REVIEW ARTICLE

https://doi.org/10.5125/jkaoms.2022.48.2.71
pISSN 2234-7550 · eISSN 2234-5930

# Use of stem cells in bone regeneration in cleft palate patients: review and recommendations

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**Abstract** (J Korean Assoc Oral Maxillofac Surg 2022;48:71-78)

This study was conducted to review the efficacy of different sources of stem cells in bone regeneration of cleft palate patients. The majority of previous studies focused on the transplantation of bone marrow mesenchymal stem cells. However, other sources of stem cells have also gained considerable attention, and dental stem cells have shown especially favorable outcomes. Additionally, approaches that apply the co-culture and co-transplantation of stem cells have shown promising results. The use of different types of stem cells, based on their accessibility and efficacy in bone regeneration, is a promising method in cleft palate bone regeneration. In this regard, dental stem cells may be an ideal choice due to their efficacy and accessibility. In conclusion, stem cells, despite the lengthy procedures required for culture and preparation, are a suitable alternative to conventional bone grafting techniques.

**Key words:** Cleft palate, Tissue engineering, Bone regeneration, Craniofacial abnormalities, Stem cells

*[paper submitted 2021. 1. 20 / revised 2021. 3. 8 / accepted 2021. 3. 9]*

## I. Introduction

Cleft lip and palate is the most common orofacial congenital defect. The prevalence of this deformity among the Iranian population is 1 per 1,000 births[1]. This congenital deformity encompasses a range of defects from cleft lip to bilateral cleft lip and cleft palate. In addition to environmental factors, several genes and signaling pathways have been discovered to have an association with cleft lip and palate.

Many surgical procedures have been proposed for the treatment of cleft lip and palate patients. Bone grafting is widely used to fill the defect area in cleft palate patients. Bone grafts can be harvested from several sites that have proved to have different outcome quality. Although the anterior iliac crest is considered as the gold standard for secondary alveolar bone grafting (SABG), Wu et al.[2] concluded that a mandibular graft is the most favorable graft for SABG cases. The underlying explanation of this conclusion is that the mandible and maxilla have the same origin and they are both developed by the intramembranous ossification process[2,3]. However, it is noteworthy that although the cranium is developed by the same ossification process, the outcomes of cranial bone grafting have not been as favorable as mandibular and iliac bone grafting[2]. Despite the quality of mandibular bone grafts, the low volume of bone obtained from the mandible may be insufficient. Therefore, Kilinc et al.[4] recommended more precise preoperative evaluations before using this technique. To overcome this obstacle, Weijs et al.[5] proposed a mandible graft enriched with β-tricalcium phosphate (β-TCP) for defects that have larger volumes than the mandible graft. As mentioned above, the anterior iliac crest is the gold standard because of its high spongy bone volume and sufficient bone needed to fill the defects. However, there are several drawbacks for this technique, such as long hospitalization time, considerable resorption rate, and surgical risks[6,7].

## II. Stem Cell Therapy

To address the morbidities of the previous therapeutic strate-

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gies, new therapeutic approaches like stem cell therapy have been developed. Here, we will discuss the current advancements in the use of stem cell therapy in cleft palate regeneration.

### 1. Umbilical cord mesenchymal stem cells

The extraction of umbilical cord mesenchymal stem cells (UCMSCs) is entirely non-invasive. These cells have been shown to survive up to 48 hours at room temperature[8]. Moreover, they can be preserved at –80°C for up to 1 year[8]. Therefore, cryopreservation seems to be a suitable approach for the storage of stem cells. In addition, cryopreservation does not seem to influence the osteogenic potential of UCMSCs. Moreover, Kotobuki et al.[9] reported that bone marrow mesenchymal stem cells (BMMSCs) that were cryopreserved for 3 years still maintained their high osteogenic potential. According to studies conducted by Mueller et al.[8] and Baba et al.[10], the subcutaneous implantation of UCMSCs with hydroxyapatite scaffold or granules does not led to mature bone formation. In contrast, Sahai et al.[11] and Sun et al.[12] demonstrated that the combination of UCMSCs with Wharton's jelly or collagen scaffold has led to favorable outcomes after 6 months. As a result, it can be concluded that UCMSCs have more osteoinductive potential, which consequently makes them ideal for stem cell co-transplantation in cases in which other sources of stems cell are not sufficient, such as stem cells from human exfoliated deciduous teeth (SHED), dental pulp stem cells (DPSCs), muscle-derived stem cells (MDSCs), and adipose-derived stem cells (ADSCs), especially in children who have a smaller number of cells for cleft regeneration.

Additionally, in a study performed by Caballero et al.[13], the outcomes of cleft treatment using UCMSCs in a poly(lactide-*co*-glycolide) (PLGA) scaffold was slightly better than the cancellous bone but the difference was not significant. However, it is noteworthy that both cancellous bone and tissue-engineered bone had superior outcomes compared to rib grafts[13]. In their next study, Caballero et al.[14] demonstrated that previously differentiated UCMSCs produced slightly more mature bone compared to undifferentiated UCMSCs in the cleft area. However, this was not significant. Therefore, the authors concluded that there was no justification for the differentiation of UCMSCs before transplantation.

### 2. Umbilical cord blood mesenchymal stem cells

Garcia et al.[15] reported the case of a patient who was treated with a combination of umbilical cord blood stem cells (UCBSCs) and a gingivoperiostioplasty technique at 5 months of age. The umbilical cord blood mesenchymal stem cells (UCBMSCs) were collected at the time of the child's birth. During the surgery, 90% of the stem cells were placed inside the gingivoperiostioplasty pocket using Gelfoam and the rest was injected into the alveolar and labial surgical wound with a syringe. Although after 18 months the result was satisfactory, large scale studies should be conducted to determine the efficacy of stem cells in this procedure. Further optimization of this procedure using stem cells may help clinicians obtain better outcomes.

In addition, Mazzetti et al.[16] reported the injection of umbilical cord blood and placenta blood stem cells for soft and hard tissue healing during the first procedure of cleft palate treatment (rhinocheiloplasty). The results on soft tissue were significant and there was less scar formation and inflammatory response in the soft tissue of the lip, but there was no bone formation. However, after the second surgery (hard palate surgery) there was less fibrous tissue formation and no palatal fistula was observed.

### 3. Adipose derived stem cells

ADSCs have been under consideration for regenerative medicine, especially for bone regeneration, due to their range of differentiation potentials. To compare the osteogenic potential of ADSCs with an autogenous bone graft, Pourebrahim et al.[17] implanted predifferentiated ADSCs, seeded on a hydroxyapatite/β-TCP scaffold in an artificially created jaw cleft of mongrel dogs, while a corticocancellous tibial bone graft was implanted on the side. The stem cell group showed inferior outcomes after 15 and 60 days. However, the rate of bone formation in the stem cell group between days 15 and 60 was markedly rapid. These data can be explained by the fact that although osteoblasts were seen on both groups on day 60, they were noticed only in the tibial graft group on day 15. Moreover, there was significantly more collagen synthesis in the stem cell group than in the bone graft group. In another study by Shahnaseri et al.[18] nearly the same procedure was applied. Although there was no statistically significant difference between the stem cell group and the tibial graft group, the time lapse was statistically significant. In this study, bone graft resorption was noticed only until day 30, but it lasted until day 45 in the stem cell group. Although bone density in both groups was the same in the second month, the radiographic view of the tissue-engineered group was not as ho-

mogenous as the tibial graft group. This delay in bone regeneration in the stem cell group might be attributed to the low angiogenic potential of the stem cell group. In addition, Sasayama et al.[19] evaluated the osteogenic potential of ADSCs and dedifferentiated fat cells (DFATs) with vacuum-heated gelatin sponges modified with epigallocatechin and vacuum-heated gelatin sponges in a congenital jaw cleft model. The highest percent of bone volume was produced by the combination of DFATs and vacuum-heated epigallocatechin gallate-gelatin sponge (vhEGCG-GS) and the vhEGCG-GS itself has shown osteoinductive potential.

A combination of ADSCs with other materials has shown favorable outcomes. According to Lee et al.[20], the combination of ADSCs with recombinant human bone morphogenetic protein-2 (BMP-2) results in superior outcomes compared with single applications of ADSCs in distraction osteogenesis. This may be due to the lack of a scaffold for the stem cells. The authors conclude that BMP-2 with or without ADSCs are effective in bone formation during distraction osteogenesis. In addition, the synergistic effect of platelet-rich plasma (PRP) and BMP-2 has been effective in osteogenic differentiation of ADSCs[21]. This may further help to optimize bone formation by ADSCs. In another study by Khojasteh et al.[22], buccal fat pad stem cells were seeded on demineralized bovine bone mineral and applied to the defect site with either the lateral ramus cortical plate or the anterior iliac crest. Moreover, anterior iliac crest was used for the control group. The first two groups exhibit superior outcomes, while the highest amount of bone fill is achieved in the group of stem cells with the anterior iliac crest. According to the results of this study[22], the combination of ADSCs with demineralized bovine bone matrix and lateral ramus cortical plate could further eliminate the need to harvest bone grafts from the iliac crest and only require an intraoral donor site.

### 4. Bone marrow mesenchymal stem cells

BMMSCs are by far the most commonly used stem cells to treat the bone defect of cleft palate patients. The results obtained from different studies vary widely, which is attributed to the type of scaffold and the type of cells, such as bone marrow mononuclear cells (BMMNCs), undifferentiated mesenchymal stem cells (MSCs), and differentiated MSCs, as well as the growth factors used. BMMNCs encompass a wide range of cells, such as BMMSCs, hematopoietic stem cells, epithelial progenitor cells, hematopoietic progenitor cells, adipocytes, macrophages, neutrophils monocytes, and platelets[23,24]. There are a couple of advantages with the use of BMMNCs instead of BMMSCs, such as there is no need to culture cells, which makes it more time-saving and less expensive. Additionally, this procedure can be performed during surgery. The other advantage of BMMNCs is their wide variety of cells with cell-cell communication that can contribute to better osteogenesis[25]. According to Al-Ahmady et al.[26], the use of BMMNCs within a collagen scaffold and nanohydroxyapatite particles, covered by the two platelet-rich fibrin (PRF) membranes, has resulted in better outcomes, compared to anterior iliac crest bone grafting. Complete closure was achieved along with less operation time and postoperative complications. In contrast, Du et al.[27] concluded that BMMNCs seeded on β-TCP have similar outcomes compared with iliac bone grafting after one year.

The use of BMMSCs has also been successful in several cases. Zhang et al.[28] demonstrated that the rate of mineralization in a group treated by BMMSCs seeded on a β-TCP scaffold is similar to a group treated with autogenous bone until the eighth week, but the mineralization rate in the stem cell group increases at the 20th week. Nevertheless, Bajestan et al.[29] reported less satisfactory outcomes of stem cell therapy in both cleft palate patients and trauma patients compared with the mandibular bone grafting technique. However, the authors argue that there is no preclinical data on the ixmyelocel-T adherence to β-TCP scaffolds. Therefore, this could have affected the results. It is also important to note that the rate of bone regeneration has been shown to be higher in trauma patients compared with cleft patients[29].

The use of platelet rich products has also been promising. In this regard, Mossaad et al.[6] reported higher bone density with the use of BMMSCs combined with a PRF membrane than with an autologous iliac graft 6 months after surgery. Also, autologous bone graft resorption has been observed, which is a common characteristic of bone grafts. In a case study by Stanko et al.[30], the combination of MSCs and a platelet gel has also resulted in oronasal fistula closure 10 weeks after surgery.

The difference between differentiated and undifferentiated BMMSCs has also been investigated in a few studies. According to Korn et al.[31], the application of undifferentiated MSCs with bovine hydroxyapatite/collagen, seems to be more efficient than differentiated cells with bovine hydroxyapatite/collagen after 12 weeks. However, neither of the groups have shown an increase in bone formation in rodent models. This lack of increase is attributed to the use of a non-resorbable scaffold that prevents bone formation. In another

study by Korn et al.[32], the same outcome is achieved. Resorptions are more significant in the undifferentiated group than the differentiated one after 1 week. Further data at the third and sixth week indicates more efficiency of undifferentiated BMMSCs compared to differentiated BMMSCs. As has been demonstrated by Bara et al.[33], osteogenically or chondrogenically differentiated BMMSCs lead to less angiogenic and neurogenic capacity of the cells and also inhibits angiogenesis. This can be satisfactory for forming avascular cartilage tissue, but it may be of concern for bone regeneration. This *in vitro* study can somehow explain the reason underlying the lower capacity of differentiated BMMSCs in alveolar cleft repair.

Scaffolds also play an important role in the process of bone regeneration, which can affect the efficiency of stem cells. The use of non-resorbable scaffolds has not been successful as there is no place for the newly regenerated bone[31]. Ideally the rate of scaffold resorption should be equal to the amount of bone formation. As mentioned above, the combination of MSCs with bovine hydroxyapatite does not enhance bone regeneration[31]. However, the use of hydroxyapatite particles in combination with PRP and BMMSCs has been successful[30]. Correspondingly, Mossaad et al.[6] also reported favorable outcomes with the combination of nanohydroxyapatite powder and a collagen scaffold. An alternative to this bone substitute is the combination of hydroxyapatite and β-TCP, which according to two studies has resulted in more satisfactory outcomes[32,34]. Another alternative material used as a scaffold is carbonated hydroxyapatite (CAP) which has a higher solubility and less stable crystals[35]. The addition of BMMSCs with CAP has resulted in perfect closure of an artificial bilateral cleft in dogs after 6 months[35]. Notably, the absence of CAP particles in the stem cell–CAP combination after 6 months conveys that all the implant was substituted by natural bone. This observation is due to the metabolic activity of stem cells, which has rendered significant angiogenesis and scaffold degradation. Also the rate of tooth movement in the area grafted with BMMSCs with CAP has been more consistent than the area grafted with only CAP[36]. Moreover, unlike hydroxyapatite the CAP scaffold does not disturb tooth movement[36]. Hydrogels also have been used for cleft palate closure. In a study by Naudot et al.[37] using a group of allogenic BMMSCs with alginate-based hydrogel scaffolds, bone formation is observed in the middle of the implant in addition to in the margins. However, the rate of mineralization in the scaffold-only group is higher in the 8th and 12th week. This may be due to the instability and higher degradation rate of hydrogels, which is also accelerated by the metabolic activity of MSCs. Consequently, there may not be enough scaffold for the regeneration of MSCs. Moreover, the use of three-dimensional (3D) scaffolds may be a proper choice for bone regeneration. In this regard, the addition of BMMSCs to a 3D polycaprolactone scaffold has resulted in 45% bone fill and 75% bone mineral density compared to the surrounding bone tissues after 6 months[38]. This outcome can be optimized by the addition of proper growth factors. Gimbel et al.[39] also suggested collagen as a scaffold for BMMSCs. This has resulted in less bleeding, wound dehiscence, and postoperative pain. The cost of this procedure has been reported to be 58% less than iliac bone grafting[39].

The use of growth factors has to some extent been explored in the regenerative effects of BMMSCs. Behnia et al.[34] concluded that platelet-derived growth factor is not a suitable growth factor by itself as its addition to BMMSCs on hydroxyapatite/β-TCP has resulted in 51.3% bone fill 3 months after surgery. However, the addition of PRP, PRF membrane, and PRP gel has resulted in better outcomes[26,40]. These materials contain a variety of growth factors, such as vascular endothelial growth factor (VEGF), transforming growth factor-β, and insulin-like growth factor[41]. PRF seems to be the material of choice because it can be used as scaffold and it has time-dependent growth factor release[42,43].

Aside from all the materials and approaches mentioned above to enhance the efficacy of the regenerative capabilities of BMMSCs, new approaches have been proposed. For example, mechanical stimulations such as rapid maxillary expansion has resulted in higher bone graft height and significant bone mineralization[44]. It is also hypothesized that endosseous implants can retard bone resorption of the bone graft[45]. Chung et al.[46] proposed the addition of the BMP-2 gene to BMMSCs through adenovirus to be more efficient for bone formation and periodontal tissue regeneration. The advantage of this technique over the addition of BMP-2 as a growth factor is its prolonged BMP-2 release from the cells, which eliminates the need for addition of BMP-2 to the scaffold. This new approach can be used in future clinical studies on BMMSCs aspirated from the iliac crest or the craniofacial area to see if it can be helpful in cleft palate closure. Also, the use of other types of stem cells can open new horizons for this procedure. As the children undergoing bone augmentation are in the age of mixed dentition, the use of dental stem cells can also be helpful. In a study by Lee et al.[47], the combined application of human MSCs (hMSCs) and stem cells derived from deciduous teeth have been evaluated. As the application of cell sheets seems to be a better approach

than stem cell injection into the defect, cell sheets have been applied to enhance bone formation[47]. This is due to the cell-cell connection and the extracellular matrix that exists in the cell sheets. The results show that SHED have a higher potential in osteogenesis than BMMSCs. Despite their higher osteogenic potential, the low numbers of these cells limit their use. Because of this, SHEDs have been co-cultured with BMMSCs in a recent study. Moreover, BMMSCs obtained from the craniofacial area have exhibited higher osteogenic and angiogenic potential compared with those derived from the iliac crest[48]. Additionally, they have exerted more favorable anti-aging and stemness capacities compared with the tibia BMMSCs[49]. Therefore, craniofacial BMMSCs are also suitable candidates for cleft bone regeneration.

### 5. Dental stem cells

Because stem cell augmentation is an alternative to SABG during the mixed dentition age of patients, dental stem cells obtained from deciduous teeth may be a proper choice. As mentioned earlier, SHEDs have exerted higher osteogenic potential compared with BMMSCs[47]. However, there has been no statistically significant difference between SHEDs, DPSCs, and BMMSCs in an animal model[50]. These results validate DPSCs and SHEDs as a promising alternative to BMMSCs. Also, it is important to mention that the largest osteoid and collagen fiber quantities have been observed in a group treated with SHEDs[50]. In addition, Tanikawa et al.[51] evaluated the efficacy of deciduous DPSCs in a clinical study. The results of this study suggest that the outcomes of stem cell therapy are comparable to iliac bone grafting[51]. Although alveolar reunion in the two groups is significantly higher than the group treated with BMP-2 6 months after surgery, the difference disappeared after 12 months[51]. However, Jahanbin et al.[52] reported that osteogenically differentiated deciduous DPSCs provide comparable results to iliac bone grafts in the second month, whereas iliac bone graft had better outcomes in the first month. In this regard, Wongsupa et al.[53] proposed DPSCs seeded on a polycaprolactone scaffold as a suitable model for stem cell therapy. One of the main obstacles that seems to limit the use of dental stem cells may be their insufficient number. To overcome this limitation, Lee et al.[47] co-cultured SHEDs with hMSCs. However, Tanikawa et al.[51] reported that obtaining a sufficient number of cells from the culture of deciduous DPSCs takes one month after extraction of the tooth. This can further encourage researchers to use dental tissue stem cells as an alternative for bone grafting.

## III. Future Prospects

The application of stem cells has opened new horizons in alveolar cleft repair as well as other bone defects. New approaches are also being applied to optimize the efficacy of this new treatment modality. As cleft defects are specific for each patient in size and volume, the use of 3D scaffolds and patient-specific scaffolds can ease the regeneration process. Scanning the defect size by computerized tomography scans can be applied. For the 3D construction of scaffolds, Berger et al.[54] proposed the use of computer aided design or computer aided manufacturing for 3D construction of the β-TCP scaffold. Cryogels can also be a proper choice for 3D scaffold design because of their exceptional mechanical characteristics[55]. Separately, regeneration of several tissues at the same time is the ultimate goal of regenerative medicine, which has also been investigated for cleft regeneration[56], but methods still need to be further optimized. Moreover, enhancing the osteogenic potential of stem cells in the grafted area is also of high importance. In this regard, low level laser therapy has been useful for *in vitro* deciduous DPSC osteogenesis[57]. According to Park and Park[58], *in vivo* red light radiation, with a wavelength of 647 nm, has been effective in enhancing bone formation in a subcutaneous graft of stem cells in mice. Irradiation is performed for 3 weeks, for 60-90 seconds each time[58].

Aside from the techniques mentioned above, there are new sources of stem cells that have not been thoroughly investigated. For example, MDSCs have the same behavioral and phenotypic characteristics as other stem cells in the body, which can be easily harvested from orbicularis oris during cheiloplasty in patients with cleft palate[59]. MDSCs seem to be a better option than muscle precursor cells as the latter needs BMP-2 for *in vitro* osteogenic differentiation[59]. In addition, application of induced pluripotent stem cells is a new opportunity for bone regeneration[60]. However, more studies need to be conducted to accurately confirm the efficacy of this new treatment.

Another method that has been evaluated for enhancing osteogenesis and neovascularization of augmented stem cells is co-culturing and co-transplantation of two different types of stem cells. Co-culturing and co-transplantation of ADSCs and endothelial progenitor cells (EPCs) has promoted bone regeneration and angiogenesis in a rat cranial defect[61]. In a study by Wu et al.[62], the addition of epithelial progenitor cells to BMMSCs has resulted in the upregulation of mRNA expressions of VEGF, osteonectin, osteopontin, and collagen type

1 and an increase in alkaline phosphatase activity and CD34. However, the role of VEGF seems controversial. As has been reported, the addition of VEGF to diaphyseal stem cells co-cultured with human umbilical vein endothelial cells (HUVECs) *in vitro* has reduced the amount of osteogenesis, while VEGF has resulted in a higher osteogenic potential in an *ex vivo* model[63]. However, by co-culturing BMMSCs with EPCs Seebach et al.[64] concluded that the indirect effects of EPCs is stronger than the direct effects, which are caused by releasing chemotactic factors such as VEGF to recruit the EPCs of the host to induce vascularization. HUVECs are another type of stem cell that are frequently used, and by secreting BMP-2 they have resulted in higher bone formation when co-cultured with ADSCs[65]. In addition, the novel method of Rong et al.[66] of co-culture of osteogenically differentiated HUVECs and angiogenically differentiated HUVECs is a new method of co-culture and co-transplantation of stem cells. Moreover, the optimum ratio of osteogenically differentiated HUVECs versus angiogenically differentiated HUVECs for mature bone formation and collagen formation has been reported as 3:1[66]. Also, Kim et al.[67] reported that although HUVECs co-cultured with BMMSCs enhance osteogenesis, their effect is less than that of nanotopography. Therefore, this method can be useful for higher bone formation in the graft area. To make this technique more applicable for osteogenesis in alveolar clefts, co-culture and co-transplantation of human exfoliated deciduous teeth stem cells or deciduous DPSCs with HUVECs or EPCs can be a proper method. However, further studies are needed to evaluate the efficacy of this method in cleft palate repair.

In conclusion, various stem cells have been recruited for alveolar bone formation. The most frequently used stem cells are BMMSCs. However, dental stem cells, due to their satisfactory osteogenic potential and accessibility, hold great promise for future applications. The following conclusions can be drawn from this review. 1) The use of stem cells with proper growth factors and scaffolds has comparable outcomes to iliac bone grafting. 2) There are fewer postoperative complications with the use of stem cells. 3) There is no superiority of differentiated stem cells over undifferentiated stem cells. 4) Dental stem cells are proper alternatives to BMMSCs. 5) To enhance the osteogenic potential of the mechanical stimulation of stem cells, red light irradiation and nanotopography has been proposed. 6) The stem cell co-culture and co-transplantation technique also enhances bone formation and vascularization. 7) A 3D scaffold design for seeding stem cells and multilayer tissue regeneration is another goal for more perfect bone repair, which requires further investigation.

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## Authors' Contributions

M.A.A. and F.L. were involved in designing and writing the manuscript. H.D. was involved in an advisory role and study design. All the authors read and confirmed the final manuscript.

## Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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**How to cite this article:** Amiri MA, Lavaee F, Danesteh H. Use of stem cells in bone regeneration in cleft palate patients: review and recommendations. J Korean Assoc Oral Maxillofac Surg 2022;48:71-78. https://doi.org/10.5125/jkaoms.2022.48.2.71