

# Bone Marrow and Adipose Tissue Derived Mesenchymal Stem Cells in Regeneration of Cleft Lip and Alveolus: A Review of Literature

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## Abstract

Congenital abnormalities are worldwide major health problem. One of these abnormalities is; cleft lip and palate. Such a condition when occur, it can affect the child and provoke physiological and psychological consequences. One of the desired and sparkling line of treatment is stem cells engineering. There are many different types of stem cells. The most popular stem cells used in craniofacial treatment are; bone marrow derived mesenchymal stem cells (BM-MSCs) and adipose tissue derived mesenchymal stem cells (AT-MSCs). Although both showed positive results, still there are few comparative studies that evaluate the effectiveness of AT-MSCs over BM-MSCs. The purpose of this review was to provide an overview of stem cells and their clinical uses, and to compare the effectiveness of BM-MSCs and AT-MSCs in cleft lip and alveolus patients. Electronic search of English scientific papers from 2000 to 2017 was accomplished using PubMed, Cochrane Database and Google Scholar search engines. The following search terms used were, Cleft Lip and Palate, Bone Marrow Derived Mesenchymal Stem Cells, Adipose Tissue Derived Mesenchymal Stem Cells, Clinical Applications in Dentistry, Animal Studies. Stem cells offer a new era for craniofacial reconstruction. Both of adipose and bone marrow derived stem cells show effective results in the level of restoring function and esthetic. However, the AT-MSCs were easier to obtain without any harm to the patients.

**Keywords:** Cleft lip and palate; Bone marrow derived mesenchymal stem cells; Adipose tissue derived mesenchymal stem cells; Clinical applications in dentistry; Animal studies

## Introduction

Clefts of lip; alveolus and palate (CLAP) are the most widespread craniofacial birth defects and the second most popular birth anomaly; second to clubfoot [1]. The approximately overall global birth prevalence of CLAP is one affected individual in every 700 newborn babies [2]. CLAP differ between races [3]. In Saudi Arabia; the prevalence varied by ten folds from 0.3 to 2.19 per 1000 live births. Males are more often affected than females [4].

In patients with cleft lip and alveolus; bone grafting in the mixed dentition in the residual alveolar cleft has become a well-established procedure. The key of early intervention as follows: maxillary arch stabilization; clear the way for the eruption of the canine and sometimes of the lateral incisor eruption; as well as allowing bony support to the teeth adjacent to the cleft; lifting the alar base of the nose; assisting of closure of an oro-nasal fistula; and to obtain suitable periodontal conditions of the teeth within and adjacent to the cleft [5]. Autogenous bone graft has been the gold standard of bone replacement for many years; regardless its benefits it has major disadvantages which include limited amount of bone and donor-site morbidity such as post-operative pain; changes in the sensation; and donor site infections and scars [6].

The combined cell therapy and tissue engineering approaches can potentially avert these problems; and develop more safe and effective therapy for such defects. Moreover; it could be a valid alternative to autogenous bone graft therapy [7]. The attention of investigators has been directed to bone regeneration through the bone marrow stem cells (BMSCs). Advantages of this approach is as follow: 1-Osteoblast's differentiation from MSCs is very well reported and standardized in

many protocols. 2- Mesenchymal bone marrow stem cells (MBMSCs) can also be isolated by means of minimally invasive procedures from Bone Marrow (BM); 3- restore bone defects without donor site morbidity [8]. Lately; it has been reported that adipose tissue can be collected in large amount with negligible amount morbidity compared to bone harvesting. As well as; adipose tissue contains a population of mesenchymal stem cells that can be isolated and differentiated into various cell lines including osteocytes; adipocytes; and myocytes depending on the culture conditions. These cells are called Adipose Derived Stem Cells (ASCs) [6].

Suitable scaffolds should be chosen with specific criteria as: resorbable; biocompatible and inert; moreover; it should promote revascularization and serve as positive matrix for loaded cells [9].

Collagen scaffolds act as a three-dimensional bone engineering framework for bone marrow stem cells and adipose stem cells nesting [9]. Collagen is a remarkable component of extracellular matrix. The scaffold made of collagen had been used in different purpose such as homeostasis; guided bone regeneration. It has low capacity to induce

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an immune response to produce antibodies; and good mechanical characteristics; promoting cell and tissue attachment and growth [10].

The aim of this review was to provide an overview of stem cells and their clinical uses; and to compare the effectiveness of BM-MSCs and the AT-MSCs in cleft lip and alveolus patients.

## Materials and Methods

Electronic search of English scientific papers and literatures from 2000 to 2017 was accomplished using “PUBMED”, “Cochrane Database” and “Google Scholar”. The scientific papers were then assessed for their relevance to the intended objectives. The following keywords were used: cleft lip and palate; bone marrow derived mesenchymal stem cells; adipose tissue derived mesenchymal stem cells; clinical applications in dentistry; and an animal studies.

## Discussion

### Different methods of CLP reconstruction

The reconstruction of such defects is one of the main concern and challenges to both the oral-maxillofacial surgeon and the family. To the surgeon; it is important to interfere early to provide alveolar bone continuity and support for tooth eruption and suitable periodontal ligaments around the tooth; as well as support the lip and nose; and to close the oro-nasal fistula for proper feeding and swallowing. To the family; the early intervention consequently improves the quality of the life of their children [11].

The earliest method of repair was by suturing the two palatal halves together but this resulted in immobile parts and consequently affects the speech and the swallowing of patients [12]. In the last 50 years; the reconstructive techniques combined the use of prosthetic; allogeneic or autologous cancellous bone grafting were the gold standard for the clefts repair. The common donor sites for bone grafting were iliac crest; tibia; mandible; and rib [13]. The procedure is performed by the terminal stage of mixed dentition; at the age of 9 to 11 years old; most preferably before the permanent canine eruption; this procedure allows adequate periodontal support for canine eruption and preservation of the teeth near to the cleft area [13]. Another principle of bone grafting is the tertiary or the late grafting of bone. The procedure is performed after the onset of the permanent tooth; at twelve to fourteen years old and after the completion of orthodontic treatment. It is carried out to support the prosthodontic and periodontal therapy as well as to promote the closure of the oro-nasal fistulae [14]. In 2009; Nguyen et al. [15] reported 41 to 73% success rate of primary alveolar cleft repair and unfortunately most of them needed second surgeries. Meyer and Molsted; [16] reported a successful rate of 82% after long-term follow-up in a cohort study. Facial growth disturbance in the form of a retrusion midface calls for other treatments for normal occlusion establishment [17]. Moreover; the development of the oro-nasal fistula is still a challenge for surgeries intended for reconstruction. Studies have reported an incidence of the development of the oro-nasal fistula ranging from 11% to 23% and presumably at the location of the soft and hard palate junction [18]. Other disadvantages include: 1) Continuous pain at the site of the surgery; 2) long time healing; 3) hypersensitivity; 4) pelvic instability; and 5) increase risk of infection. As well as many grafts may undergo a certain level of resorption; loss of sensation at the site of the surgery; disease transmission and scars; long hospital stay; an emotional and social burden on the patients' family [13]. Consequently; the surgeon started to use the allografts to decrease the donor site morbidity and other consequences of autografts. But this alternative came up with other disadvantages; in form of very less degree

of cellularity; less ability to vascularize the site of surgery; and higher resorption rate than the autograft [19]. Multiple alloplastic materials were used to replace autologous tissue as silicone; titanium but all have displayed mechanical failure; infection; extrusion and limited capability to recreate previous bony form [20]. Therefore surgeons; had to think of another approach that can minimize the side effects associated with the reconstructive techniques.

### Tissue engineering

Tissue engineering is defined as an integrative field that comprises a combination of the principles of engineering; material and biological sciences headed toward the development of therapeutic techniques and biological alternatives that aims to restore; conserve; substitute or enhance biological functions [21]. Schimming and Schmelzeisen [22] stated that it takes only 3-month post-surgery for the lamellar bone to develop; and can provide a suitable room for the implant to be implanted.

**Tissue engineering in dentistry:** Tissue engineering came up and lighten the world of dentistry by focusing on the enhancement of materials and approaches in the replacement of the impaired tissues for biological resources by combining concepts; procedures and knowledge in all the concerning fields of science in tissue engineering [23]. Many dental researches reported an important advancement in dentistry focusing on the regeneration of: 1) temporomandibular joint [24]. They reported that the new strategies in treating the temporomandibular joint defects have resulted in very successful outcomes which are: no infections at the site of surgery; reduction of the donor site morbidity; and functional bony support for future implants. 2) Tooth-supporting tissues; that are destroyed due to trauma or systemic diseases as diabetes; or due to surgical resection. Another study; a customized periodontal scaffold with genetically redesigned human cells were fabricated for the formation of human tooth peridontium *in vivo*. The results showed newly formed parallel- and obliquely-aligned fibers that grow forming tooth ligament for oral and dental applications [25]. 3) The dentin; Sakai et al. [26] showed that the exfoliated deciduous teeth stem cells (SHED) can differentiate into angiogenic endothelial cells and odontoblast which can generate tubular dentin. Tissue engineering has shown a great potential and grateful results in biological regeneration of craniofacial defects. These defects include complex structures as bone; cartilage; soft tissue; nerves; muscles and blood vessels. So; the ideal repair should replicate the lost structure; reinstall the function; as well as it must be harmless; biodegradable and reliable [27]. To obtain good and ideal results; we need a scientific orchestration of the three fundamental elements: 1- stem cells (osteogenesis) (osteoprogenitor cells); 2-scaffold (osteoconduction) is an approach in which the scaffold support the bone growth and 3-cell signaling (osteoinduction) in which the non-osseous cells can be transferred into bone- forming cells by Bone Morphogenetic Proteins (BMPs) and other growth factors [21].

### The three important elements in tissue engineering:

**Stem cells:** Stem cells are colonogenic cells which have the capability to self-renew as well as to produce different predecessors. They are present in normal tissues functioning for regeneration and healing upon injuries [28]. These cells are known as readily available in high amounts; shows no immune refusal; no host disease versus graft; shows no tumorigenesis; little ethical issues; predictable differentiation potential and tissue-integration [29]. There are two main types of stem cell depending on their origin.

**Embryonic stem cells (ESCs):** ESCs are originated from an

embryo that has been fertilized *in vitro* fertilization clinic (100–200 cell blastocyst stage). They are known as pluripotent cell population and are characterized by the ability to maintain karyotype. ESCs can be differentiated into the three embryonic germ layers (ectoderm; endoderm; and mesoderm); and in a culture; they are formed in colonies. These key features make ESCs favorable for future use in regenerative medicine [30]. Still; the ethical discussions over the use of human embryos in research are unlikely ever to be fully determined; as it is handled by religious laws and philosophical reflections of the nature of human life; in addition to scientific and practical issues [31].

**Adult stem cells:** Adult stem cells are found in fetus and postnatal tissues; they are more specialized (multipotent) cells that are involved in tissue repair and regeneration as well as aging processes [32]. There are many sources for adult stem cells as bone marrow; adipose tissues; liver; umbilical cord; and muscles [32]. The intra-oral sources are dental pulp; periodontal ligaments can also be a source of adult stem cells [33].

Mesenchymal stem cells (MSCs) belong to the adult stem cells group; and are recognized as multi-potent stem cells that possess the self-renewal ability and the capacity to differentiate to numerous types of cells in mesoderm origin. There are many sources of MSCs including trabecular bone; deciduous teeth; adipose tissue; and other sources suggesting that MSCs niches are not only confined to bone marrow [34]. MSCs are fibroblast-like cells that can move quickly to the site of injury and can synthesize a high number of cytokines and growth factors that allow proper healing and tissue regeneration [35]. International Society for Cellular Therapy (ISCT) has recommended three main criteria to define MSCs regardless of the tissue origin in 2010 [36]. First; MSCs in a standard culture condition must adhere to the plastic culture flasks. Second; they must show positive staining for surface antigens of CD44; CD37; CD90 and CD105 and on the other hand must show negative staining for CD34 and CD45 which are human hematopoietic stem cells' markers as well as negative staining for CD11b; CD14; CD19; CD79- $\alpha$ . Third; they must be able to self-renewal and multi-differentiation into osteoblast; adipocyte and chondroblast [36]. Most of the of clonally expanded MSCs can differentiate *in vitro* into bone nodules rich in proteoglycan that results positive when stained by alizarin red and upon subjecting to Von Kossa techniques [37]. Others under right culture conditions differentiate into lipid droplets within their cytoplasm and stain positive by Oil Red O staining [38]. As well as some of the MSCs can generate glycosaminoglycan proteins and differentiation into mature chondrocytes that stain positive by Alcian blue staining [39]. The MSCs are also known to have a paracrine effect. They have immunomodulation; anti-apoptosis; anti-scarring; chemo-attraction effect and they also support cell growth and differentiation [40]. They secrete many cytokines and growth factors such as: transforming growth factor- $\alpha$  TGF- $\alpha$  transforming growth factor- $\beta$  TGF- $\beta$ ; hepatocyte growth factor HGF; fibroblast growth factor FGF and vascular endothelial growth factor VEGF [41]. All these unique characteristics and biological properties highlight its significance in cell-based therapy; regenerative medicine as well as tissue engineering [42]. The two types of MSCs that were most commonly reported in regenerative studies in CLAP are: 1) Bone Marrow Derived Mesenchymal Stem Cells (BM-MSCs) and 2) Adipose tissue derived Mesenchymal Stem Cells (AT-MSCs).

**Clinical trials of using BM-MSCs in craniofacial defects:** Over 25 years; the stromal cells of the bone marrow; also known as marrow stromal cells or BM-MSCs have become the concern of many researchers [43]. The typical assay employed that identification of BM-MSCs is through the colony-forming unit-fibroblast (CFU-FB);

they are spindle-shaped cells that are capable of proliferating and adhering on the surface to form colonies after the primary culture [44]. BM-MSCs reported positive and significant results in multiple types of therapies as in treating children suffering from osteogenesis imperfecta [45]; hematopoietic reconstruction [46] and bone tissue reconstruction [47]. Many other reports showed that the BM-MSCs are viable treatment option avoiding iliac harvesting; and less donor side morbidity [48]. Gimbel et al. [49] reported that the pain scored from the bone marrow aspirate in cleft patients was significantly lower than those cleft patients who underwent conventional separation of iliac bone marrow. Nevertheless; there are several drawbacks with the use of BM-MSCs in the clinical purposes mainly. The low number of cells obtained in comparison to the amount of bone harvested has longer culture time to obtain a significant number of cells numbers to use in clinical application [43]. Moreover; the life span of the BM-MSCs decreases with increasing age [50].

**Clinical trials of using AT-MSCs in craniofacial defects:** Considering what is mentioned about BM-MSCs, Adipose Tissue Derived Mesenchymal Stem Cells (AT-MSCs) have been studied for tissue engineering and regenerative medicine. AT-MSCs in the human body; are abundant and can easily be isolated in high amounts with less pain and donor site morbidity [51]. Adipose tissues were first recognized as MSCs; however in 2001 they are considered as a great pool of different cells for tissue engineering applications compared to MSCs from bone marrow [52]. These cells can easily be retrieved via liposuction or the "waste" of surgical procedures [53]. Furthermore; AT-MSCs showed great potential to differentiate into osteoblast; chondroblast; endothelial cells and neuronal cells [54]. AT-MSCs can be derived from different sources as the upper arm; thoracic; medial thigh; or subcutaneous that is the clinically most relevant source; as well as abdominal fat tissues without any major clinical risk [55]. Several different terminologies exist for the stem cells derived from adipose tissue; such as pre-adipocytes; adipose – derived stromal tissue; adipose-derived mesenchymal stem cells; adipose-derived adult stem cells; as well as processed lipoaspirated cells. Finally in 2014; it was termed as adipose-derived stem cells (ADSCs) [51].

Adipose tissue consists predominantly of adipocytes (fat cells) making more than 90% of the tissue volume. In addition; these tissues contain fibroblasts; pericytes; vascular endothelial cells as well as an extracellular matrix [56]. The adipose tissues are well known as highly vascularized tissues [57]. Araña et al. [58] showed that the processed harvested fat includes three layers: (i) the superficial layer; containing fat that is discarded; (ii) the lower layer; which include blood and tissue fluids; and (iii) the medial layer; is the Stromal Vascular Fraction (SVF) that contain the connective tissue cells (stromal cells); vascular endothelial cells as well as the vascular smooth cells and pericytes (mural cells) which are responsible for normal vasculature and responsible to vascular endothelial growth factors.

The AT-MSCs have sparked the surgeons towards its use in craniofacial surgeries due to their valuable characteristics such as the ability toward angiogenesis; restrict apoptosis; modify the immune response and multiple differentiation potentialities [59]. Liu et al. [60] utilized the allogenic AT-MSCs from the dogs seeded on a coral scaffold; and apply it to a cranial critical sized defect created surgically in the dogs. The results showed that the defect healed with proper bone without the need of immunosuppressive therapy and these results revealed that AT-MSCs have an immunomodulatory property. In mandibular defects; Warnke et al. [61] reported novel method in preparing mandible *in vivo*. They embedded a prefabricated titanium mesh with stem cells

in a scaffolding base loaded with growth factor; and then they have been allowed to grow into the latissimus dorsi muscle in the patient's body. After 7 weeks; the formed mandible was transplanted into the mandibular defect. Wilson et al. [62] reported bone development after 2 and 4 weeks following injecting the ramus of the pig's mandible with AT-MSCs. In the major maxillary defects; Mesimaki et al. [63] reported promising outcomes up to 8 months post-operation in treating large maxillary defects. Taylor [64] reported the use of AT-MSCs in treating the bilateral orbit-zygomatic defects in a 14 years old boy with Treacher Collin Syndrome. After 4 months postoperative; the CT scan showed a complete bone formation of the bilateral orbit-zygomatic bone as well as in the histology; it was noted that the bone was rich in vascular blood supply similar to the natural bone.

**Bone marrow derived mesenchymal stem cells (BM-MSCs) vs adipose derived mesenchymal stem cells (AT-MSCS):** AT-MSCs became more popular and sparkle in regenerative medicine due to their angiogenic; release of various cytokines with an immunomodulatory effect; and wound healing properties [65]. In addition; a high number of cells resulted in adipose tissue aspirate with less pain and donor site morbidity. In contrast; MSCs harvested from the bone aspirate at volume of 10-40 ml of marrow resulted in a low number of cells with lots of pain and donor side morbidity [66]. Cowan et al. [67] showed that greater attachment and proliferation property were displayed on culture dishes of AT-MSCs than BM-MSCs cells. Freshly gathered BM-MSCs need 2 to 3 weeks to merge; while freshly gathered AT-MSCs merged together in only 2-3 days. Cell-counting and Western blot analysis was done to detect the proliferating cellular nuclear antigen (PCNA); a protein whose presence correlates with proliferation rate. AT-MSCs proliferated substantially more than BM-MSCs over a period of 7 days. The AT-MSCs showed the ability to multiple lineage differentiation into different cell types as osteoblast; chondroblast; etc.; under certain conditions as well as high adherence property [68]; these characteristics that attract the tissue engineering team toward its use and its beneficial results in reconstructive surgeries. Zuk et al. [69] stated that the AT-MSCs could be a promising alternative source for BM-MSCs. Researchers showed that a single gram of adipose tissue aspirate approximately yields  $3.5 \times 10^5$  to  $1 \times 10^6$  stem cells; which means 500- fold greater than MSCs which can be yielded from bone marrow harvesting [70].

Some studies have come up with a conclusion that the AT-MSCs have several advantages when compared to BM-MSCs. First; AT-MSCs are available in large quantities; almost unlimited; and easily can be obtained in high volumes of cellular population with less invasive methods such as subcutaneous adipose tissue fragments or liposuction aspirates [71]. Moreover; AT-MSCs have an extensive self-renewal capacity; and can easily be expanded *in vitro* [72] and are easily handled in the lab; cheaper; and readily can be isolated by differential sedimentation [73].

However; they share same morphology and immunophenotypically cells marker expression as CD44; CD105; CD90 and CD 73. Both can be differentiated into osteoblast; chondroblast and adipocytes as well as both are plastic adherent [74].

**The scaffold:** The scaffold is a solid framework that allows the growth of the cell and its differentiation at a local area. It can be implanted alone; allowing the host cells to migrate and proliferate at the site of injury; or it can be used as a carrier for cells-replacement therapy [75]. Its main function is to mimic the extracellular matrix component for cell adhesion; proliferation; migration; and differentiation [76].

The scaffold's ideal design should have the following characteristic: (i) biocompatible; (ii) absorbable with the rate of resorption proportional with the rate of new bone formation; (iii) have a high mechanical stability; (iv) easy to manufacture; (v) properly fit the shape of the anatomical defect; (vi) sterile; (vii) can be easily managed in operating room [29]; (viii) have sufficient porosities to accommodate the osteoblast or osteo-progenitor cells to enhance their growth and proliferation as well as their differentiation into a new bone; (ix) promote vascularization; and (x) there should be high connections between the porosities to allow the nutrients to and metabolites out between the scaffolds and the osteo-progenitor cells [77].

The physical characteristic of the scaffold as biodegradable; porosity; stiffness; strength can readily affect the osteo-conduction and therefore the overall clinical success of the graft [78].

In craniofacial tissue engineering; more importance is given in the focusing on the presence of porosities in the scaffolds design with adequate permeability that allow vascular infusion; as well as the transportation of nutrients and cells inside the scaffolds; at the same time the design must be able to endure bearing load states that the craniofacial bone experienced due to the various oral functions such as mastication; deglutition and speech [79].

A scaffold is of two types: (i) natural (those derived from bone and demineralized bone matrix; in the form of powder; small fragments or blocks) [80]. Examples of natural scaffolds include collagen type I; chitosan; composite; calcium alginate; and hyaluronic acid; all which showed osteo-conductive activities in both *in vitro* and *in vivo* bone formation. Nevertheless; their main disadvantages; they have poor mechanical integrity [81]. (ii) Synthetic scaffolds are an alternative to the natural scaffolds as they have high mechanical stability; and their content can support bone tissue differentiation and formation [82]. Examples include ceramic; coral hydroxyl-apatite; titanium; or combination; polyglycolic acid; and polylactic acid [83]. The scaffolds are either permanently placed or temporarily three-dimensional porous in the form of porous solid mesh; spongy; fibers injectable gel networks; or foam [84]. In craniofacial deformities; the most favorable is the injectable type that fills the irregularities more easily; as well as can be administered harmlessly and easily can be mixed with the therapeutic agents or stem cells [85].

The best scenario for bone formation is to occur on a collagen matrix with fiber wrap ranging from 50 to 500 nm in diameter [86]. That's why; the nanofiber scaffolds are best for bone formation; as they mimic the morphology of the collagen fibers; allow for more bone tissue differentiation and enhance mineral deposition with better cellular attachment compared with solid-walled scaffold [87]. Collagen scaffold is worthy for BM-MSCs and AT-MSCs nesting and acts as a three-dimensional bone-engineering framework; *in vivo* and *in vitro* [9]. Collagen is an integral part of the extracellular component. It has been used for several purposes such as homeostasis; guided bone regeneration. It is characterized by the presence of good mechanical properties; low antigenicity as well as promoting cell and tissue growth and attachment [10]. Absorbable Hemostatic Gelatin Sponge SURGISPON<sup>®</sup>; is one type of many manufactured gelatin sponge that is certified in India by Food and Drug Committee (Aegis Lifesciences; 2015). It is nontoxic; non-immunogenic; non-allergic as well as non-pyrogenic. It is manufactured in India from highly purified first extract gelatin intended for utilization in multiple procedures in surgery. It approximately absorbs 40-50-fold of its water or blood weight; and holds fast quickly to the location of bleeding. It is characterized by

its uniform porosity for favorable hemostatic results. These results achieved by attracting the thrombocytes to be associated with the sponge matrix; and this instigates the release of substances by the thrombocytes; allowing the formation of aggregates as well as the change in their surface character hence allowing them to represent as a fibrin formation catalyst. During *in vivo* implantation; the absorption is accomplished within the span of 3 to 4 weeks. The sponge can be used in multiple surgeries as in neurosurgery; orthopedic surgeries as well as in dental surgeries; etc. In dental surgeries; it is mainly used in oral surgery after extraction and in retrograde root canal treatments more often in patients who can bleed extensively [88]. Gupta et al. [89] showed clinical and radio graphical satisfactory results when the collagen is applied immediately after tooth extraction. They concluded that the use of collagen allowed the formation of sound bone quality for future successful implant placement. Another study; concluded that by use of collagen scaffold there were no significant reports about excessive or prolonged bleeding and no any significant wound infection [90].

**Biochemical signals:** Biochemical signals are part of a complicated system of communication between cells and they orchestrate their interactions [91]. In bone engineering; the Bone Morphogenetic Proteins (BMP-2 and BMP-7) showed potential osteoinductive property [92]. The main action of BMP-2 is to enhance the differentiation of mesenchymal stem cells into osteoblasts; as well as it plays an indirect role in the proliferative behavior of these cells and the chemotactic effect [93]. In different animal models; the application of recombinant human BMP-2 (rhBMP-2) has been shown to accelerate healing of long bone defects; critical-sized defects [94] promote healing of diaphysis bone fractures [95]. On the other hand; Platelet-rich plasma (PRP) becomes an innovation toward the regeneration of tissue; and it is turning out to be a treasured appendage promoting healing in several dental and oral surgery procedures. PRP can be collected from patient's blood and undergo centrifugation with varying speed. Growth factors are stored in the  $\alpha$ -granules; includes: - platelet-derived growth factor (PDGF); transforming growth factor- $\beta$  (TGF- $\beta$ ); vascular endothelial growth factor (VEGF); insulin-like growth factor I and II (IGF I and II); and fibroblast growth factor (FGF) with different functions with regards to cell proliferation; angiogenesis and chemotaxis [96]. Growth factors accelerate bone repair for osteogenesis; allowing fibroblast proliferation and increasing the tissue vascularity for better wound healing [97]. In dentistry; PRP has been used in sinus lift procedures; cleft alveolus; cleft lip; oral/nasal fistula repair; and mandibular fractures. On the other hand; it has been utilized in periodontal surgery for gingival grafting; periodontal regeneration and crown lengthening [98]. Marukawa et al. [99] concluded that PRP used in cleft alveolus resulted in a reliable bone formation when seeded in the autologous cancellous bone collected from iliac bone. Ouyang and Qiao; [100] reported an excellent bone formation and soft tissue healing after 3 months when (PRP) was added to the scaffold and stem cells in patients with severe maxillary atrophy. Hibi et al. [48] reported valued results when they used the tissue engineering technique and (PRP) as a signaling molecule in cleft alveolus osteoplasty of a 9-year-old girl. The results showed that after serial computed tomogram they reported healthy bone formation after 3 months and extension of the bony wall in the defect after 6 months. It was also observed that 79.1% of the defect was covered with bone allowing the canine and the lateral incisor to erupt in a healthy and well-formed alveolar ridge.

**Soft tissue engineering:** The main objectives of the soft tissue treatment include resorting the defect with almost the same anatomical structure and the physiological function to imitate the epithelial barrier; probable wound healing without granulation tissue; scarring

and infection; as well as to restore esthetics and speech [101]. Large and open defects as in cleft lip repair; the healing process is by secondary intention that causes dramatic granulation tissue formation and scar formation [102]. Various practices have been tried as primary closure; graft and flap procedures. These procedures caused donor site morbidity; volume reduction; damage in the function; poor aesthetic appearance; and psychological well-being of patients [103]. Synthetic materials were also frequently used for soft tissue reconstruction but ended up with many drawbacks; mainly poor biocompatibility "allergic reactions;" local chronic edema; lymphadenopathy; scarring; and ulcerations [104]. Preferably; the resource material to be used in the restoration of soft tissue should be: non-allergic; non-expensive; shows stability upon injection or implantation; can be substituted by host tissue in the long run; allow for angiogenesis; and that there is a convenience in handling it in the operating room and in the outpatient situations [104]. In the case of fairly minute malformations; it is preferred that the material be easily bent upon subjecting through small-gauge needles while still possessing the capacity to be molded into a solid implant upon injection [105]. Scientists continue to explore and try to find something that can maintain the soft tissue shape; dimension and biocompatibility. Tissue engineering strategies involving seeding cells with suitable tissue induction; differentiation; and maintenance factors in a 3-D natural; artificial; or hybrid scaffold with a proper vascularization to develop biological and functional substitutes that restore tissue function and aesthetics [106]. Granero-Molto et al. [107] explained the role of MSCs in the healing at the site of injury. MSCs help in repair in two ways. First; by differentiating into tissue cells to restore both the morphology and the function of the damaged area. Second is by secreting an extensive range of bioactive factors that have an anti-apoptotic effect; immunoregulatory function and the stimulation of endothelial progenitor cell proliferation that create an environment for repair. The BM-MSCs were harvested from the bone marrow since its discovery; and it has been known as the "gold standard" for tissue; but this technique resulted in several drawbacks including pain; morbidity; and low cell number upon harvest [33]. In soft tissue engineering; AT-MSCs are presently one of the well-utilized adult stem cell populations in several studies; as well as an interesting alternative to BM-MSCs. It showed several advantages than other adult stem cells; including variable autologous source; minimal intrusive harvesting (liposuction); maximum number of proliferative cells in culture and multi-lineage potential [108]. Alhadlaq et al. [109] concluded that the MSCs in human could turn into AT-MSCs when they are subjected to adipogenic inducing medium. AT-MSCs can secrete angiogenic growth factors that can enhance the wound healing and regeneration [110]. Furthermore Zhu et al. [111] reported that after 6 and 9 months the AT-MSCs increased the expression of different growth factors which include the insulin growth factor-1; and the vascular endothelial growth factor thus allowing for more angiogenesis as well as adipose differentiation and prevent apoptosis. All these factors increase the longevity by twofold of the AT-MSCs when compared with adipose free grafts. Alamoudi et al. [112] conducted an experimental study where they treated oral ulcers in dogs using AT-MSCs. The results showed that AT-MSCs group showed significant healing of oral ulcer in contrast with the control groups and with the Dexamethasone. The expression of vascular endothelial growth factor (VEGF); epidermal growth factor (EGF); platelets derived growth factor (PDGF); and collagen genes has been observed to increase in AT-MSCs treated ulcers compared with Dexamethasone and controls.

**Tissue engineering in children:** Many considerations must be undertaken when using tissue engineering in children: 1) children

have better reparative property than adults; their cells have a greater reparative capacity. The ratio of mesenchymal stem cells in newly born is about 1MSC per 10;000 mononuclear cells in bone marrow [113]. While in teenagers 1 MSC per 100;000 mononuclear cells in bone marrow. In persons in their 50s 1 MSC per 400;000 mononuclear cells in bone marrow; while in persons in their 80s 1 MSC per 2;000;000 mononuclear cells in bone marrow. 2) The engineered fabricate must allow the continuous normal growth of the bone after the reconstruction. 3) Amount of bone marrow available is limited in children; in general it is agreed to have 1-5 milliliters of bone marrow per kilogram of the weight of the patient. Children have relatively lower weight than the adults. To solve this problem an extensive amplification of stem cells is required in tissue engineering [114].

### Animal studies in CLP

Researchers focused on animal models because these models share some physiological and pathological similarities with human as well as being able to follow up in a relatively short period of time frame [115].

Many veterinarian studies done on craniofacial engineering using MSCs on dogs [116-118]; rabbits [119]; pigs [120] and sheep [121]; all concluded that MSCs formed better bone regeneration than the conventional bone substitute.

MSCs seem to represent a significant and powerful tool in regenerative therapy. Tissue engineering involve triad of: stem cells; suitable scaffolds and growth factors which provide an attractive alternate to facilitate both hard and soft tissue healing.

### Conclusion

According to the literature review both undifferentiated AT-MSCs and BM-MSCs have the osteogenic; chondrogenic and lipogenic differentiation potential. They both secrete markers that help in defect repair and have same morphology; spindle shape cells [122]. They both showed positive results when used in reconstructive therapies. Some differences exist between them; but the most important one is; the ease of collection as well as less donor site morbidity [51]. These two factors were very important feature in choosing adipose versus bone marrow in repair. AT-MSCs and BMMSCs may be an appropriate substitute to the conventional intrusive procedure in future. Further understanding of the stem cells types, different scaffolds and how growth factors affect the tissue engineering pathway will construct chances to manipulate bone and soft tissue restoration and offer innovative treatment option for patients with cleft lip/palate and alveolus in the future.

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