Chapter 9

Mesenchymal Stem Cells for Optimizing Bone Volume at the Dental Implant Recipient Site

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Additional information is available at the end of the chapter

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Abstract

Inadequate bone volume at the implant recipient site presents a clinical challenge for many dental practitioners. To overcome these problems, several approaches have been developed and are currently used, including bone grafting strategies and distraction osteogenesis. Mesenchymal stem cells (MSCs) have gained their popularity within the last two decades, with regard to promising clinical results in improving the bone architecture at the implant recipient site. The aim of this chapter was to briefly outline the accessibility properties, differentiation capacities, isolation, and characterization of MSCs with regard to optimizing bone volume in dental implantology. Additionally, potential benefits and pitfalls are discussed in comparison with the conventional bone augmentation techniques.

Keywords: bone, dental, implantology, mesenchymal stem cells, platelet-rich plasma

1. Introduction

Dental implant therapies became an integral part of the daily dental practice. The success rate of implants is related to the correct position and angulation of implants in residual crest, so that height and thickness of bone augmentation can allow predictable results [1]. Therefore, the qualitative and quantitative characteristics of the surrounding tissues at the implant recipient site play a key role in the success of the procedure. Systemic diseases such as osteoporosis, changes in vitamin D metabolism, diabetes and adverse pregnancy outcomes, and local factors such as periodontitis, infections, pre-existing cysts or tumors, and traumatic extractions might result in the loss of both alveolar bone volume and quality and complicates the feasibility and long-term clinical outcomes of dental implant rehabilitation.

Various reconstructive surgical interventions could be necessary to regenerate bone defects prior to implant placement. In the literature, there are numerous clinical and experimental studies presenting techniques with different results that overcome the problems related to the insufficient bone volume at the edentulous alveolar ridge. Among these, the mostly performed surgical procedures to obtain bone augmentation are guided bone regeneration techniques via synthetic materials, xenografts or allografts, distraction osteogenesis of the alveolus, and the augmentation with autogenous bone blocks, which is thought to be the gold standard to obtain accurate bone volume and morphology with long-term predictable results. All techniques described above have their own advantages and pitfalls.

2. Conventional bone grafting strategies

2.1. Synthetic bone graft materials

A variety of artificial bone substitutes were used to reconstruct bone defects of the jaws. Synthetic bone grafts at most possess only osseoconductive characteristics and ideally should be biocompatible, show minimal fibrotic reaction, undergo remodeling, support new bone formation, and should have a similar strength or similar mechanical characteristics to that of the cortical/cancellous bone being replaced [2]. Availability of synthetic bone graft materials would eliminate the need for invasive graft-harvesting procedure and the dangers of pathogen transmission from immunogenic reaction to bank bone [2, 3]. In the maxillofacial reconstruction, the mostly used synthetic bone graft materials are:

- calcium phosphates,
- calcium phosphate cements,
- beta-tricalcium phosphates,
- synthetic hydroxyapatites,
- coralline hydroxyapatites, and
- bioactive glasses.

It is obvious that synthetic bone substitutes only have osteoconductive properties, and there is a need for improvement in their mechanical and degradation properties to ensure the replacement of the graft material with the living bone.

2.2. Allografts

The term allograft describes transplants between two subjects of the same species. Complications associated with the harvesting of autogenous bone have led to gain in their popularity as a treatment option in maxillofacial reconstruction. Allografts might offer the same characteristics as autograft; however, they do not present same osteogenic cells and therefore fulfill only the demand of osteoconductivity and serve mostly as a scaffold for new bone formation [2].

The advantages of allografts include availability and avoidance of morbidity associated with autogenous bone graft harvesting.

It is obvious that tissue safety is a major concern in transplantation. The major risk and disadvantage related to the use of allografts are the transmission of infectious agents from donor to recipient, which could result in microbial contamination from an infected donor, during collection of the tissue from donors or the environment and during processing of the tissues [4].

Viral transmission is a potential risk that is historically and serologically reported in association with allografts. Despite the exceedingly low risk, the transmission of human immunodeficiency virus (HIV-1) from seronegative cadaveric donors has reported in Refs. [4, 5]. During the history of allogenic tissue transfer, many sterilization techniques have been used to prevent infection through allografts which include gamma irradiation, ethylene oxide gas, thermal treatment with moist heat, beta-propiolactone, chemical processing, and antibiotic soaks [4]. Among these, gamma irradiation offers a clear advantage in terms of safety compared with other sterilization techniques.

2.3. Xenografts

Xenograft is a term used to describe a surgical graft of tissue from one species to an unlike species such as coral, bovine, and porcine and are used as calcified matrices generally. The processing of xenografts is reported to remove organic components such as cells and proteinaceous materials, leaving an inert absorbable bone scaffold, which assists in revascularization, osteoblast migration, and new bone formation [2, 6].

The use of xenografts has been demonstrated to be effective for increasing bone height and bone volume especially in sinus augmentation procedures (**Figure 1**). Xenogeneic bone is available in greater supply and larger sizes, and their physical properties are comparable to human cancellous bone [2, 6–8]. In the literature, it has been suggested that the resorption of xenografts and their replacement with new bone appears to be slow [9] and consideration must be given to the risk of cross contamination with bovine spongiform encephalopathy or porcine endogenous retroviruses [10].

2.4. Autografts

In the reconstruction of bony defects of the jaws, autogenous cancellous bone grafts are stated to be the most effective bone graft material considering their osteoinductive effects and predictable long-term results. Autogenous bone contains all of the elements necessary to promote vital bone formation, including mineral, collagen matrix, growth factors, and particularity vital cells [2]. Following the transplantation, few mature osteoblasts survive the procedure, but adequate numbers of precursor cells which have the osteogenic potential remain [2, 11]. Considering the bone volume needed, the donor sites for the reconstruction of the defects of the jaws are anterior or posterior iliac crest, mandibular ramus, mandibular symphysis, tibia, and parietal bone.

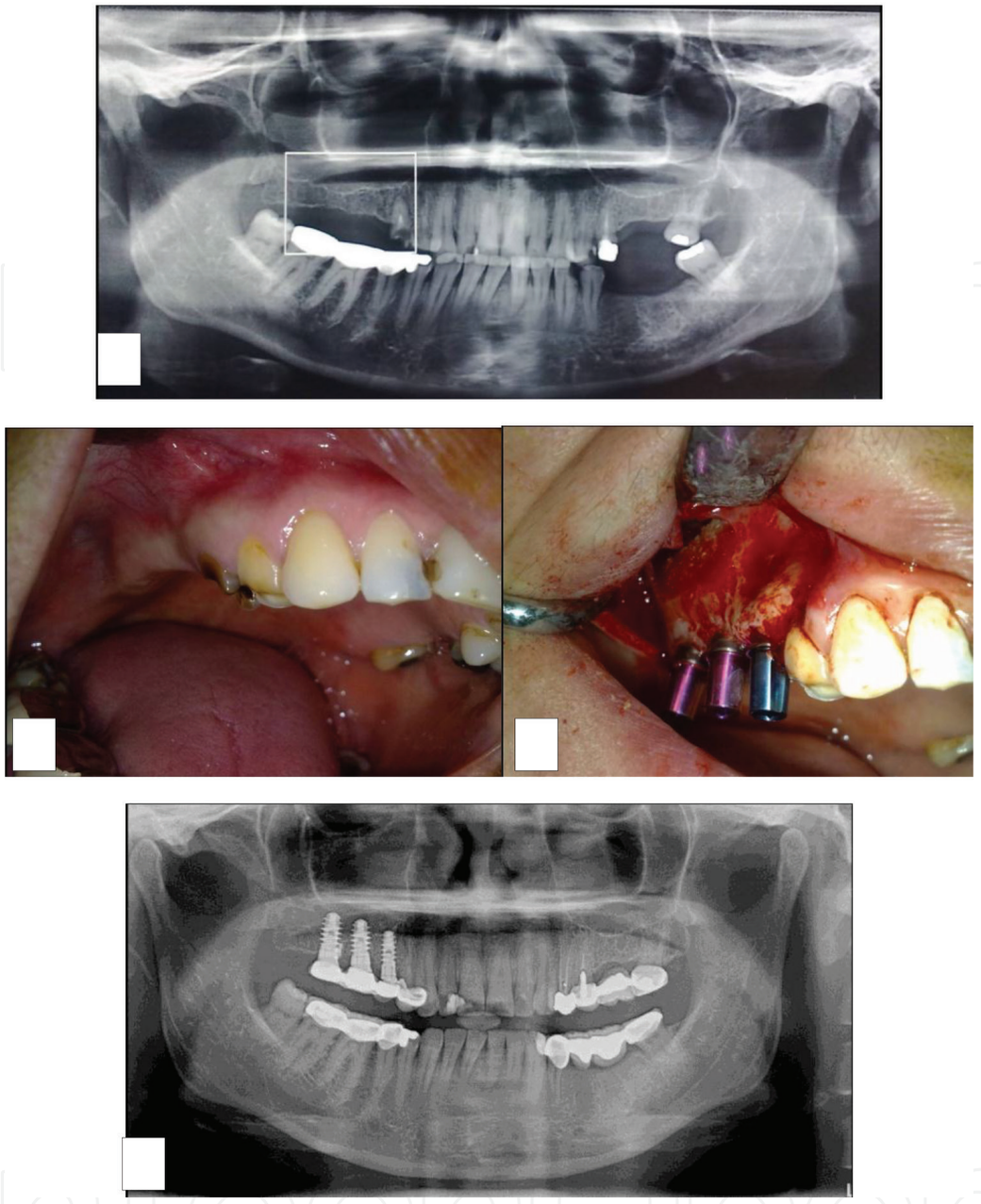

Figure 1. Augmentation of the atrophic posterior maxilla (a) The insufficent bone volume at the right posterior maxilla. (b) Intraoral clinical view (c) Sinus bone grafting with xenograft (Bioss®, Geistlich Germany). Implants were inserted simultaneously with sinus membrane elevation. (d) Panoramic radiograph after 1 year.

Limitations of the use of autogenous bone graft harvesting differ from the selected donor site, however, are mostly related to the so-called "donor site morbidity." The complications related to bone graft harvesting are [12]:

- increased operative time,
- limited availability and significant morbidity related to the intraoperative blood loss,
- wound complications,

- possible neurosensory alterations at the harvesting site, and
- chronic pain which is mainly attributed to the dissections performed during the graft harvesting.

An ultimate bone formation occurs only as a consequence of osteogenic/osteoinductive/osteoblastic cellular activity. In recent years, experimental studies followed by successfully clinical series have led to gaining the popularity of osteogenic precursors such as mesenchymal stem cells (MSCs) in bony reconstruction of the defects of the jaws and added a new dimension to the bone-gaining procedures in dental implantology.

The aim of this chapter was to describe the isolation methods regarding the MSCs used in oral implantology and briefly describe their clinical applications in peri-implantary surgical interventions.

3. Mesenchymal stem cells

Stem cells are unspecialized cells with the ability to proliferate and differentiate to multiple cell types when stimulated by both internal and external signals. They can be either embryonic stem cells, which are found in blastocysts or adult stem cells, which are called as pluripotent cells and can be found in bone marrow in the form of hematopoietic, endothelial, and MSCs.

The first successful isolation of bone marrow MSCs, then called colony-forming fibroblast-like cells, was described in 1968 by Friedenstein et al. [13] Today, MSCs are defined as nonhematopoietic progenitor cells that have the ability to differentiate into distinct mesodermal lineages (adipogenic, chondrogenic, osteogenic, or myogenic), which can produce bone, cartilage, fat, or fibrous connective tissue depending on their differentiation process [14].

Sources of MSCs in adult patients are [15, 16]:

- bone marrow [16] (**Figure 2**),
- peripheral blood [17],
- adipose tissue [18],
- muscle [19],
- periosteum [20],
- synovium [21], and
- teeth (perivascular niche of dental pulp and periodontal ligament) [22].

Since the first description of MSCs, various studies aimed to identify an ideal source for MSC harvesting. In 2006, Zhu et al. [23] have performed a study on the investigation of donor cell-related differences in tissue-engineered bone and examined bone marrow MSCs, alveolar bone cells, and periosteal cells for their in vivo potential to form bone.

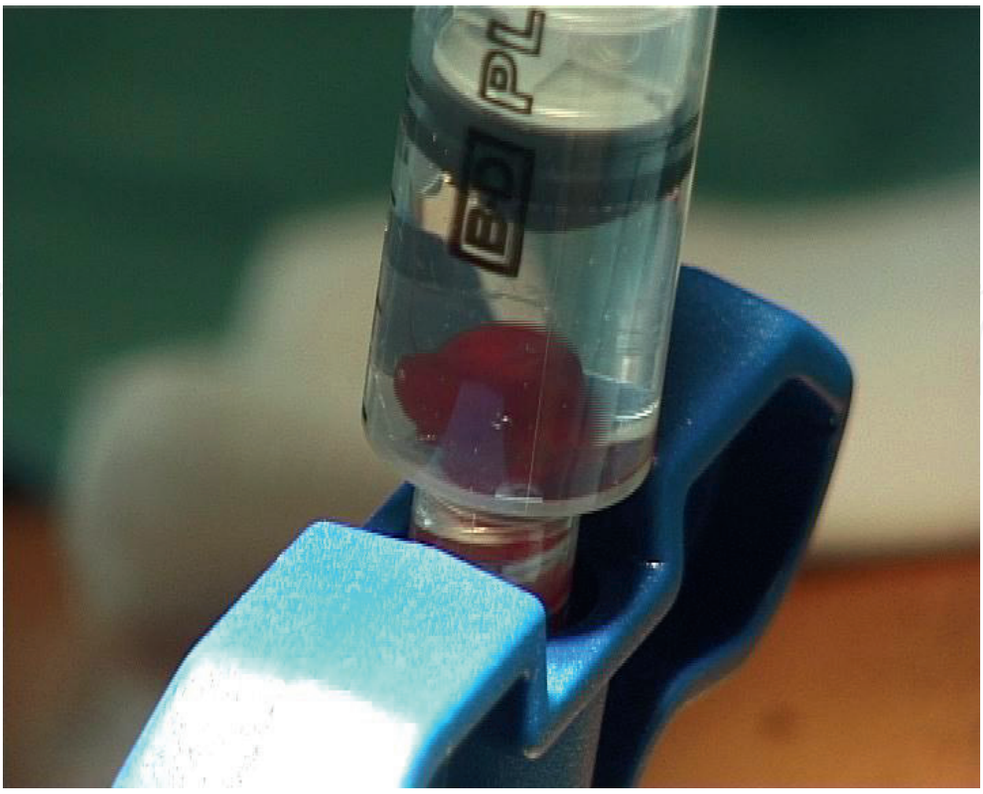

Figure 2. Bone marrow aspiration.

They have demonstrated that periosteal cells are the best choice for enhancing bone formation in tissue engineering of bone regeneration. In addition, recent studies showed a lower osteogenic differentiation potential of adipose tissue–derived stromal cells (ASCs) compared to bone marrow–derived mesenchymal stromal cells. According to Açil et al. [24], a careful reconsideration of the use of ASCs in bone tissue engineering application should be given.

3.1. Characterization of mesenchymal stem cells

Surface antigen expression, which allows for a rapid identification of a cell population, has been extensively used in experimental studies focusing on the identification of MSCs. For analysis of surface antigen expression, flow cytometry analysis and immunocytochemistry are efficient methods that reveal the marker profile of individual cells. In addition, fluorescence-activated cell sorting (FACS) is a valuable protocol for sorting isolation of MSCs. (**Figure 3**)

All techniques described above rely on both positive and negative selection by cell antigen surface markers, as well as physical properties of cells such as forward and side scatter characteristics [25].

According to the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy [26], minimal criteria to define human MSCs are as follows:

- MSC must be plastic-adherent when maintained in standard culture conditions.

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- MSC must express CD105, CD73, and CD90 and lack expression of CD45, CD34, CD14 or CD11b, CD79a, or CD19 and human leukocyte antigen-D-related (HLA-DR) surface molecules.
- MSC must differentiate to osteoblasts, adipocytes, and chondroblasts in vitro.

Since the first description of the above mentioned criteria by Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy in 2006, many studies have investigated the surface antigen expression of human MSCs in order to increase the confidence in their identification and verification. Lee et al. [27] have demonstrated that CD14, CD31, CD34, CD45, CD49d, CD49f, CD51, CD54, CD71, CD106, CD133, major histocompatibility complex (MHC II), cytokeratin, and desmin were absent from human MSCs, whereas CD13, CD29, CD44, CD59, CD73, CD90, CD105, CD166, MHC I,a-SMA, and vimentin were present on human MSCs. For human bone marrow stromal cells, common targets of negative antigene expression include CD2, CD3, CD11b/Integrin alpha M, CD14, CD15/Lewis X, CD16/$F_c$gamma RIII, CD19, CD38, CD56/NCAM-1, CD66b/CEACAM-8, CD123/IL-3 R alpha, and CD235a/Glycophorin.

For the positive selection of MSCs, CD271/NGF R, CD105/Endoglin, STRO-1, ganglioside GD2, and SUSD2 are relatively newly identified surface markers. In addition, STRO-1, CD271, CD200, ganglioside GD2, and frizzled-9 tissue non-specific alkaline phosphatase (TNAP) are suggested to be the latest markers used to verify MSC Identity [28, 29]. Identification of both positive and negative novel antigen surface markers would lead to modifications in the future.

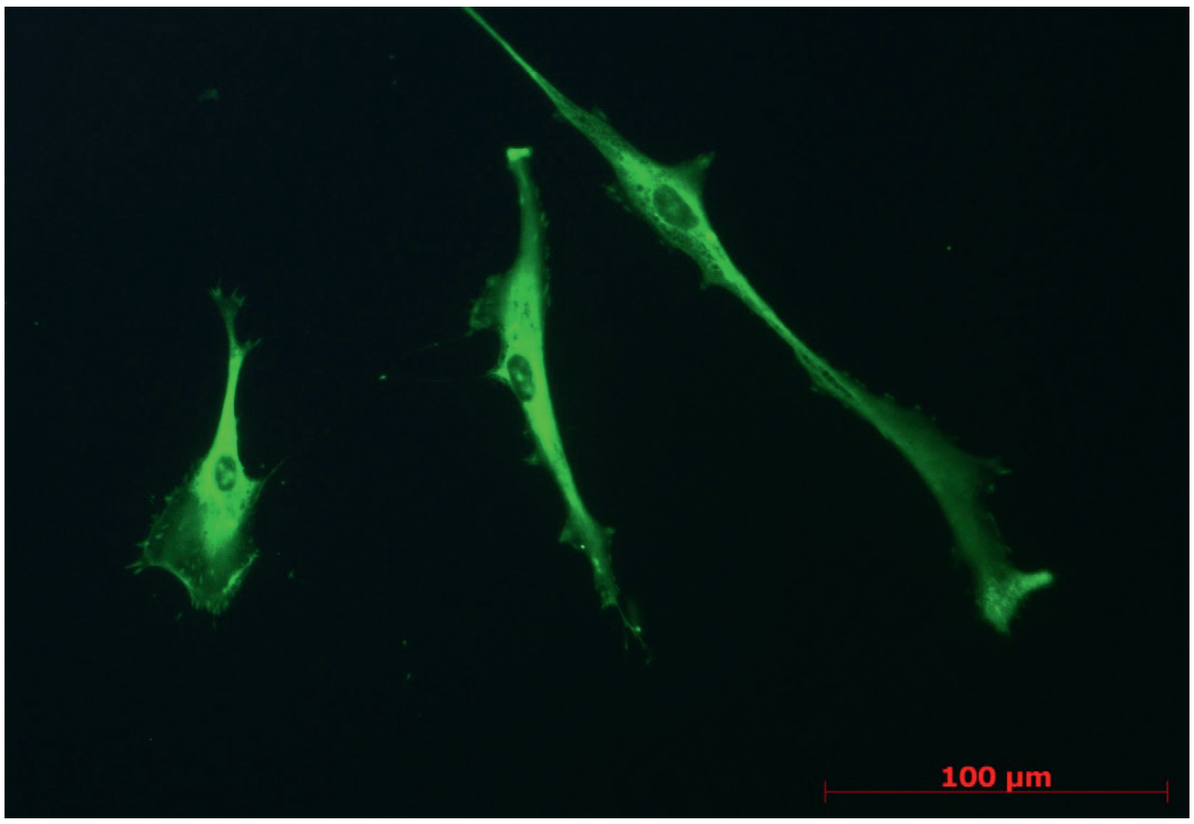

Figure 3. Morphology of MSCs obtained from bone marrow transfected with fluorescent protein. (Scale 100 µm).

4. Current concepts in mesenchymal stem cell harvest

4.1. Periodontal ligament

Shi et al. [14] have showed that periodontal ligament regeneration involves the recruitment of progenitor cells or stem cells, differentiating into either fibroblasts, cementoblasts, or osteoblasts, securing the teeth in the sockets between the cementum and adjacent alveolar bone. Seo et al. [30] have isolated stem cells from periodontal ligament for the first time and gave us new strategy to reconstruction of periodontium. According to Seo et al. [30], periodontal ligament stem cells (PDLSCs) share similar characteristics with other adult stem cells, including the ability to self-renew and multi-lineage differentiation potential. All these results suggested that PDLSCs might belong to a unique population of postnatal mesenchymal cells.

A literature survey could reveal that third molar teeth were mostly used for PDLSC isolation. Briefly [31], impacted third molars were surgically extracted, and periodontal dental ligament was gently scraped from the middle root surface. Coronal and apical portions of the ligament were not used in order to avoid contamination by gingival and pulpal cells. Periodontal dental ligament tissues were then minced then digested in a solution containing 3 mg/ml collagenase type I and 2.5 mg/ml dispase II for 1 h at 37°C. After digestion, tissue was seeded into culture flasks with alpha-modification of Eagle's Medium supplemented with 10% fetal bovine serum, 2-mM Glutamine, 100-U/mL penicillin and 100-μg/mL: streptomycin solution at 37°C in 5% CO_2 in a humidified atmosphere. After single cells were attached on the plastic bottom of the flask, non-adherent cells were removed by changing the medium [31].

Hakki et al. [32] have suggested that BMP-2, -6, and -7 are potent regulators of periodontal ligament stem cell gene expression and bio mineralization. BMPs with periodontal ligament stem cell isolated from periodontal ligament tissues provide a promising strategy for bone tissue engineering. According to a recent study performed by Açil et al. [51], BMP-7 triggers periodontal dental ligament cells to differentiate toward an osteoblast/cementoblast phenotype.

4.2. Adipose tissue

According to Açil et al. [24], ASCs could be easily isolated by using the modified technique that has been previously described by Zuk et al. [33]. Briefly description of the technique is; the adipose tissue, which could be obtained from liposuction procedures or from the subcutaneous tissue at the surgical access to the iliac crest during reconstructive maxillofacial surgical procedures.

Recent studies indicated a lower osteogenic differentiation potential of ASCs compared to bone marrow–derived mesenchymal stromal cells. As we have mentioned before, Açil et al. [24] have evaluated the effects of potent combinations of highly osteogenic BMPs in order to enhance the osteogenic differentiation potential of ASCs and indicated a restricted osteogenic differentiation potential of ASCs and suggest careful reconsideration of their use in bone tissue engineering applications.

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4.3. Bone marrow

The superior iliac crest is usually preferred as a donor site due to its ease in access and trabecular structure. As described by Hernigou et al. [34] and later by Shapiro et al. [35], briefly, appropriate local anesthesia of the skin and subcutaneous soft tissues should be administered. Then, a 1-cm stab incision was performed over iliac crest. An 11-gauge, 11-cm Jamshidi needle was used to aspirate the bone marrow. Effort was taken to use a parallel approach, with the needle directed parallel to the iliac wing between the inner and outer tables, and the needle was subsequently withdrawn and repositioned [34, 35] (**Figure 4**). The marrow aspirates was then passed through a sterile filter into a separate compartment to remove particulate matter. The material was transferred for centrifugation resulting marrow cell concentration [34, 35].

Recent literatures have showed the potential benefits of using a cocktail of mononuclear cells without expanding them in vitro before reimplantation [36] (**Figure 5**). Therefore, there are also various systems developed for harvesting of MSCs from bone marrow. One of these is the bone marrow–derived MNCs isolation by synthetic poylsaccharid (FICOLL), technique, which is currently accepted as the gold standard [36, 37]. The FICOLL method might present a useful

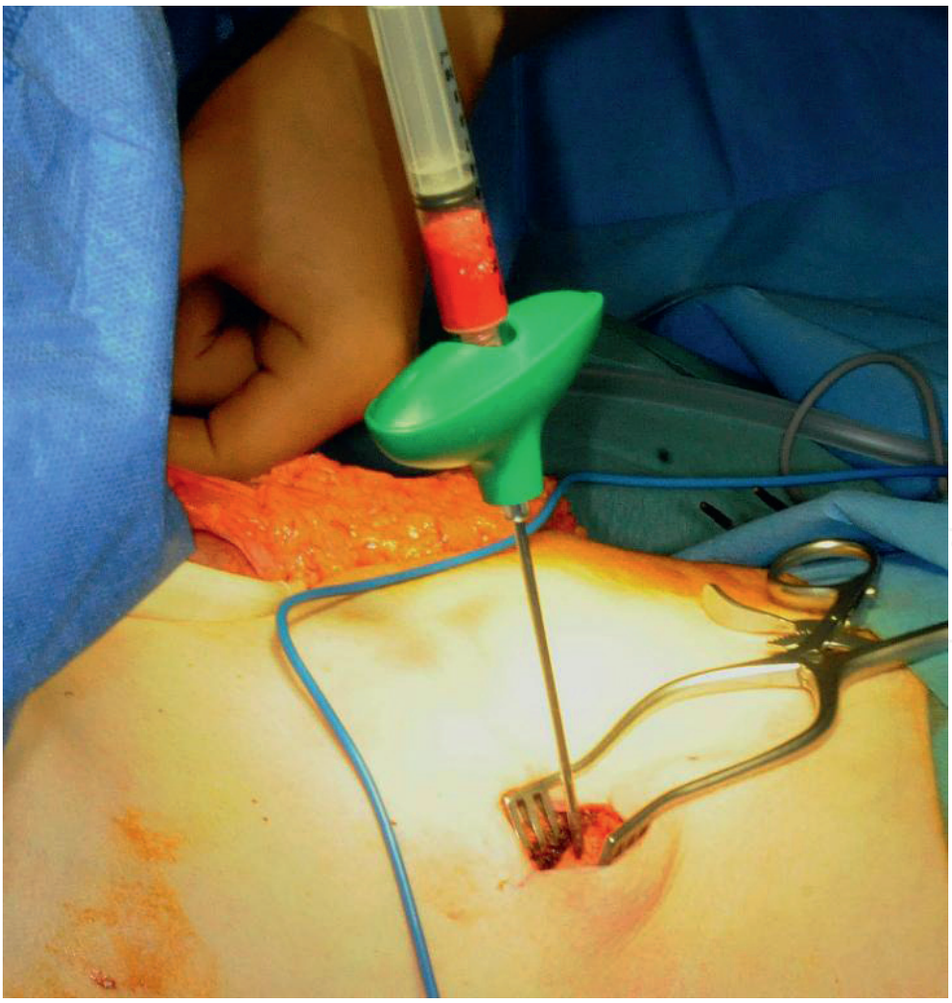

Figure 4. Bone marrow aspiration from the superior iliac crest.

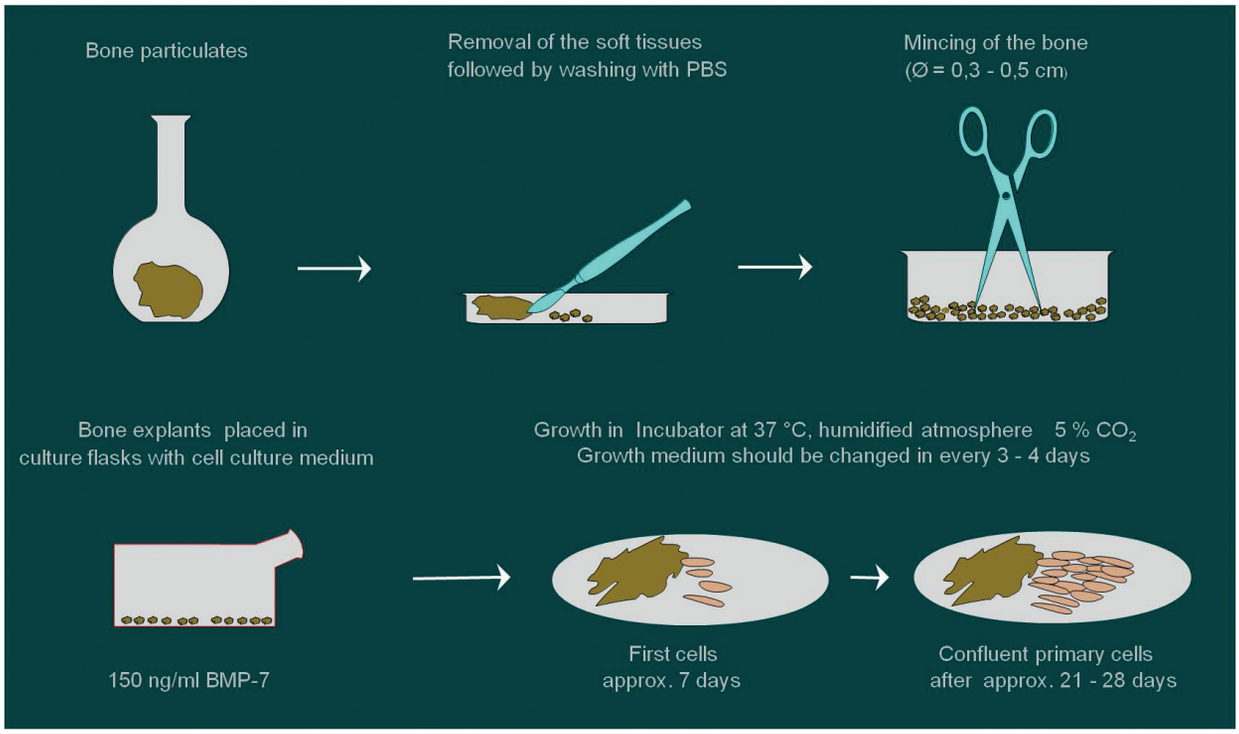

Figure 5. Diagrammatic illustration of the steps in osteoblast cell culture. Bone particles were obtained, the soft tissues were removed, and washed with PBS. The bone particles were minced and placed in culture flasks. After 3–4 weeks in incubation, cells have reached confluence.

technique for hospitals; however, the system is a time-consuming one, and a good manufacturing practice (GMP) laboratory is required. To ensure the clinical use in operating facilities without GMP possibilities, closed systems such as closed bone marrow aspirate concentrate (SmartPReP2 Bone Marrow Aspirate Concentrate System; BMAC; Harvest Technologies GmbH) system were developed [38]. Saurbier et al. [36] have compared new bone formation in maxillary sinus augmentation procedures using biomaterial associated with MSCs separated by FICOLL and BMAC and observed a higher proportion of hard tissue in the BMAC group.

Marx et al. [39] have compared the histologic parameters and outcomes of two types of grafts in large vertical maxillary defects: a composite graft of recombinant human bone morphogenetic protein-2/acellular collagen sponge (rhBMP-2/ACS), crushed cancellous freeze-dried allogeneic bone (CCFDAB), and platelet-rich plasma (PRP) and size-matched 100% autogenous grafts in 20 patients. According to their findings, the composite graft of rhBMP-2/ACS-CCFDAB-PRP regenerates bone in large vertical ridge augmentations as predictably as 100% autogenous graft with less morbidity, equal cost, and more viable new bone formation without residual nonviable bone particles but with more edema which might be attributed to the incisional release of the periosteum intraoperatively.

4.4. Peripheral blood

According to the material and methods of the experimental study performed by Sato et al. [40], peripheral blood could be obtained by jugular vein puncture, collected into syringes containing 0.5-ml sodium heparin and should be transported at 4°C to the laboratory within 3 h. To isolate peripheral blood-derived mononuclear cells, undiluted blood layered onto 12-ml Lympholyte

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in a 50-ml tube and centrifuged at 300 g for 40 min without braking [59]. The mononuclear cells were collected and washed twice with phosphate buffered saline (PBS) by centrifuging at 300 g for 5 min followed by an additional wash with PBS. After that, cells were re-suspended in culture medium which consists of Dulbecco's modified Eagle's medium with 5% separated autologous plasma, 10% fetal bovine serum and 10-μl/ml 100-units/ml Penicillin/Streptomycin solution. Subsequently, cells obtained from each 12 ml of blood were seeded onto a 100-mm^2 tissue culture dishes and incubated in a humidified atmosphere at 37°C with 5% CO_2. Nonadherent cells were removed by washing the mononuclear cells twice with PBS after 72 h of incubation. After 2 weeks, colonies of adherent fibroblast-like cells could be noticed. When the colony reached the approximate size of 5 cm^2, cells are detached and seeded in a new flask. The MSCs maintained in growth medium until ~70% confluence. The cells were then treated with 0.05% EDTA solution and could be cultured for subsequent passage in 100 mm^2 dishes at 7500 cells/cm^2 in base medium. This procedure was repeated as many times as possible.

Kassis et al. [41] evaluated the ability of fibrin microbeads (FMB) to separate human MSC from different sources other than bone marrow, with special emphasis on granulocyte colony-stimulating factor (G-CSF)–mobilized peripheral blood of healthy individuals. According to their material and methods, fibrin microbeads that bind matrix-dependent cells were produced from concentrated fibrinogen by a stirred heated oil emulsion technique and used to isolate MSC from the mononuclear fraction of mobilized peripheral blood of adult healthy human donors treated with G-CSF. Based on their results, FMB may have special advantage in isolating MSC from mobilized peripheral blood.

The isolation of MSCs from peripheral blood is a relatively new method with the main advantage of the ease in access, and further studies are needed to clarify the most appropriate technique. In addition, the introduction of platelet aggregates in oral and maxillofacial surgery has changed the approach toward extensive reconstruction of resorbed maxillae (**Figure 6**) and mandibles for implant reconstruction [42].

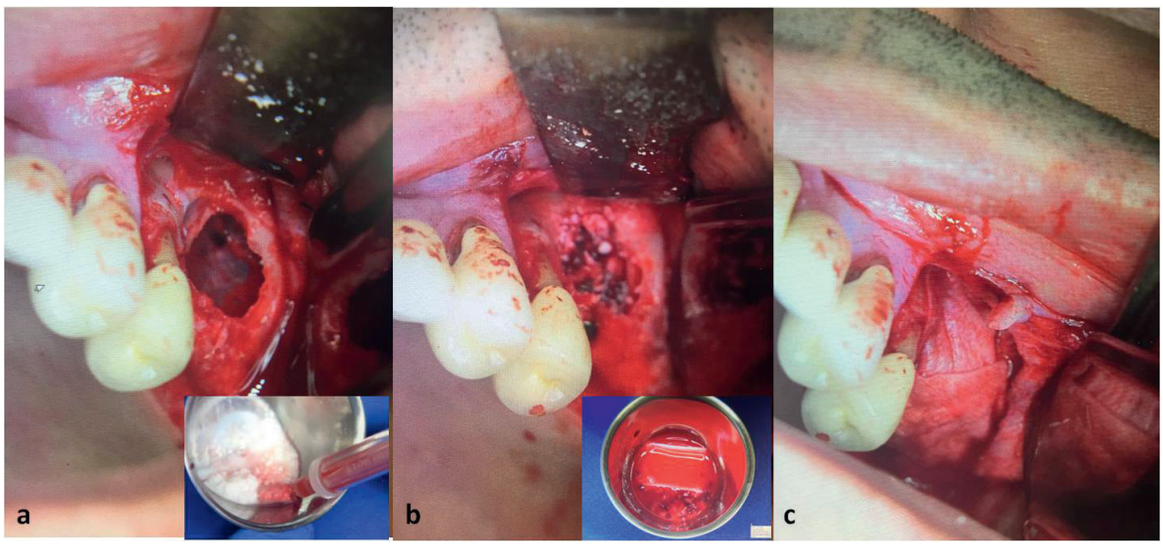

Figure 6. Second generation platelet aggregate (platelet rich fibrin) application in augmentation of the posterior maxilla. (a) Elevation of the membrane and preparation of PRF combined with Xenograft (Bioss®, Geistlich Germany) (b) Platelet rich fibrin (PRF) and Xenograft in situ. Preparation of the PRF membrane. (c) Placement of the membrane.

A possible role of platelet aggregates in local regulation of fracture healing and bone regeneration was attributed to the synergic effect of growth factors such as isomers of platelet-derived growth factor, transforming growth factor A1 and A2, insulin like growth factor > and A, and vascular endothelial growth factor. From this point of view, platelet aggregates could help in the differentiation and chemotactic and mitogenic stimulation of MSCs, which leads to an enhancement of bone repair and regeneration. Moreover, Marx [43] have recently confirmed that platelet rich plasma (PRF) contains an amount of 250×10^3 -400×10^3 per mL, which are positive for CD 44, CD 90, CD 105, and CD 34.

5. Mesenchymal stem cells in the reconstruction of the osseous defects of the jaws

Tissue-engineering therapy is a recent treatment modality in dental field to rehabilitate quantitative and qualitative properties of both soft and hard tissues with the use of cells with regenerative potential signaling molecules such as growth factors and a biocompatible matrix serving as a scaffold [44, 45].

During the past 2 decades, various experimental studies focusing on the osteogenic properties of MSCs have been performed. In 2001, Cooper et al. [46] have studied the relationship between bone sialoprotein (BSP) expression and osteocalcin expression with subsequent osteogenesis occurring in MSC-based implants and suggested that culture-expanded, cryopreserved human MSCs have osteogenic potential and demonstrated that implanted cell gene expression can reveal the early onset of bone formation.

In 2003, De Kok et al. [47] have evaluated MSC-based alveolar bone regeneration in a canine alveolar saddle defect model and observed that equivalent amounts of new bone were formed within the pores of the matrices loaded with autologous MSCs or MSCs from an unrelated donor, confirming the hypothesis that MSCs have the capacity to regenerate bone within craniofacial defects In addition, they have also stated that neither autologous nor allogeneic MSCs induced a systemic response by the host. Gutwald et al. [48] compared the osteogenic potential of mononuclear cells harvested from the iliac crest combined with bovine bone mineral (BBM) with that of autogenous cancellous bone alone and studied bilateral augmentations of the sinus floor in 6 adult sheep and reported that MSCs, in combination with BBM as the biomaterial, have the potential to form bone.

In the literature, there are also numerous studies focusing on the stimulating effects of various growth factors, most notably BMPs, on the osteogenic differentiation of MSCs [49, 50]. Açil et al. [51] have compared the most potent growth factors in regard to their osteoinductive potential and stated that the combined addition of BMP-2, BMP-6, and BMP-9 to the osteoinductive culture medium containing dexamethasone, β-glycerophosphate, and ascorbate-2-phosphate produces more potent osteoblast differentiation of human MSCs in vitro.

Following various experimental studies, the number of the clinical prospective studies has also increased steadily, and good cases of translational research from basic research to clinical

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application have arisen. In a groundbreaking study, Wiltfang et al. [52] have reconstructed a mandibular discontinuity defect after ablative surgery using the gastrocolic omentum as a bioreactor for heterotopic ossification via a titanium mesh cage filled with bone mineral blocks, infiltrated with 12 mg of recombinant human BMP2, and enriched with bone marrow aspirate. The scaffold was implanted into the gastrocolic omentum, and 3 months later, a free flap was harvested to reconstruct the mandibular defect. In vivo single-photon-emission computed tomography/computed tomography revealed bone remodeling and mineralization inside the mandibular transplant during prefabrication. They have reported that the quality of life of the patient significantly increased with acquisition of the ability to masticate and the improvement in pronunciation and aesthetics.

It is well known that MSCs can be directed to differentiate into an osteoblastic lineage in the presence of growth factors. Furthermore, platelet-rich plasma (PRP), which can be easily isolated from whole blood, was often used for bone regeneration, wound healing, and bone defect repair [53]. Marx [43] have stated that PRP contains an amount of 250×10^3 –400×10^3 per ml which are positive for CD 44, CD 90, CD 105, and CD 34.

Yamada et al. [54] investigated as basic research tissue-engineered bone regeneration using MSCs and PRP in a dog mandible model and confirmed the correlation between osseointegration in dental implants and the injectable bone. After that, same authors applied this injectable tissue-engineered bone to onlay plasty in the posterior maxilla or mandible in three human patient with simultaneous implant placement and reported stable and predictable results in terms of implant success [55]. In 2005, Ueda et al. [56] have used MSCs in a clinical study undertaken to evaluate the use of tissue-engineered bone, MSCs, platelet-rich plasma, and beta-tricalcium phosphate as grafting materials for maxillary sinus floor augmentation and proclaimed that tissue-engineered bone provided stable and predictable results in terms of implant success.

In order to increase the amount of available bone where dental implants must be placed, Filho Cerruti et al. [57] evaluated PRP and mononuclear cells (MNCs) from bone marrow aspirate and bone scaffold in 32 patients and have concluded that the process of healing observed in the patients was due to the presence of mesenchymal stem cell in MNC fraction in the bone grafts. Schmelzeisen et al. [58] reported a simplified method of using to regenerate hard tissue and suggested that bone marrow aspirate concentrate combined with a suitable biomaterial can form sufficient bone within 3 months for further implants to be inserted and at the same time minimize morbidity at the donor site. Similarly, Ricket et al. [59] have assessed whether differences occur in bone formation after maxillary sinus floor elevation surgery with bovine bone mineral mixed with autogenous bone or autogenous stem cells and stated that MSCs seeded on bovine bone mineral particles can induce the formation of a sufficient volume of new bone to enable the reliable placement of implants within a time frame comparable with that of applying either solely autogenous bone or a mixture of autogenous bone and bovine bone mineral particles.

Not only the defects at implant recipient sites, peri-implantar bone loss has also become a point of interest for some researchers, and efforts have been made over the last few decades to produce reliable and predictable methods to stimulate bone regeneration in bone defects resulting from peri-implant diseases [60]. Ribeiro et al. [61] have investigated the effect of bone marrow–derived cells associated with guided bone regeneration in the treatment of dehiscence

bone defects around dental implants and suggested that bone marrow–derived cells provided promising results for peri-implantar bone regeneration, although the combined approach seems to be relevant, especially to bone formation out of the implant threads. Similarly, Kim et al. [62] evaluated the potential of periodontal ligament stem cells and bone marrow stem cells on alveolar bone regeneration in a canine peri-implant defect model and demonstrated the feasibility of using stem cell–mediated bone regeneration to treat peri-implant defects.

6. Conclusion

A growing number of studies indicate that stem applications are feasible protocols with clinically successful results in restoration of the bone architecture of the maxillofacial region. Composite grafts of MSCs, BMP, PRP, and bone graft combinations are able to achieve clinical results equivalent to autogenous grafts in large vertical ridge augmentations without donor bone harvesting.

Continued and extended experimental studies are needed to exactly determine the isolation, characterization, and differentiation properties of MSCs. In addition, development of chairside protocols would be beneficial in order to adapt MSC applications to the daily dental practice.

Abbreviations

ASC	Adipose tissue-derived stromal cells
BMAC	Bone marrow aspirate concentrate
BMP	Bone morphogenetic protein
CCFDAB	Crushed cancellous freeze-dried allogeneic bone
CD	Cluster of differentiation
CEACAM	Carcinoembryonic antigen-related cell adhesion molecule
FACS	Fluorescence-activated cell sorting
FMB	Fibrin microbeads
GMP	Good manufacturing practice
HIV	Human immunodeficiency virus
HLA-DR	Human leukocyte antigen-D-related
MHC	Major histocompatibility complex
MSC	Mesenchymal stem cell
NCAM	Neural cell adhesion molecule
PBS	Phosphate buffered solution

PDLSC	Periodontal ligament stem cell
PRF	Platelet rich plasma
PRP	Platelet-rich fibrin
rhBMP	Recombinant human bone morphogenetic protein
TNAP	Tissue non-specific Alkaline Phosphatase

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