about one-third of the defect size.

Bioactive glass
Saçak et al.[100] investigated bone regeneration in calvarial bone defect of mice using ADSCs seeded bioactive glass. The authors divided the animals into four groups either untreated, treated with autologous bone graft, treated with bioactive glass, or treated with ADSC-seeded bioactive glass. Bone regeneration in the ADSC-seeded bioactive glass group and autologous bone graft treatments were equivalent.

Jing et al.[101] doped 45S5 Bioglass with Icariin, a flavonoid glucoside isolated from the plant Herba Epimedii and then seeded the implant with ADSCs. Implantation of the Icariin-doped, ADSC-seeded scaffold resulted in the complete repair of the rat calvarial defect in 12 wk. Groups receiving no scaffold, Bioglass only, and ADSC-seeded Bioglass without Icariin only exhibited partial repair. The authors reported that when cultured with Icariin, ADSCs upregulate their expression of VEGF, thus promoting angiogenesis which was the mechanism behind their enhanced osteogenic potential.

Polymers
Caetano et al.[102] evaluated the use of polycaprolactone, a semi-crystalline biodegradable polymer, as a scaffold for human ADSCs to treat critical-size calvarial bone defects in rats. They compared undifferentiated hADSCs with hADSCs cultured in osteogenic conditions. The authors found osteoid tissue larger in size and more organized in groups treated with both types of ADSCs. Immunohistochemical staining revealed that the undifferentiated ADSCs group showed the highest percentage of cells with BMP-2 expression. The two groups with ADSCs showed equivalent angiogenesis, assessed via CD31 staining, which was significantly higher than no ADSCs groups.

Platelet-rich plasma as carrier material
Cruz et al.[103] evaluated the use of platelet-rich plasma activated with calcium chloride as the carrier for ADSCs to treat 10 mm wide, beagle dog tibial bone defects. Four defects were introduced in each animal. The defects were treated with clot, PRP only, autogenous bone graft, or ADSCs-seeded PRP. Histological analysis showed that the PRP-ADSCs group induced significantly more bone formation when compared to control, autogenous bone graft, and PRP only.

Tajima et al.[104] similarly explored activated PRP as the scaffold for delivering ADSCs to rat calvarial defects. Based on micro-CT results at 4 and 8 wk following the surgery, the authors found that ADSCs-seeded PRP demonstrated significantly larger regenerated bone area and volume compared to treatment with ADSCs-seeded type 1 collagen, PRP only, type 1 collagen only, and PBS control. The authors also confirmed that ADSCs transplanted by this manner differentiated into osteoblasts, by creating a construct using GFP expressing ADSCs-seeded PRP and observing cells staining positive for both GFP as well as osteocalcin.

Hybrid materials
Liao et al.[105] used hyaluronic acid-g-chitosan-g-poly(N-isopropylacrylamide) (HA-CPN) embedded with biphasic calcium phosphate microparticles and PRP as the organic matrix for delivery of rabbit ADSCs to critical-size cranial bone defects in rabbits. This experimental implant induced significant bone formation, almost completely covering the defect area while the control showed only negligible bone formation at 16 wk.

Wan et al.[106] designed a construct involving multi-layer stacking of three ADSCs-seeded polycaprolactone/gelatin electro-spun membranes. The construct was implanted into a model of calvarial defects in rats on bilateral parietal bones. The authors reported that the ADSCs seeded multilayer membrane group showed significantly more bone regeneration at higher density than those found in control and scaffold-only groups.
Park et al\cite{107} evaluated a paper-based multi-layer scaffold for delivery of ADSCs to a mouse calvarial defect model. Based on their \textit{in vitro} results, the authors determined that a scaffold of commercial weighing paper coated with 1H,1H,2H,2H-perfluorodecyl acrylate (97\%) and glycidyl methacrylate was most suitable. The authors compared two stacks of scaffolds only, two stacks of ADSCs-seeded scaffolds, three stacks of alternating ADSCs-seeded scaffolds (A) and HUVEC seeded scaffolds (H), and finally five stacks of alternating A-H-A-H-A scaffolds. All ADSCs seeded scaffolds increased bone healing after 8 wk compared to the blank control and scaffold-only groups. HUVEC-seeding did not show any statistically significant difference but there was a trend of increased bone healing.

SURFACE RECEPTORS EXPRESSION-BASED SELECTION OF SUB-POPULATIONS OF ADSCS AS A RELIABLE STRATEGY TO IMPROVE THE THERAPEUTIC POTENTIAL OF ADSCS

Since investigations utilizing ADSCs in their un-purified and unaltered form have given mixed results, strategies to create ADSCs-based formulation that can enhance bone healing, unambiguously and reliably are necessary. Therefore, the search for the osteogenic sub-population of ADSCs has been initiated worldwide. Since a common molecular marker for all osteogenic progenitors has not been discovered and the precise identity of true skeletal stem cells, required for homeostasis and repair of the postnatal skeleton, remains elusive, investigators have used various surface markers for the selection of osteogenic sub-populations of ADSCs. The results of this investigation are summarized in this section and in Table 3.

CULTURED CELLS VS UNCULTURED SVF

Culture-expanded, horse ADSCs showed superior osteogenic ability when implanted in nude rats than that induced by the un-cultured SVF\cite{108}.

CD146

Interestingly, a mixture of two distinct FACS-purified hADSCs populations (CD146\(^+\) CD34\(^-\) and CD146\(^+\)CD34\(^+\)) induced ectopic bone formation and also healed 60\% of calvarial defect created in immunodefective mice\cite{109-111}. When FACS-purified CD146\(^+\)CD34\(^-\) cells were compared with un-purified SVF for their bone-forming ability using the ectopic bone formation assay and in the calvarial defect model, cells showed greater bone formation. Moreover, BMP2 treated cells showed more bone formation but with a massive adipogenic response. Usage of Nel-1 in place of BMP2 avoided adipogenesis to selectively promote only bone formation\cite{110}. A study has shown that both CD146\(^+\)CD34\(^-\) and CD146\(^+\)CD34\(^+\) subpopulations from hADSCs undergo tri-lineage differentiation and express adult stem cell markers CD105, CD90, CD73. CD34\(^+\) cells pre-cultured in an osteogenic medium for 3 d could induce bone formation in calvarial defects\cite{112}.

CD90

FACS-purified CD90\(^+\) hADSCs, but not un-purified hADSCs, induced bone healing in calvarial defects of nude mice\cite{113}. However, when CD90\(^+\)CD34\(^-\) hADSCs were implanted in nude mice using a collagen sponge, they generated only adipose tissue after 4 wk\cite{114}. This indicates the relevance of CD34 expression. In another study, mADSCs were FACS-purified into CD90\(^+\)CD105\(^-\), CD90\(^+\)CD105\(^+\), CD90\(^-\)CD105\(^-\), and CD90\(^-\)CD105\(^+\) populations. Marker expression of cells in basal medium, in osteogenic medium, and BMP2 transfected cells were determined. BMP2 transfection and culturing in an osteogenic medium were found to decrease the expression of CD105 \cite{115}. CD105\(^+\) and CD90\(^+\) subpopulations were purified from hADSCs and compared with each other for their osteogenic potential. CD90\(^+\) cells were found to be more osteogenic compared to CD105\(^+\) cells \textit{in vitro} as measured by ALP, Alizarin Red staining, and mRNA expression of Runx2, Ocn, Opn. When sorted cells were
Table 3 Specific markers used for selection of sub-populations of adipose derived stem cells showing superior bone forming ability

| Ref. | Marker | Study outcome and salient findings |
|------|--------|-----------------------------------|
| James et al. [10] | CD146 | Implantation of human CD90/CD146/FACS-sorted, human, CD105 Marker protein; TCP: Tricalcium phosphate; PLGA: Polylactic glycolic acid; HA: Hydroxyapatite; DBM: Demineralized bone matrix. |
| James et al. [109] | CD146/CD34/CD45 (Pericytes) + CD146/CD34/CD45 (Adventitial cells) | Human pericytes + adventitial cells together make up around 40% of SVF from human liposapce (60 patients tested) both types representing around 20% and these numbers do not change with age, gender, or body mass index; FACS purified, human, pericytes + adventitial cells induce significantly more healing in mouse calvarial defect than SVF; 250000 cells were implanted to critical size (3 mm) calvarial defect in SCID mice for 8 wk using PLGA |
| Meyers et al. [112] | CD146/CD34/CD45 (Pericytes) + CD146/CD34/CD45 (Adventitial cells) | It was feasible to purify human pericytes + adventitial cells using a multi-column approach of magnetic beads; Purified pericytes + adventitial cells could enhance critical size (4 mm) calvarial defect created in SCID mice; 25000 cells were implanted to critical size (4 mm) calvarial defect in SCID mice for 8 wk using PLGA |

**Legend:**
- **ADSCs:** Adipose-derived stem cells
- **FACS:** Fluorescence-activated cell sorting
- **SVF:** Stromal vascular fraction
- **CD:** Cluster of differentiation
- **CD105** ( $$\alpha$$ + - CreER): Mouse skeletal stem cells that give rise to bone were identified as Alpha V $$\alpha$$+ -CreER mice for 8 wk and were implanted intramuscularly in nude mice for 4 wk using collagen sponge or DBX+
- **CD105** high: Cells isolated from hard tissue but not the soft tissues showed a strikingly high tendency for skeletogenesis; This corresponded to high CXCR4 signaling in periosseal cells; Inhibition of CXCR4 signaling abrogated bone-forming potential of CD146/CD34/CD45 periosseal cells; CXCR4+ cells from soft tissue (adipose) derived CD146/CD34/CD45 cells represented osteostatic/non-adipocytic precursor cells; 1 × 10⁶ cells were implanted subcutaneously in nude mice for 4 wk using Matrigel
- **CD105** low: Cells isolated from the femoral growth plate; They were not present in adipose tissue; however, when a collagen sponge loaded with BMP-2 was implanted in adipose tissue, the authors reported de novo formation of Alpha V/CD200/CD105/D90 in the adipose tissue; Subcutaneous implantation of 10 µg BMP2+ Collagen Sponge in nude mice for 4 wk was successful.
- **CXCR4**: CD146/CD34/CD45 cells were FACs-purified from hard (human periosteum) and soft (adipose and dermal tissue) isolated from hard tissue but not the soft tissues showed a strikingly high tendency for skeletogenesis; This corresponded to high CXCR4 signaling in periosseal cells; Inhibition of CXCR4 signaling abrogated bone-forming potential of CD146/CD34/CD45 periosseal cells; CXCR4+ cells from soft tissue (adipose) derived CD146/CD34/CD45 cells represented osteostatic/non-adipocytic precursor cells; 1 × 10⁶ cells were implanted subcutaneously in nude mice for 4 wk using Matrigel
- **PDGFRα**: Lineage tracing using PDGFRα reporter mice showed that PDGFRα expression marks different sub-populations in the adipose tissue; PDGFRα+ and PDGFRα- fractions both are multipotent progenitor cells, however, PDGFRα+ ADSCs-derived ectopic implants ossify to a greater degree than PDGFRα- cell fractions; 1 × 10⁶ PDGFRα+ or PDGFRα- cells were implanted intramuscularly in nude mice for 8 wk using HA-β-TCP; Or Subcutaneous implantation of 2.5 µg BMP2 + Matrigel into the inguinal fat pad of PDGFRα+ -CreER for 8 wk

ADSC: Adipose-derived stem cells; FACS: Fluorescence-activated cell sorting; SCID: Severe combined immunodeficiency; BMP: Bone morphogenetic protein; TCP: Tricalcium phosphate; PLGA: Polylactic glycolic acid; HA: Hydroxyapatite; DBM: Demineralized bone matrix

Implanted into calvarial defects of nude mice, CD90+ cells showed more bone formation [113].
CD105, TGF-β1 SIGNALING AND BMP-RESPONSIVENESS

While ADSCs have been conventionally characterized by positive expression of CD105 [39,40,59,116,117], many groups have also observed considerable amounts of phenotypic variability within ADSCs during in vitro expansion [118,119]. Our group and other laboratories are actively investigating the role of CD105 in determining the osteogenic potential of ADSCs. CD105 is the co-receptor of the TGF-β1 signaling pathway and is known to enhance signaling of the main receptors Alk1 and Alk5 through phosphorylation of the downstream mediators - Smads2/3.

It has been shown that FACs-purified CD105+ hADSCs possess more osteogenic differentiation potential than CD105+ and unsorted cells in vitro, and also show decreased TGF-β1 and Smad2 phosphorylation. Treatment with TGF-β1 significantly reduces the osteogenic differentiation of CD105+ ADSCs in vitro. In contrast, treatment with the Alk5 inhibitor enhances osteogenic differentiation. Moreover, CD105 knockdown promoted the bone-forming potential of ADSCs in immunodeficient animals in vivo [120-122].

We FACs-purified 4 different sub-populations of mADSCs; CD105+CD34+, CD105-CD34+, CD105 CD34+ and CD105+CD34+ and tested their BMP-responsiveness in vitro. Only CD105+CD34+ cells, showing the classical MSCs phenotype, responded to BMPs while others did not show significant response. We hypothesized that the ADSCs population maximally responding to BMPs in vitro would possess the ability to induce bone formation, and therefore investigated the bone-forming potential of CD105+CD34+ ADSCs in immunocompetent mice. Our hypothesis was clearly refuted and CD105+CD34 ADSCs could not induce any bone formation [123]. Although we did not test the bone-forming ability of other three FACs-purified populations in that study, we found that bone marrow-derived D1 osteoprogenitors and also that bone marrow-derived D1 osteoprogenitors isolated from the same Balb/c mouse strain, did not express CD105 and did not respond to BMPs in vitro, but showed robust ability to induce bone formation [123,124]. Data from our group and others suggest that CD105 population represents true osteoprogenitors and inhibition of TGF-β1 signaling can improve the bone-forming ability of ADSCs. However, the bone-forming ability of CD105- ADSCs is not yet established in immunocompetent hosts. FACs purified CD105+ human bone marrow-derived MSCs showed superior osteogenic efficacy when compared to CD105- cells in vitro. In critical-size defects created in the tibia of canine, CD105+MSCs implantation led to superior bone healing with complete bone remodeling, while CD105- MSCs implants failed to remodel resulting in the defect site filled with fibrocartilaginous tissue [125]. In sum, these studies showed that CD105- cells have more osteogenic potential in vitro as well as in vivo.

We have shown that simultaneously inhibiting TGF and BMP signaling pathways by using small chemical inhibitors induces neuronal differentiation of hADSCs in vitro and neurite outgrowth in vitro [126]. Previously this was demonstrated in ESCs and iPSCs, but not in adult ADSCs. It is well established that activin/nodal signaling contributes to the maintenance of pluripotency of hESCs. Activin/nodal/TGF-β and BMP pathways naturally antagonize each other because they compete for a common signal transducer Smad4. Inhibition of activin/nodal/TGF-β signaling results in trophoblast differentiation, similar to induction of trophoblast differentiation by BMP-4 [127]. These findings reveal the crucial roles of TGF-β and BMP signaling in deciding the fate of ADSCs.

In a recent discovery, the phenotype of mouse skeletal cells (mSSC) has been described as the CD45+ Tier19+ Tie2+ AlphaV+ Thy6C+ CD105+CD200+ cells which were isolated from femoral growth plates of the mice [128]. These CD105+ cells were able to form bone in vivo when implanted beneath the kidney capsule of T-cell deficient mice. Surprisingly, these cells were not efficiently engrafted, suggesting their requirement for a supportive niche. When these cells were transplanted with unsorted cells, they could form both bone and cartilage. Blocking VEGF signaling promoted chondrogenesis. Subcutaneous implantation of BMP2 in a collagen sponge in mouse inguinal pad formed ectopic bone; however, it did not originate from circulating SSCs recruited to implanted sites but SSCs formation was induced in the adipose tissue. It is not clear whether the CD105+ or CD105- population of adipose tissue contributed to SSCs formation and this ectopic bone formation. Co-delivery of BMP2 with VEGF inhibitor into adipose tissue favored cartilage formation over bone [128]. We have shown that the crosstalk between BMP and VEGF signaling pathways enhances osteogenic differentiation of hADSCs through the p38 signaling pathway. Mineralization was abrogated when the p38 signaling pathway was inhibited [129]. We also found that VEGF could crosstalk with a downstream signal mediator of BMP, LIM mineralization protein I (LMP1) to enhance cell mineralization and ectopic bone formation mediated
by osteoprogenitors[130]. Similar to mSSCs, human skeletal stem cells (hSSCs) formation has also been reported, by the discoverers of mSSCs, in BMP2 treated adipose tissue. hSSCs displayed the phenotype PDPN⁺ CD73⁺ CD164⁺ CD146⁻ [131].

### CD271

CD34⁺CD271⁺ hADSCs showed increased osteogenic differentiation compared to CD34⁺CD271⁻ and SVF whereas adipogenic and chondrogenic differentiation were similar[132].

### CXCR4

FACS purified CD146⁺CD31⁻CD45⁻ hADSCs isolated from different origins such as the periosteum, adipose, and dermal tissue display different degrees of osteogenic capabilities. Periosteal cells also express standard adult stem cell markers (CD105, CD90, CD73), Gli1, PDGRFa, and CXCR4; and are known to be more osteogenic in vitro as well as in vivo unlike soft tissue-derived CD146⁺CD31⁺CD45⁻ ADSCs. Inhibition of CXCR4 expression abolishes the ability of these ADSCs to induce ectopic bone formation. Unsorted ADSCs as well as CD146⁺ ADSCs further selected for CXCR4+ show enhanced osteogenic potential in vitro and in vivo[133].

### PDGFRα

PDGFRα⁺CD34⁺, PDGFRα⁺CD34⁻, PDGFRα⁻CD34⁺, and PDGFRα⁻CD34⁻ were sorted from SVF of mouse adipose tissue from PDGFRα⁺CreER and PDGFRα⁻CreER mice. The authors found that PDGFRα⁺CD34⁺ ADSCs displayed more osteogenic potential in vitro. They also found that subcutaneously implantation of PDGFRα⁻ cells and subcutaneous implantation of BMP2 into inguinal fat pads of PDGFRα⁺CreER mice formed more bone as compared to controls[134].

### CD105 AND SSEA3 EXPRESSING MUSE CELLS

Multilineage-differentiating stress-enduring (Muse) cells were first identified from bone marrow, which are of interest. These cells are positive for mesenchymal and embryonic stem cell markers CD105 and SSEA3. Muse cells comprise a small population of MSCs in BM-MSCs (1%-2%) and ADSCs (5%). 250000-500000 cells can be obtained from one gram of lipoaspirate. Adipose-derived Muse cells spontaneously differentiate into all three germ layers: mesodermal, endodermal, and ectodermal cell lineages and have non-tumorigenic and immunomodulatory properties. Muse cells have been successfully used for regeneration of skin, muscle, liver, kidney in different animal disease models however it has not been tested for its osteogenic differentiation potential[135].

Thus, the selection of subpopulations of ADSCs can harness abundantly available ADSCs for applications in bone regeneration.

### CONCLUSION

The safety of ADSCs is reasonably established since they have been tested in 79 clinical trials including 580 patients total and there have been no serious adverse events reported. However, the clinical trials, as well as the pre-clinical studies investigating the potential of ADSCs in enhancing bone regeneration, have given confounding outcomes. In some cases, they were reported to enhance bone healing whereas, in others, they have failed to do so. It is also difficult to compare outcomes of different studies as investigators have used different animal models, delivery methods, and genetic manipulation of ADSCs. In many of the pre-clinical studies, T cell-deficient hosts were used. This transplant scenario is unlikely to provide a realistic picture of the osteogenic potential of ADSCs since T cells are likely to modulate bone regeneration induced by exogenously added adult stem cells. After careful review of
all the published reports, it is safe to conclude that ADSCs in their unaltered and unpurified form cannot be considered as reliable therapy for bone repair yet. Two major steps can be taken to solve this problem - first is to develop potency assays for each batch of ADSCs used in clinical and pre-clinical studies to allow comparison of outcomes of different studies and second is to search for a unique and reliable set of surface markers to define ADSCs. The current definition of adult stem cells can no longer be applied to ADSCs since both CD105+ as well as CD105+ fractions of ADSCs have been shown to possess bone forming potential. Surface markers such as CD146, AlphaV, CD200, PDPN, CD164, CXCR4, and PDGFRα will play an important role in defining osteogenic population within ADSCs in coming years. Areas such as the role of endogenous bone-progenitors in bone regeneration induced by exogenously added ADSCs and BMP-responsiveness of ADSCs also need immediate attention. Most of the studies published so far have not evaluated the survival and differentiation of transplanted ADSCs as well as recruitment of endogenous bone-progenitors to investigate whether the regenerated bone is donor stem cells-derived or originates from endogenous precursors. While BMPs are thought to promote differentiation of stem cells into the osteogenic lineage and BMP-overexpression has increased bone-forming potential of ADSCs in certain animal models, some investigators have also reported that ADSCs do not respond to BMPs. This observation and recent findings that implantation of BMP in adipose stroma leads to skeletal reprogramming and de novo formation of skeletal stem cells in adipose tissue, together, demand urgent attention of the scientific community to signaling pathways of ADSCs during osteogenic differentiation and after BMP stimulation. VEGF, BMP and TGF-β signaling pathways are the most important ones in this regard. Although the current clinically tested ADSC therapies do not yet appear to induce bone repair reliably, the ADSC optimizations described in this manuscript, based on cell subset purification and stimulus/activation, show great promise, and could potentially dominate stem cell-based therapies such as bone regeneration in the future.

REFERENCES

1. Babu S, Sandford NA, Vrahhs M. Use of Teriparatide to improve fracture healing: What is the evidence? World J Orthop 2015; 6: 457-461 [PMID: 26191492 DOI: 10.5312/wjo.v6.i6.457]
2. Bishop GB, Einhorn TA. Current and future clinical applications of bone morphogenetic proteins in orthopaedic trauma surgery. Int Orthop 2007; 31: 721-727 [PMID: 17668207 DOI: 10.1007/s00264-007-0424-8]
3. Tatara AM, Mikos AG. Tissue Engineering in Orthopaedics. J Bone Joint Surg Am 2016; 98: 1132-1139 [PMID: 27385607 DOI: 10.2106/JBJS.16.00299]
4. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop DJ, Horwitz E. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 2006; 8: 315-317 [PMID: 16923606 DOI: 10.1080/14653240600855905]
5. Lin CS, Ning H, Lin G, Lue TF. Is CD34 truly a negative marker for mesenchymal stromal cells? Cytotherapy 2012; 14: 1159-1163 [PMID: 23066784 DOI: 10.3109/14653249.2012.729817]
6. Bourin P, Bunnell BA, Castella L, Dominici M, Katz AJ, March KL, Redl H, Rubin JP, Yoshimura K, Gimble JM. Stromal cells from the adipose tissue-derived stromal vascular fraction and culture expanded adipose tissue-derived stromal/stem cells: a joint statement of the International Federation for Adipose Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ISCT). Cytotherapy 2013; 15: 641-648 [PMID: 23570660 DOI: 10.1016/j.jcyt.2013.02.006]
7. Mendicino M, Bailey AM, Wendacott K, Puri RK, Bauer SR. MSC-based product characterization for clinical trials: an FDA perspective. Cell Stem Cell 2014; 14: 141-145 [PMID: 24506881 DOI: 10.1016/j.stem.2014.01.013]
8. Hernigou P, Poignard A, Beaujean F, Rouard H. Percutaneous autologous bone-marrow grafting for nonunions. Influence of the number and concentration of progenitor cells. J Bone Joint Surg Am 2005; 87: 1430-1437 [PMID: 15995108 DOI: 10.2106/JBJS.D.02215]
9. Hernigou P, Housset V, Dubory A, Rouard H, Auregan JC. Early injection of autologous bone marrow concentrates decreases infection risk and improves healing of acute severe open tibial fractures. Injury 2020 [PMID: 33423770 DOI: 10.1016/j.injury.2020.12.007]
10. Labibzadeh N, Emadedin M, Fazeli R, Mohseni F, Hosseini SE, Mardpour S, Azimian V, Ghobari Liastani M, Mirazimi Baghi F, Baghban Esalamnejad M, Aghdami N. Mesenchymal Stromal Cells Implantation in Combination with Platelet Lysate Product Is Safe for Reconstruction of Human Long Bone Nonunion. Cell J 2016; 18: 302-309 [PMID: 27602311 DOI: 10.22074/cellj.2016.4557]
11. Quarto R, Mastrogiacomo M, Cancetta R, Kutelev SM, Mukhachev V, Lavroukou A, Kon E, Marucci M. Repair of large bone defects with the use of autologous bone marrow stromal cells. N Engl J Med 2001; 344: 385-386 [PMID: 11195802 DOI: 10.1056/NEJM200102103440501]
12 Marceci M, Kon E, Moukhachev V, Lavroukov A, Katepov S, Quarto R, Mastrogiacomo M, Cancetta R. Stem cells associated with macroporous bioceramics for long bone repair: 6- to 7-year outcome of a pilot clinical study. *Tissue Eng 2007;* 13: 947-955 [PMID: 17484701 DOI: 10.1089/tc.2006.0271]

13 Connolly JF. Clinical use of marrow osteoprogenitor cells to stimulate osteogenesis. *Clin Orthop Relat Res* 1998; S257-S266 [PMID: 9917645 DOI: 10.1097/00003086-199810010-00026]

14 Dallari D, Savarino L, Stagni C, Cenni E, Cenacchi A, Fornarsari PM, Alabisni U, Rimondi E, Baldini N, Giunti A. Enhanced tibial osteotomy healing with use of bone grafts supplemented with platelet gel or platelet gel and bone marrow stromal cells. *J Bone Joint Surg Am 2007;* 89: 2413-2420 [PMID: 1794883 DOI: 10.2166/JBJS.F.01026]

15 Schmid U, Thielemann F, Weise K, Ochs BG. [A novel therapeutic approach to bone replacement: vivalisation of industrial processed allogenic bone graft with autologous bone marrow]. *Z Orthop Unfall 2007;* 145: 221-229 [PMID: 1792564 DOI: 10.1055/s-2007-96204]

16 Jäger M, Herten M, Fochtmann U, Fischer J, Hernigou P, Zilkens C, Hendrich C, Krauspe R. Bridging the gap: bone marrow aspiration concentrate reduces autologous bone grafting in osseous defects. *J Orthop Res* 2011; 29: 173-180 [PMID: 20740672 DOI: 10.1002/jor.21230]

17 Younger EM, Chapman MW. Morbidity at bone graft donor sites. *J Orthop Trauma 1989;* 3: 192-195 [PMID: 2809818 DOI: 10.1097/00005131-198909000-00002]

18 Oryan A, Alidadi S, Moshiri A, Maffulli N. Bone regenerative medicine: classic options, novel strategies, and future directions. *J Orthop Surg Res 2014;* 9: 18 [PMID: 24628910 DOI: 10.1186/1749-799X-9-18]

19 Delimar D, Smoljanovic T, Bojanic I. Could the use of bone morphogenetic proteins in fracture healing do more harm than good to our patients? *Int Orthop 2012;* 36: 683; author reply 685 [PMID: 22052478 DOI: 10.1007/s00264-011-1397-1]

20 Nauth A, Ristinemi J, McKee MD, Schemitsch EH. Bone morphogenetic proteins in open fractures: past, present, and future. *Injury 2009;* 40 Suppl 3: S27-S31 [PMID: 20082787 DOI: 10.1016/S0260-3833(09)70008-7]

21 Garrison KR, Shemilt I, Donnell S, Ryder JJ, Mugford M, Harvey I, Song F, Alt V. Bone morphogenetic protein (BMP) for fracture healing in adults. *Cochrane Database Syst Rev 2010;* CD006950 [PMID: 2055677 DOI: 10.1002/14651858.CD006950.pub2]

22 Olsen TR, Ng KS, Lock LT, Ahsan T, Rowley JA. Peak MSC-Are We There Yet? *Front Med (Lausanne)* 2015; 5: 178 [PMID: 29977893 DOI: 10.3389/fmed.2018.00175]

23 Strioga M, Viswanathan S, Darwinskas A, Slaby O, Michalek J. Same or not the same? vs21: 2724-2752 [PMID: 22468918 DOI: 10.1089/scd.2011.0722]

24 Kern S, Eichler H, Stoeve J, Kilter H, Bieback K. Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. *Stem Cells 2006;* 24: 1293-1301 [PMID: 16410387 DOI: 10.1634/stemcells.2005-0342]

25 De Ugarte DA, Morizono K, Elbarbary A, Alfonso Z, Zak PA, Zhu M, Dragoo JL, Ashjian P, Thomas B, Benhamin P, Chen I, Fraser J, Hedrick MH. Comparison of multi-lineage cells from human adipose tissue and bone marrow. *Cells Tissues Organs 2003;* 174: 101-109 [PMID: 12835573 DOI: 10.1159/000071150]

26 Sipp D, Robey PG, Turner L. Clear up this stem-cell mess. *Nature 2018;* 561: 455-457 [PMID: 30258150 DOI: 10.1038/s41586-018-06756-9]

27 Galipeau J, Sensébé L. Mesenchymal Stromal Cells: Clinical Challenges and Therapeutic Opportunities. *Cell Stem Cell 2018;* 22: 824-833 [PMID: 29859173 DOI: 10.1016/j.stem.2018.05.004]

28 Skovrj B, Guzman JZ, Al Maahia M, Cho SK, Iatridis JC, Qureshi SA. Cellular bone matrices: viable stem cell-containing bone graft substitutes. *Spine J 2014;* 14: 2763-2772 [PMID: 24929059 DOI: 10.1016/j.spinee.2014.05.024]

29 Pak J, Chang JJ, Lee JH, Lee SH. Safety reporting on implantation of autologous adipose tissue-derived stem cells with platelet-rich plasma into human articular joints. *BMJ Musculoskeletal Disord* 2013; 14: 337 [PMID: 24289766 DOI: 10.1186/1477-2580-13-337]

30 Saxer F, Schererbach A, Todorov A, Studer P, Miot S, Schreiner S, Güven S, Tchang LA, Haug M, Herten M, Fochtmann U, Fischer J, Hernigou P, Zilkens C, Hendrich C, Krauspe R. Implantation of Stromal Vascular Fraction Progenitors at Bone Fracture Sites: From a Rat Model to a First-in-Man Study. *Stem Cells 2016;* 34: 2956-2966 [PMID: 27538760 DOI: 10.1002/stem.2478]

31 Thesleff T, Lehtimäki K, Niskakangas T, Mannerström B, Miettinen S, Suuronen R, Öhman J. Cranioplasty with adipose-derived stem cells and biomaterial: a novel method for cranial reconstruction. *Neurosurgery* 2011; 68: 1555-1560 [PMID: 21356223 DOI: 10.1227/NEU.0b013e31820e024c]

32 Thesleff T, Lehtimäki K, Niskakangas T, Huovinen S, Mannerström B, Miettinen S, Seppänen-Kajiansinko R, Öhman J. Cranioplasty with Adipose-Derived Stem Cells, Beta-Tri calcium Phosphate Granules and Supporting Mesh: Six-Year Clinical Follow-Up Results. *Stem Cells Transl Med 2017;* 6: 1576-1582 [PMID: 28504874 DOI: 10.1002/stcm.16-0410]

33 Sándor GK, Numminen J, Wolff J, Thesleff T, Miettinen A, Tuovinen VJ, Mannerström B, Patriksson M, Seppänen R, Miettinen S, Rautiainen M, Öhman J. Adipose stem cells used to reconstruct 13 cases with cranio-maxillofacial hard-tissue defects. *Stem Cells Transl Med 2014;* 3: 530-540 [PMID: 24558162 DOI: 10.5966/stemcells.2013-0173]

34 Dufrane D, Docquereau P, Deloye C, Poirel H, André W, Aouassar N. Scaffold-free Three-
dimensional Graft From Autologous Adipose-derived Stem Cells for Large Bone Defect Reconstruction: Clinical Proof of Concept. Medicine (Baltimore) 2015; 94: e2220 [PMID: 26683933 DOI: 10.1097/MD.00000000000002220]

35 Prius HJ, Schultz EA, Ten Bruggenkate CM, Klein-Nulend J, Helder MN. Bone Regeneration Using the Freshly Isolated Autologous Stromal Vascular Fraction of Adipose Tissue in Combination With Calcium Phosphate Ceramics. Stem Cells Transl Med 2016; 5: 1362-1374 [PMID: 27388241 DOI: 10.5966/stcm.2015-0369]

36 Khojasteh A, Kheiri L, Behnia H, Tehranchi A, Nazeman P, Nadjini N, Soleimani M. Lateral Ramus Cortical Bone Plate in Alveolar Cleft Osteoplasty with Concomitant Use of Buccal Fat Pad Derived Cells and Autogenous Bone: Phase I Clinical Trial. Biomed Res Int 2017; 2015: 6502034 [PMID: 29379800 DOI: 10.1155/2017/6502034]

37 Castillo-Cardiel G, López-Echaury AC, Saucedo-Ortiz JA, Fuentes-Orozco C, Michel-Espinosa LR, Irusteta-Jiménez L, Salazar-Parra M, González-Ojeda A. Bone regeneration in mandibular fractures after the application of autologous mesenchymal stem cells, a randomized clinical trial. Dent Traumatol 2017; 33: 38-44 [PMID: 27513920 DOI: 10.1111/det.12303]

38 Myerson CL, Myerson MS, Coetzee JC, Stone McGaver R, Giveans MR. Subtalar Arthrodesis with Use of Adipose-Derived Cellular Bone Matrix Compared with Autologous Bone Graft: A Multicenter, Randomized Controlled Trial. J Bone Joint Surg Am 2019; 101: 1904-1911 [PMID: 31567665 DOI: 10.2106/jbjs.18.01300]

39 Freitas GP, Lopes HB, Almeida ALG, Albuna RPF, Gimenes R, Souza LEB, Covas DT, Beloti MM, Rosa AL. Potential of Osteoblastic Cells Derived from Bone Marrow and Adipose Tissue Associated with a Polymer/Ceramic Composite to Repair Bone Tissue. Calcif Tissue Int 2017; 101: 312-320 [PMID: 28451713 DOI: 10.1007/s00223-017-0282-3]

40 Niemeyer P, Fechner K, Milz S, Richter W, Suedkamp NP, Mehlhorn AT, Pearce S, Kasten P. Comparison of mesenchymal stem cells from bone marrow and adipose tissue for bone regeneration in a critical size defect of the sheep tibia and the influence of platelet-rich plasma. Biomaterials 2010; 31: 3572-3579 [PMID: 20153047 DOI: 10.1016/j.biomaterials.2010.01.085]

41 Pourrebrinah N, Hashemibenib B, Shahnasereib S, Torabiniib M, Houssibiib S, Adibib S, Heidariib F, Alavib MJ. A comparison of tissue-engineered bone from adipose-derived stem cell with autogenous bone repair in maxillary alveolar cleft model in dogs. Int J Oral Maxillofac Surg 2013; 42: 562-568 [PMID: 23219713 DOI: 10.1016/j.ijom.2012.10.012]

42 Godoy Zanieotti D, Decestre DE, Duncan WJ. In vivo bone regeneration on titanium devices using serum-free grown adipose-derived stem cells, in a sheep femur model. Clin Oral Implants Res 2017; 28: 64-75 [PMID: 26853552 DOI: 10.1111/ctr.12761]

43 Brennan MA, Renaud A, Guilloton F, Mebarki M, Trichet V, Sensèbe L, Deschaseaux F, Chevallier N, Layrolle P. Inferior In Vivo Osteogenesis and Superior Angiogenesis of Human Adipose-Derived Stem Cells Compared with Bone Marrow-Derived Stem Cells Cultured in Xeno-Free Conditions. Stem Cells Transl Med 2017; 6: 2160-2172 [PMID: 29052365 DOI: 10.1002/stcm.17-0133]

44 Xu L, Liu Y, Sun Y, Wang B, Xiong Y, Lin W, Wei Q, Wang H, He W, Li G. Tissue source determines the differentiation potentials of mesenchymal stem cells: a comparative study of human mesenchymal stem cells from bone marrow and adipose tissue. Stem Cell Res Ther 2018; 9: 275 [PMID: 29208029 DOI: 10.1186/s13287-017-0716-x]

45 Reinsch A, Eichart N, Thomas D, Hofmann NA, Freuehцhrt W, Sinha S, Chan CK, Seranath-Yapa K, Seo EY, Wearda T, Hartwig UF, Beham-Schmid C, Trajanoski S, Liu Q, Wagner W, Dullin C, Alves F, Andreeff M, Weissman IL, Longaker MT, Schallmoser K, Majeti R, Strunk D. Epigenetic and in vivo comparison of diverse MSC sources reveals an endochondral signature for human hematopoietic niche formation. Blood 2015; 125: 249-260 [PMID: 25406351 DOI: 10.1182/blood-2014-04-722525]

46 Ma J, Yang F, Both SK, Prins HJ, Helder MN, Pan J, Cui FZ, Jansen JA, van den Beucken J. Bone forming capacity of cell- and growth factor-based constructs at different ectopic implantation sites. J Biomed Mater Res A 2015; 103: 439-450 [PMID: 24737694 DOI: 10.1002/jbm.a.35192]

47 Fennema EM, Tchhang LAH, Yuan H, van Blitterswijk CA, Martin I, Schererich A, de Boer J. Ectopic bone formation by aggregated mesenchymal stem cells from bone marrow and adipose tissue: A comparative study. J Tissue Eng Regen Med 2018; 12: e150-e158 [PMID: 28485099 DOI: 10.1002/term.2453]

48 Levi B, James AW, Nelson ER, Vistnes D, Wu B, Lee M, Gupta A, Longaker MT. Human adipose derived stromal cells heal critical size mouse calvarial defects. PLoS One 2010; 5: e11177 [PMID: 20565750 DOI: 10.1371/journal.pone.0011177]

49 Chou VF, Zuk PA, Chang TL, Benhaim P, Wu BM. Adipose-derived stem cells and BMP2: part 1. BMP2-treated adipose-derived stem cells do not improve repair of segmental femoral defects. Connect Tissue Res 2011; 52: 109-119 [PMID: 20701464 DOI: 10.3109/03008207.2010.484514]

50 Zuk P, Chou VF, Mussano F, Benhaim P, Wu BM. Adipose-derived stem cells and BMP2: part 2. BMP2 may not influence the osteogenic fate of human adipose-derived stem cells. Connect Tissue Res 2011; 52: 119-132 [PMID: 20701465 DOI: 10.3109/03008207.2010.484515]

51 Runyan CM, Ali ST, Chen W, Calder BW, Rumburg AE, Billmire DA, Taylor JA. Bone tissue engineering by way of allograft revitalization: mechanistic and mechanical investigations using a porcine model. J Oral Maxillofac Surg 2014; 72: 1000.e1-1000.11 [PMID: 24742484 DOI: 10.1016/j.joms.2014.01.017]

52 Keibl C, Füg G, Zanoni S, Tangl S, Wolbank S, Redl H, van Griensven M. Human adipose derived
stem cells reduce callus volume upon BMP-2 administration in bone regeneration. *Injury* 2011; 42: 814-820 [PMID: 21457972 DOI: 10.1016/j.injury.2011.03.007]

53 Qing W, Guang-Xing C, Lin G, Liu Y. The osteogenic study of tissue engineering bone with BMP2 and BMP7 gene-modified rat adipose-derived stem cell. *J Biomed Biotechnol* 2012; 2012: 410879 [PMID: 22778550 DOI: 10.1155/2012/410879]

54 Jeong W, Kim YS, Roh TS, Kang EH, Jung BK, Yun IS. The effect of combination therapy on critical-size bone defects using non-activated platelet-rich plasma and adipose-derived stem cells. *Childs Nerv Syst* 2020; 36: 145-151 [PMID: 30879128 DOI: 10.1007/s00381-019-04109-2]

55 Mazzoni E, D’Agostino A, Iaquinta MR, Bononi I, Trevisiol L, Rotondo JC, Paternagni S, Giorgi C, Gunson MJ, Arnett GW, Nocini PF, Tognon M, Martini F. Hydroxyapatite-collagen hybrid scaffold induces human adipose-derived mesenchymal stem cells to osteogenic differentiation in vitro and bone regrowth in patients. *Stem Cells Transl Med* 2020; 9: 377-388 [PMID: 31834992 DOI: 10.1002/sctm.19-0170]

56 Kang BJ, Ryu HH, Park SS, Koyama Y, Kikuchi M, Woo HM, Kim WH, Kweon OK. Comparing the osteogenic potential of canine mesenchymal stem cells derived from adipose tissues, bone marrow, umbilical cord blood, and Wharton’s jelly for treating bone defects. *J Vet Sci* 2012; 13: 299-310 [PMID: 23000587 DOI: 10.4142/jvs.2012.13.3.299]

57 Toplu G, Ozcelik D, Serin M, Erdem H, Topacoglu AT. Adipose Tissue-Derived Stromal Vascular Fraction Increases Osteogenesis in an Experimental Design Zygomatic Bone Defect Model. *J Craniofac Surg* 2017; 28: 2179-2182 [PMID: 28938327 DOI: 10.1097/SCS.0000000000002980]

58 Kim A, Kim DH, Song HR, Kang WH, Kim HJ, Lim HC, Cho DW, Bae JH. Repair of rabbit ulna segmental bone defect using freshly isolated adipose-derived stromal vascular fraction. *Cytotherapy* 2012; 14: 296-305 [PMID: 22091832 DOI: 10.1016/j.jcyt.2011.07.019]

59 Cui L, Liu B, Liu G, Zhang W, Cen L, Sun J, Yin S, Liu W, Cao Y. Repair of cranial bone defects with adipose-derived stem cells and coral scaffold in a canine model. *Biomaterials* 2007; 28: 5477-5486 [PMID: 17885208 DOI: 10.1016/j.biomaterials.2007.08.042]

60 Shah AR, Cornejo A, Guda T, Sahar DE, Stephenson SM, Chang S, Krishnegowda NK, Sharma R, Wang HT. Differentiated adipose-derived stem cell cocultures for bone regeneration in polymer scaffolds in vivo. *J Craniofac Surg* 2014; 25: 1504-1509 [PMID: 24943502 DOI: 10.1097/SCS.0000000000000755]

61 Sahar DE, Walker JA, Wang HT, Stephenson SM, Shah AR, Krishnegowda NK, Wenke JC. Effect of endochondral differentiated adipose-derived stem cells on vascularity and osteogenesis in poly(D,L-lactide) scaffolds in vivo. *J Craniofac Surg* 2012; 23: 913-918 [PMID: 22627404 DOI: 10.1097/SCS.0b013e1821e5cd8]

62 Zhang H, Kot A, Lay YE, Fierro FA, Chen H, Lane NE, Yao W. Acceleration of Fracture Healing by Overexpression of Basic Fibroblast Growth Factor in the Mesenchymal Stromal Cells. *Stem Cells Transl Med* 2017; 6: 1880-1893 [PMID: 28792122 DOI: 10.1002/stem.10036]

63 Kim I, Lee SS, Kim SHL, Bae S, Lee H, Hwang NS. Osteogenic Effects of VEGF-Overexpressed Human Adipose-Derived Stem Cells with Whitlockite Reinforced Cryogel for Bone Regeneration. *Macromol Biosci* 2019; 19: e1800460 [PMID: 30821921 DOI: 10.1002/mabi.201800460]

64 Behr B, Tang C, Germann G, Longaker MT, Quarto N. Locally applied vascular endothelial growth factor A increases the osteogenic healing capacity of human adipose-derived stem cells by promoting osteogenic and endothelial differentiation. *Stem Cells* 2011; 29: 286-296 [PMID: 21732486 DOI: 10.1002/stem.581]

65 Du J, Xie P, Lin S, Wu Y, Zeng D, Li Y, Jiang X. Time-Phase Sequential Utilization of Adipose-Derived Mesenchymal Stem Cells on Mesoporous Bioactive Glass for Restoration of Critical Size Bone Defects. *ACS Appl Mater Interfaces* 2018; 10: 28340-28350 [PMID: 30080385 DOI: 10.1021/acsami.8b08563]

66 Rindone AN, Kachniziar B, Achebe CC, Riddle RC, O'Sullivan AN, Dorafshar AH, Grayson WL. Heparin-Conjugated Decellularized Bone Particles Promote Enhanced Osteogenic Signaling of PDGF-BB to Adipose-Derived Stem Cells in Tissue Engineered Bone Grafts. *Adv Healthc Mater* 2019; 8: e1801565 [PMID: 30941920 DOI: 10.1002/adhm.201801565]

67 Park S, Heo HA, Lee KB, Kim HG, Pyo SW. Improved Bone Regeneration With Multiporous PLGA Scaffold and BMP-2-Transduced Human Adipose-Derived Stem Cells by Cell-Permeable Peptide. *Implant Dent* 2017; 26: 4-11 [PMID: 27893514 DOI: 10.1177/1049249817695223]

68 Lin L, Shen Q, Wei X, Hou Y, Xue T, Fu X, Duan X, Yu C. Comparison of osteogenic potentials of BMP4 transduced stem cells from autologous bone marrow and fat tissue in a rabbit model of calvarial defects. *Culcis Tissue Int* 2009; 85: 55-65 [PMID: 19424739 DOI: 10.1007/s00223-009-9520-x]

69 Hao W, Dong J, Jiang M, Wu J, Cui F, Zhou D. Enhanced bone formation in large segmental radial defects by combining adipose-derived stem cells expressing bone morphogenetic protein 2 with sHA/RHLC/PLA scaffold. *Int Orthop* 2010; 34: 1341-1349 [PMID: 20140671 DOI: 10.1007/s00264-009-0946-3]

70 Lin CY, Wang YH, Li KC, Sung LY, Yeh CL, Lin KJ, Chen TC, Chang YH, Hu YC. Healing of massive segmental femoral bone defects in minipigs by allogenic ASCs engineered with FLPe/Frt-based baculovirus vectors. *Biomaterials* 2015; 50: 98-106 [PMID: 25736500 DOI: 10.1016/j.biomaterials.2015.01.052]

71 Fan J, Im CS, Cui ZK, Guo M, Bezouglia O, Fastash A, Lee JY, Nguyen J, Wu BM, Aghaloo T, Lee M. Delivery of Phenamid Enhances BMP-2-Induced Osteogenic Differentiation of Adipose-
Le Q et al. Adipose-derived stem cells for bone regeneration

Derived Stem Cells and Bone Formation in Calvarial Defects. Tissue Eng Part A 2015; 21: 2053-2065 [PMID: 25869476 DOI: 10.1089/teng.TEA.2014.0489]

72 Li KC, Lo SC, Sung LY, Liao YH, Chang YH, Hu YC. Improved calvarial bone repair by hASCs engineered with Cre/LoxP-based baculovirus conferring prolonged BMP-2 and MiR-148h co-expression. J Tissue Eng Regen Med 2017; 11: 3068-3077 [PMID: 27687795 DOI: 10.1002/tcr.2208]

73 Weimin P, Zheng C, Shuaiuqin J, Dan L, Jianchang Y, Yue H. Synergistic enhancement of bone regeneration by LMP-1 and HIF-1α delivered by adipose derived stem cells. Biotechnol Lett 2016; 38: 377-384 [PMID: 26564407 DOI: 10.1007/s10529-015-1988-9]

74 Levi B, Hyun JS, Nelson ER, Li S, Montoro DT, Wan DC, Jia FJ, Glotzbach JC, James AW, Lee M, Huang M, Quarto N, Gurtner GC, Wu JC, Longaker MT. Nonintegrating knockdown and customized scaffold design enhances human adipose-derived stem cells in skeletal repair. Stem Cells 2011; 29: 2018-2029 [PMID: 21997852 DOI: 10.1002/stem.757]

75 Fan J, Park H, Lee MK, Bezouglaia O, Farata A, Kim J, Aghaloo T, Lee M. Adipose-derived stem cells and BMP-2 delivery in chitosan-based 3D constructs to enhance bone regeneration in a rat mandibular defect model. Tissue Eng Part A 2014; 20: 2169-2179 [PMID: 24524819 DOI: 10.1089/teng.TEA.2013.0523]

76 Wang Y, Liu Y, Zhang M, Lv L, Zhang X, Zhang P, Zhou Y. Inhibition of PTGIS1 promotes osteogenic differentiation of adipose-derived stem cells by suppressing NF-κB signaling. Stem Cell Res Ther 2019; 10: 57 [PMID: 30760327 DOI: 10.1186/s13287-019-1167-3]

77 Xie Q, Wang Z, Zhou H, Yu Z, Huang Y, Sun H, Bi X, Wang Y, Sh i W, Gu P, Fan X. The role of miR-135-modified adipose-derived mesenchymal stem cells in bone regeneration. Biomaterials 2016; 75: 279-294 [PMID: 26513420 DOI: 10.1016/j.biomaterials.2015.10.042]

78 Gao L, Huang Z, Yan S, Zhang K, Xu S, Li G, Cui L, Yin J. Sr-HA-graft-Poly-(b enzyl-l-glutamate) Nano composite Microcarriers: Controllable Sr²⁺ Release for Accelerating Osteogenesis and Bone Nonunion Repair. Biomacromolecules 2017; 18: 3742-3752 [PMID: 28960963 DOI: 10.1021/acs.biomac.7b01101]

79 Doğan A, Demirci S, Bayır Y, Halıcı Z, Karakus E, Aydin A, Cadirci E, Albayrak A, Demirci E, Karahan A, Ayan AK, Gundogdu C, Sahin F. Boron containing poly(lactide-co-glycolide) (PLGA) scaffolds for bone tissue engineering. Mater Sci Eng C Mater Biol Appl 2014; 44: 246-253 [PMID: 25280703 DOI: 10.1016/j.msec.2014.08.035]

80 Shin J, Cho JH, Jin Y, Yang K, Lee JS, Park HJ, Han HS, Lee J, Jeon H, Shin H, Cho SW. Mussel Adhesion-Inspired Reverse Transfection Platform Enhances Osteogenic Differentiation and Bone Formation of Human Adipose-Derived Stem Cells. Small 2016; 12: 6266-6278 [PMID: 27717233 DOI: 10.1002/smll.201601868]

81 Wang CC, Wang CH, Chen HC, Cheng JH, Chang SJ, Wang YW, Chang A, Yeh JZ, Huang YH, Liu CC. Combination of resveratrol-containing collagen with adipose stem cells for craniofacial tissue-engineering applications. Int Wound J 2018; 15: 660-672 [PMID: 29336622 DOI: 10.1111/iwj.12910]

82 Man Y, Wang P, Guo Y, Xiang L, Yang Y, Qu Y, Gong P, Deng L. Angiogenic and osteogenic potential of platelet-rich plasma and adipose-derived stem cell ladden alginate microspheres. Biomaterials 2012; 33: 8802-8811 [PMID: 22981779 DOI: 10.1016/j.biomaterials.2012.08.054]

83 Wang CZ, Chen SM, Chen CH, Wang CK, Wang GJ, Chang JK, Ho ML. The effect of the local delivery of alendronate on human adipose-derived stem cell-based bone regeneration. Biomaterials 2010; 31: 8674-8683 [PMID: 20719378 DOI: 10.1016/j.biomaterials.2010.07.096]

84 Deng B, Zhu W, Duan Y, Hu Y, Chen X, Song S, Yi Z, Song Y. Exendin4 promotes osteogenic differentiation of adipose-derived stem cells and facilitates bone repair. Mol Med Rep 2019; 20: 4933-4942 [PMID: 31661134 DOI: 10.3892/mmr.2019.10764]

85 Li KC, Chang YH, Hsu MN, Lo SC, Li WH, Hu YC. Baculovirus-Mediated miR-214 Knockdown Shifts Osteoprotic ASCs Differentiation and Improves Osteoprotic Bone Defects Repair. Sci Rep 2017; 7: 16225 [PMID: 29176755 DOI: 10.1038/s41598-017-16547-3]

86 Gu H, Xiong Z, Yin X, Li B, Mei N, Li G, Wang C. Bone regeneration in a rabbit ulna defect model: use of allogeneic adipose-derivedstem cells with low immunogenicity. Cell Tissue Res 2014; 358: 453-464 [PMID: 25064029 DOI: 10.1007/s00441-014-1952-3]

87 Wen C, Yan H, Fu S, Qian Y, Wang D, Wang C. Allogeneic adipose-derived stem cells regenerate bone in a critical-sized ulna segmental defect. Exp Biol Med (Maywood) 2016; 241: 1401-1409 [PMID: 25819682 DOI: 10.1177/1535370215576298]

88 Liu G, Zhang Y, Liu B, Sun J, Li W, Cui L. Bone regeneration in a canine cranial model using allogeneic adipose derived stem cells and coral scaffold. Biomaterials 2013; 34: 2655-2664 [PMID: 23343633 DOI: 10.1016/j.biomaterials.2013.01.004]

89 Liu J, Zhou P, Long Y, Huang C, Chen D. Repair of bone defects in rat radii with a composite of allogeneic adipose-derived stem cells and heterogeneous deproteinized bone. Stem Cell Res Ther 2018; 9: 79 [PMID: 29587852 DOI: 10.1186/s13287-018-0817-1]

90 Wagner JM, Conze N, Lewig K, Wallner C, Brune JC, Dittfeld S, Laurich H, Becerikli M, Dadras M, Harati K, Fischer S, Lehnhardt M, Behr B. Bone allografts combined with adipose-derived stem cells in an optimized cell/volume ratio showed enhanced osteogenesis and angiogenesis in a murine femur defect model. J Mol Med (Berl) 2019; 97: 1439-1450 [PMID: 31367858 DOI: 10.1007/s00109-019-01822-9]

91 Zhang C, Li M, Jia Z, Luo F, Zhao J. Enhanced bone repair induced by human adipose-derived
stem cells on osteogenic extracellular matrix ornamented small intestinal submucosa. Regen Med 2017; 12: 541-552 [PMID: 28718708 DOI: 10.2217/rme-2017-0024]

92 Ko E, Alberti K, Lee JS, Yang K, Jin Y, Shin J, Yang HS, Xu Q, Cho SW. Nanostructured Tendon-Derived Scaffolds for Enhanced Bone Regeneration by Human Adipose-Derived Stem Cells. ACS Appl Mater Interfaces 2016; 8: 2219-22829 [PMID: 27502160 DOI: 10.1021/acsami.6b05358]

93 Wu PH, Chung HY, Wang JH, Shih JC, Kuo MY, Chang PC, Huang YD, Wang PC, Chang CC. Amniotic membrane and adipose-derived stem cell co-culture system enhances bone regeneration in a rat periodontal defect model. J Formos Med Assoc 2016; 115: 186-194 [PMID: 26073611 DOI: 10.1016/j.jfma.2015.02.002]

94 Ben-David D, Fishman B, Rubin G, Novak A, Laevsky I, Kadouri A, Nishi Katz Y, Burger O, Zaretsky A, Bor N, Tzur E, Meretzki S, Rozen N. Autologous cell-coated particles for the treatment of segmental bone defects—a new cell therapy approach. J Orthop Surg Res 2019; 14: 198 [PMID: 31262323 DOI: 10.1186/s13018-019-1219-5]

95 Arrigoni E, de Girolamo L, Di Giancamillo A, Stano C, DellaViva C, Carnelli D, Campagnol M, Donneneghini C, Brini AT. Adipose-derived stem cells and rabbit bone regeneration: histomorphometric, immunohistochemical and mechanical characterization. J Orthop Sci 2013; 18: 331-339 [PMID: 23344932 DOI: 10.1007/s00776-012-0349-y]

96 de Girolamo L, Arrigoni E, Stano C, Dopa S, Di Giancamillo A, Addis A, Borgonovo S, DellaViva C, Donneneghini C, Brini AT. Role of autologous rabbit adipose-derived stem cells in the early phases of the repairing process of critical bone defects. J Orthop Surg Res 2011; 29: 100-108 [PMID: 20607837 DOI: 10.1002/jor.21184]

97 Xia L, Lin K, Jiang X, Fang B, Xu Y, Liu J, Zeng D, Zhang M, Zhang X, Chang J, Zhang Z. Effect of nano-structured bioceramic surface on osteogenic differentiation of adipose derived stem cells. Biomaterials 2014; 35: 8514-8527 [PMID: 25002263 DOI: 10.1016/j.biomaterials.2014.06.028]

98 Calabrèse G, Giaufreda F, Forte S, Fabbi C, Figallo E, Salvatorelli L, Parenti R, Gulisano M, Gulino R. Human adipose-derived mesenchymal stem cells seeded into a collagen-hydroxyapatite scaffold promote bone regeneration after implantation. Sci Rep 2017; 7: 7110 [PMID: 28769083 DOI: 10.1038/s41598-017-07672-0]

99 Probst FA, Fliefel R, Burian E, Probst M, Eddicks M, Cornelissen M, Riedl C, Seitz H, Aszödi A, Schieker M, Otto S. Bone regeneration of minipig mandibular defect by adipose derived mesenchymal stem cells seeded tri-calcium phosphate-poly(D,L-lactide-co-glycolide) scaffolds. Sci Rep 2020; 10: 2062 [PMID: 32029875 DOI: 10.1038/s41598-020-59038-8]

100 Saçak B, Certel F, Akdemir Z, Karademir B, Ercan F, Özkan N, Akpinar İN, Çelebiler Ö. Repair of critical size defects using bioactive glass seeded with adipose-derived mesenchymal stem cells. J Biomed Mater Res B Appl Biomater 2017; 105: 1002-1008 [PMID: 26888652 DOI: 10.1002/jbmr.33634]

101 Jing X, Yin W, Tian H, Chen M, Yao X, Zhu W, Guo F, Ye Y. Icariin doped bioactive glasses as a combination of adipose-derived stem cells and platelet-rich plasma improved bone regeneration and maturation in vivo. Dent Traumatol 2015; 31: 42-48 [PMID: 25336206 DOI: 10.1111/det.12134]

102 Tajima S, Tobita M, Orbay H, Hyakusoku H, Mizuno H. Direct and indirect effects of a combination of adipose-derived stem cells and platelet-rich plasma on bone regeneration. Tissue Eng Part A 2015; 21: 895-905 [PMID: 29471105 DOI: 10.1089/ten.TEA.2018.02.026]

103 Caetano G, Wang W, Murashima A, Passarini JR Jr, Bagne L, Leite M, Hyppolito M, Al-Deysab S, El-Newehy M, Bärtolo P, Frade MAC. Tissue Constructs with Human Adipose-Derived Mesenchymal Stem Cells to Treat Bone Defects in Rats. Biomaterials 2014; 35: 9811-9823 [PMID: 25241138 DOI: 10.1016/j.biomaterials.2014.09.002]

104 Cruz AC, Caon T, Menin Á, Granato R, Bouabaid F, Simões CM. Adipose-derived stem cells incorporated into platelet-rich plasma improved bone regeneration and maturation in vivo. Dent Traumatol 2015; 31: 42-48 [PMID: 25336206 DOI: 10.1111/det.12134]

105 Liao HT, Tsai MJ, Brahmayya M, Chen JP. Bone Regeneration Using Adipose-Derived Stem Cells in Injectable Thermo-Gelling Hydrogel Scaffold Containing Platelet-Rich Plasma and Biphasic Calcium Phosphate. Int J Mol Sci 2018; 19 [PMID: 30150580 DOI: 10.3390/ijms19092537]

106 Wan W, Zhang S, Ge L, Li Q, Fang X, Yuan Q, Zhong W, Ouyang J, Xing M. Layer-by-layer paper-stacking nanofibrous membranes to deliver adipose-derived stem cells for bone regeneration. Int J Nanomedicine 2015; 10: 1273-1290 [PMID: 25709448 DOI: 10.2147/IJN.S77118]

107 Park HJ, Yu SJ, Yang K, Jin Y, Cho AN, Kim J, Lee B, Yang HS, Im SG, Cho SW. Paper-based bioactive scaffolds for stem cell-mediated bone tissue engineering. Biomaterials 2014; 35: 9811-9823 [PMID: 25241138 DOI: 10.1016/j.biomaterials.2014.09.002]

108 Cheung WEEKS, Working DM, Galuppo LD, Leach JK. Osteogenic comparison of expanded and uncultured adipose stromal cells. Cytotherapy 2010; 12: 554-562 [PMID: 20370535 DOI: 10.1177/14634203103790694]

109 James AW, Zara JN, Corselli M, Askarinam A, Zhou AM, Hourfar A, Nguyen A, Megerdichian S, Zara JN, Corselli M, Askarinam A, Zhou AM, Hourfar A, Nguyen A, Megerdichian S. An abundant perivascular source of stem cells for bone tissue engineering. Stem Cells Transl Med 2012; 1: 673-684 [PMID: 23197874 DOI: 10.5966/scm.2012-0053]

110 James AW, Zara JN, Zhang X, Askarinam A, Goyal R, Chiang M, Yuan W, Chang L, Corselli M, Shen J, Pangan B, Stoker D, Wu B, Ting K, Pault B, Soo C. Perivascular stem cells: a prospectively purified mesenchymal stem cell population for bone tissue engineering. Stem Cells Transl Med 2012; 1: 510-519 [PMID: 23197855 DOI: 10.5966/scm.2012-0002]
Le Q et al. Adipose-derived stem cells for bone regeneration

111 James AW, Zara JN, Corselli M, Chiang M, Yuan W, Nguyen V, Askarinam A, Goyal R, Siu RK, Scott V, Lee M, Ting K, Peault B, Soo C. Use of human perivascular stem cells for bone regeneration. J Vis Exp 2012; e2952 [PMID: 22664543 DOI: 10.3792/jvisexp2952]

112 Meyers CA, Xu J, Zhang L, Chang L, Wang Y, Asatryan G, Ding C, Yan N, Zou E, Broderick K, Lee M, Peault B, James AW. Skeletogenic Capacity of Human Perivascular Stem Cells Obtained Via Magnetic-Activated Cell Sorting. Tissue Eng Part A 2019; 25: 1658-1666 [PMID: 31020920 DOI: 10.1089/ten.TEA.2019.0031]

113 Ferraro GA, De Francescò F, Nicoletti G, Piazzo F, Desiderio V, Tirino V, D'Andrea F. Human adipose CD34+/CD90+ stem cells and collagen scaffold constructs grafted into vivo fabricate loose connective and adipose tissues. J Cell Biochem 2013; 114: 1039-1049 [PMID: 23129214 DOI: 10.1002/jcb.24443]

114 Yamamoto M, Nakata H, Hao J, Chou J, Kasugai S, Kuroda S. Osteogenic Potential of Mouse Adipose-Derived Stem Cells Sorted for CD90 and CD105 In Vitro. Stem Cells Int 2014; 2014: 576358 [PMID: 25302065 DOI: 10.1155/2014/576358]

115 Chung MT, Liu C, Hyun JS, Lo DD, Montoro DT, Hasegawa M, Li S, Sorkin M, Rennert R, Keeney M, Yang F, Quarto N, Longaker MT, Wan DC. CD90 (Thy-1)-positive selection enhances osteogenic capacity of human adipose-derived stromal cells. Tissue Eng Part A 2013; 19: 989-997 [PMID: 23216074 DOI: 10.1089/ten.TEA.2012.0370]

116 Gronthos S, Franklin DM, Ledyd HA, Robey PG, Storms RW, Gimble JM. Surface protein characterization of human adipose tissue-derived stem cells. J Cell Physiol 2001; 189: 54-63 [PMID: 11573204 DOI: 10.1002/jcp.1113]

117 Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang JI, Misono H, Alfonso ZC, Fraser JK, Benhaim P, Hedrick MH. Human adipose tissue is a source of multipotent stem cells. Mol Biol Cell 2002; 13: 4279-4295 [PMID: 12475952 DOI: 10.1091/mbc.e02-02-0105]

118 Katz AD, Tholpady A, Tholpady SS, Shang H, Ogle RC. Cell surface and transcriptional characterization of human adipose-derived adherent stromal (hADAS) cells. Stem Cells 2005; 23: 412-423 [PMID: 15749936 DOI: 10.1634/stemcells.2004-0021]

119 Rada T, Gomes ME, Reis RL. A novel method for the isolation of subpopulations of rat adipose stem cells with different proliferation and osteogenic differentiation potentials. J Tissue Eng Regen Med 2011; 5: 655-664 [PMID: 21268288 DOI: 10.1002/tjem.364]

120 Levi B, Wan DC, Glotzbach JP, Hyun J, Januszyk M, Montoro D, Sorkin M, James AW, Nelson ER, Li S, Quarto N, Lee M, Gurtner GC, Longaker MT. CD105 protein depletion enhances human adipose-derived stromal cell differentiation by TGF-beta1 based on species of derivation. Stem Cells 2011; 29: 655-664 [PMID: 21479275 DOI: 10.1002/stem.1126]

121 Madhu V, Kilianski A, Reghu N, Dighe AS, Cui Q, Expression of CD105 and CD34 receptors controls BMP-induced in vitro mineralization of mouse adipose-derived stem cells but does not predict their in vivo bone-forming potential. J Orthop Res 2015; 33: 625-632 [PMID: 25728702 DOI: 10.1002/jor.22883]

122 Madhu V, Li CJ, Dighe AS, Balian G, Cui Q. BMP-non-responsive Scal+ CD73+ CD44+ mouse bone marrow derived osteoprogenitor cells respond to combination of VEGF and BMP-6 to display enhanced osteoblastic differentiation and ectopic bone formation. PLoS One 2014; 9: e103060 [PMID: 25048464 DOI: 10.1371/journal.pone.0103060]

123 Leyva-Leyva M, López-Díaz A, Barrera L, Camañor-Morales A, Hernandez-Aguilar F, Carrillo-Casas EM, Arríaga-Pizano L, Calderón-Pérez J, García-Alvarez J, Orozco-Hoyuela G, Piña-Barba C, Rojas-Martínez A, Romero-Díaz V, Lara-Arias J, Rivera-Bolaños N, López-Camarillo C, Moncada-Saucedo N, Galván-De los Santos A, Meza-Urzúa F, Villarreal-Gómez L, Fuentes-Mera L. Differential Expression of Adhesion-Related Proteins and MAPK Pathways Lead to Suitable Osteoblast Differentiation of Human Mesenchymal Stem Cells Subpopulations. Stem Cells Dev 2015; 24: 2577-2590 [PMID: 26230358 DOI: 10.1089/scd.2015.0070]

124 Madhu V, Dighe AS, Cui Q, Deal DN. Dual Inhibition of Activin/Nodal/TGF-β and BMP Signaling Pathways by SB431542 and Dorsomorphin Induces Neuronal Differentiation of Human Adipose Derived Stem Cells. Stem Cells Int 2016; 2016: 1035374 [PMID: 26798350 DOI: 10.1155/2016/1035374]

125 Wu Z, Zhang W, Chen G, Cheng L, Liao J, Jia N, Gao Y, Dai H, Yuan J, Xiao L. Combinatorial signals of activin/nodal and bone morphogenic protein regulate the early lineage segregation of human embryonic stem cells. J Biol Chem 2008; 283: 24991-25002 [PMID: 18596037 DOI: 10.1074/jbc.M803893200]

126 Chan CK, Seo EY, Chen JY, Lo D, Mc Ardle A, Sinha R, Tevlin R, Seita J, Vincent-Tompkins J, Wearda T, Lu WJ, Senarah-Yapa K, Chung MT, Marecic O, Tran M, Yan KS, Upton R, Walmsey GG, Lee AS, Sahoo D, Kuo CJ, Weissman IL, Longaker MT. Identification and specification of the mouse skeletal stem cell. Cell 2015; 160: 285-298 [PMID: 25594184 DOI: 10.1016/j.cell.2014.12.002]
129 Li CJ, Madhu V, Balian G, Dighe AS, Cui Q. Cross-Talk Between VEGF and BMP-6 Pathways Accelerates Osteogenic Differentiation of Human Adipose-Derived Stem Cells. *J Cell Physiol* 2015; 230: 2671-2682 [PMID: 25735222 DOI: 10.1002/jcp.24983]

130 Wang X, Cui F, Madhu V, Dighe AS, Balian G, Cui Q. Combined VEGF and LMP-1 delivery enhances osteoprogenitor cell differentiation and ectopic bone formation. *Growth Factors* 2011; 29: 36-48 [PMID: 21222516 DOI: 10.3109/08977194.2010.544656]

131 Chan CKF, Gulati GS, Sinha R, Tompkins JV, Lopez M, Carter AC, Ransom RC, Reinisch A, Wearda T, Murphy M, Brewer RE, Koepke LS, Maricic O, Manjunath A, Seo EY, Leavitt T, Lu W1, Nguyen A, Conley SD, Salhotra A, Ambrosi TH, Borrelli MR, Siebel T, Chan K, Schallmoser K, Seita J, Sahoo D, Goodnough H, Bishop J, Gardner M, Majeti R, Wan DC, Goodman S, Weissman IL, Chang HY, Longaker MT. Identification of the Human Skeletal Stem Cell. *Cell* 2018; 175: 43-56.e21 [PMID: 30241615 DOI: 10.1016/j.cell.2018.07.029]

132 Beckenkamp LR, Souza LEB, Melo FUF, Thomé CH, Magalhães DAR, Palma PVB, Covas DT. Comparative characterization of CD271⁺ and CD271⁻ subpopulations of CD34⁺ human adipose-derived stromal cells. *J Cell Biochem* 2018; 119: 3873-3884 [PMID: 29125884 DOI: 10.1002/jcb.26496]

133 Xu J, Li D, Hsu CY, Tian Y, Zhang L, Wang Y, Tower RJ, Chang L, Meyers CA, Gao Y, Broderick K, Morris C, Hooper JE, Nimmagadda S, Peault B, James AW. Comparison of skeletal and soft tissue pericytes identifies CXCR4⁺ bone forming mural cells in human tissues. *Bone Res* 2020; 8: 22 [PMID: 32509378 DOI: 10.1038/s41413-020-0097-0]

134 Wang Y, Xu J, Meyers CA, Gao Y, Tian Y, Broderick K, Peault B, James AW. PDGFRα marks distinct perivascular populations with different osteogenic potential within adipose tissue. *Stem Cells* 2020; 38: 276-290 [PMID: 31742801 DOI: 10.1002/stem.3108]

135 Fisch SC, Gimeno ML, Phan JD, Simmerman AA, Dumesic DA, Perone MJ, Chazenbalk GD. Pluripotent nontumorigenic multilineage differentiating stress enduring cells (Muse cells): a seven-year retrospective. *Stem Cell Res Ther* 2017; 8: 227 [PMID: 29041955 DOI: 10.1186/s13287-017-0674-3]
