Management of Maxillofacial Defects by Stem Cells Therapy

Hossam Abdelbaky; Mohamed Ali El Said.; Mohamed Elsaid and Haitham Elsaid Abdalla Mohamed

Department of Otorhinolaryngology, Faculty of Medicine-Benha University.
Dr.haithamabdalla@yahoo.com

Abstract: Stem cell biology is now one of the most exciting and rapidly advancing areas of scientific effort. Promises of cures of a wide variety of diseases through specific replacement of damaged or malfunctioned tissues by the use of stem cells are on the horizon in clinical practice. Stem cells are able to divide and renew themselves over long periods of time. They are not specialized and can differentiate into specialized cells. Various sources for stem cells include embryonic tissue (blastocysts), bone marrow, adipose tissue, skin, and the brain. Stem cell therapy can be used in the field of Otorhinolaryngology - Head & Neck Surgery in sensorineural hearing loss, facial nerve paralysis, tympanic membrane perforations, auricular reconstruction, tracheal resection, incisional wounds, cancer treatment and head & neck reconstructive surgery.
[Hossam Abdelbaky**;** Mohamed Ali El Said.; Mohamed Elsaid and Haitham Elsaid Abdalla Mohamed **Management of Maxillofacial Defects by Stem Cells Therapy.** *Nat Sci* 2016;14(6):33-39]. ISSN 1545-0740 (print); ISSN 2375-7167 (online). http://www.sciencepub.net/nature. 4. doi:10.7537/marsnsj14061604.

Keywords: stem cells-application of stemcells in otolaryngology (alveolar margine –septum _tympanic membrane-cranial defects_mandible)

Introduction

Cranio-maxillofacial skeletal defects arise as a con- sequence of congenital malformations such as cleft lip and palate, are due to traumatic avulsion, result from tumor resection, or follow severe infection. The use of autogenous bone is still considered to be the gold standard for the reconstruction of cranio-maxillofacial skeletal defects [(1)]. Harvesting bone for grafting, however, is associated with a second potentially unnecessary surgical site with significant donor site morbidity that also requires additional operative and anesthetic time [(2,3)]. The elimination of autogenous bone graft harvesting would spare patients from the potential suffering related to major donor site morbidity. This realization has led to a search for an ideal bone sub stitute that could mimic the osteogenic capacity of autogenous bone[(4)].

An alternative approach is that of tissue engineering. Tissue engineering was defined by **Langer and Vacanti**[(5)] as an interdisciplinary field of research that applies both the principles of en- gineering and the processes and phenomena of the life sciences toward the development of biological substitutes that restore, maintain, or improve tis- sue function. Three components are described as part of the tissue engineering of bone, including vital bone-forming cells, growth factors, and a scaf- fold to promote the formation of new bone in a desired location and with particular precise dimensions and shape[(6)].

Growth factors can induce mesenchymal cells to differentiate into osteoprogenitor cells[(7)]. Whereas several growth factors have been identified, few have been used in a clinical setting[(8)]. Scaffolds or bone substitutes such as bioactive glass (BAG) and b-tricalcium phosphate (b-TCP) can be used in combination with growth factors[(9)].

Constructs using stem cells obtained from autogenous adipose tissue, for example, can be made using scaffolds and growth factors to enhance bone regeneration [(10)]. Although major segments of human mandibular defects have been reconstructed with constructs containing growth factors, such as recombinant human bone morphogenetic protein-7 (rhBMP-7)[(11)], reports regarding cell-seeded constructs are still few in number[(12)]. Whereas there has been much laboratory study of possible techniques [(13)], most clinical reports describe the occasional single-case success with limited follow-up[(14)].

Aim of the Work

In these work, we aim to study the role of stem cells in management of different maxillofacial defects either alone or in compination with chemical material or the use of tissue engenering or growth factor.

Also we will discusses the sources of stem cells and the methods of application.

Finally we hope to provide the recent application of stem cells in our field.

Results

In these study we discuss the result and outcome of application of stem cells in the field of maxillofacial defects .the study will focus on the application of stem cells in:

1-Septal defect or perforation.

2-Alveolar margine.

3-tympanic membrane.

4-cranial defecs.

5-mandibular defects.

Nasal Septum Results

The two nasal septum patients healed uneventfully. Nasal septum patient 1 was satisfied with her nasal septal perforation repair, and the perforation has not recurred in over 28 months of follow-up. Unfortunately, nasal septum patient 2 began to resume her habitual nasal picking and was un- able to stop. This eventually led to recurrent ulceration, crusting, and finally exposure of the ASC seeded ChronOS Strip construct. The construct was removed 12 months after its initial placement.

The success criterion for the grafts at the four different sites, frontal sinus, cranium, mandible, and nasal septum, was functional, that is to say, healed hard-tissue grafts in their recipient bed functioning according to the demands of their new native sites during the follow-up period[15].

Results in alveolar margine

There was successful healing with no fistula or oro-nasal communication in all cases. The mean postoperative defect fill was measured 51.3% in 4 alveolar pre-maxillary clefts 3 months post operatively . The patients were referred back to the department of orthodontics to start orthodontic tooth alignment.

Results in tympanic membrane

Our results support the notion that the latent stem cells are key regulators of TM regeneration in acute and chronic perforations.

The epithelial layer of the TM is similar to the epidermis of the skin[16].

Althoughfurther studies of TM stem cell markers are required to identify the exact stem cells in the TM, we demonstrated that potential stem cells in the TM present high expression of epithelial stem cell markersand proliferation markers. Stem cells are responsible for tissue repair and the high proliferation of epithelial tissues[17].

Cranial Defect Clinical and Radiological Results

The wounds of the five cranial defect patients healed uneventfully following their reconstructions. Cranial patients 1 and 5 both underwent hemangioma or meningioma resections before their reconstructions. Both these patients had a titanium mesh: cranial patient 1 on the outside, and cranial patient 5 on the inside with an outer resorbable mesh. These patients showed clinical and radiographic evidence of bony defect healing, although one patient had a recurrence of the meningioma at a resection margin with positive evidence of bone healing of the repaired cranial defects at the second resection.

Cranial patient 2 had the loosened acrylic cranioplasty removed before reconstruction with an outer resorbable mesh. This patient had evidence of graft resorption and was reoperated on 1 year later with placement of a titanium mesh on the outer cranium. Cranial patient 3 had a late bone infection before the autologous ASC-seeded b-TCP reconstruction with resorbable meshes on the outer and inner cranium. This patient has shown some mild resorption at the edges of the reconstruction. Cranial patient 4 had a previously resected hemangioma with a bone flap infection before reconstruction. This patient had almost complete resorption of the ASC-seeded b-TCP reconstruction, which also used resorbable meshes on the outer and inner cranium . Cranial patient 4 also sustained an abdominal hematoma at the adipose do nor site, which required drainage on the first postoperative day.

Clinical and Radiological Results in Mandibular Defects

The reconstruction of the three mandibular defects with autolo- gous ASCs was successful in bridging the large mandibular defects averaging 8.2 cm (range, 6.0–10.0 cm) with uneventful healing. Two of the three patients opted for reconstruction with dental implants. There was a total of seven dental implant fixtures placed in two patients with six (86%) fixtures being successfully osseointegrated and then loaded in masticatory function. The three patients have been under follow-up for an av- erage of 35 months following their ASC-based mandibular recon- structions (range, 27–51 months).

Discussion

Septum

Two female patients aged 44 and 50 years with chronic nasal septum perforations were included in the study. Both patients were troubled by their constantly crusted intranasal ulcers and found this difficult to tolerate . Neither patient had a his- tory of cocaine or other drug abuse. In both cases the nasal sep tal perforations were treated with soft tissue flaps on both sides of the nasal septal perforations . An ASC-seeded re- sorbable ChronOS Strip (Synthes) was used as a scaffold and implanted sandwiched between the two flaps. The flexible ChronOS Strips contain «-polycaprolactone and differ from ChronOS Granules, which consist solely of b-TCP. The two patients were followed closely, and clinical evaluations were carried out at 1 week; 1, 6, and 12 months after surgery; and annually there after[15].

Alveolar margin

Human bone marrow derived mesenchymal stem cells (hMSCs) appear to be a popular source of adult stem cells and nowadays are widely used in various cell therapy and tissue engineering procedures[17]. These cells have not been shown to be perfectly capable of osteogenesis in an in vitro or in vivo environment, nevertheless their behaviour is different and less predictable in a living context. Several studies

carried out on animal and human models have not been able to demonstrate a significant increase in bone augmentation with the application of MSCs[18,19,20]. A previous study has shown that when compared to platelet derived growth factors, MSCs are capable of inducing new bone formation[19]. Defect size and site play an important role in the process of healing and osteogenesis and the anterior maxillary cleft seems to be a challenge. **Pradel et al.** have demonstrated the successful use of differentiated osteogenic cells for cleft repair in a case report, concluding that their method can lead to spontaneous tooth eruption on the cleft side[20-21]. Meanwhile, another case study on the effectiveness of MSCs for anterior maxillary cleft augmentation demonstrated disappointing results with insufficient amounts of bone formation[20]. The design of this study aimed to assess the use of MSCs loaded on HA/TCP scaffolds in combination with PDGF for alveolar cleft regeneration. The purpose was to make a triad for cell based tissue engineering. Parallel with cell based strategies for in vivo bone repair, the use of growth factors such as morphogenic proteins has attracted much attention in different treatment protocols[22-26].

Lee et al. proved an early positive effect of PRP on bone healing in secondary alveolar bone grafts, but failed to demonstrate the late effects[25-26]. **Klongnoi et al.** did not report a beneficial use of PRP, combined with flourohydroxyapatite in a procedure of sinus augmentation[23-24]. Various combinations of rhBMP-2 with different kinds of bone substitutes have shown promising results. Critical size animal defects have been successfully healed with rhBMP-2[22]. However, the application of rhBMP-2 in human subjects has not been as reliable. 1 year post-operation, an adult alveolar cleft treated with rhBMP-2 showed no significant bone regeneration[27]. Herford et al. reported a bone volume ratio of 71% in children with premaxillary clefts treated with a combination of absorbable collagen membrane and rhBMP-2[25]. In this study the authors found that PDGF may have an augmenting effect on the capacity of hMSCs for bone regeneration. The mean amount of regenerated bone (51.3%) achieved with the present protocol appears lower than the amount of maxillary cleft repair induced by rhBMP-2 or autogenous iliac graft[25]. In addition, the amount of graft resorption in a treated cleft area is reported to be higher than other augmented maxillofacial sites[28,29]. As a result, although the use of hMSCs with PDGF increases bone regeneration when compared with unaccompanied hMSCs[20], the results are far from being satisfactory. The high cost of rhBMP-2 and the donor site morbidities of an iliac bone graft is the driving engine for investigators to search further for an ideal method of in vivo bone engineering. The patients in this study have not subsequently been treated with any other kind of surgical procedures, so histological evaluation of the amount of newly generated bone or the remnant part of the bone substitute could not be carried out. This study can therefore not be compared to other similar studies[30] in a quantitative method. The second criticism of this study is the absence of a control group. This was unavoidable due to ethical issues but unfortunately has reduced the validity of the data.

In tympanic membrane

A broad distribution of potential stem cells was observed in both normal TMs and perforated TMs. After acute TM perforation, epidermal stem cell markers were significantly upregulated. The epidermal stem cell markers INGβ1 and CK19 were highly expressed during the period of perforation; however, their expression decreased to normal levels after the perforations healed. In the unhealed perforations in animals with chronic perforations, the increased expression of INGβ1 and CK19 remained, even at 9 weeks. A similar trend for the expression of TM stem cells in the acute and chronic perforations was observed; strong expression of stem cell markers was observed in the perforated regions, whereas the expression was significantly decreased when the TMs were regenerated. In the normal human TM, possible progenitor cells have been found in themalleus and annulus region[31].

This observation is similar to our findings in rats, where marked upregulation of INGβ1 and CK19 was observed around themalleus handle and annulus after TM perforation. These observations provide the important insight that TM stem cells continuously act on the TM surface, especially during both acute and chronic TM regeneration, as a key regulator, and thus provide a novel target for regenerationof the TM without surgical intervention. We therefore considered that these latent stem cells could play a critical role in the acute TM regeneration mediated by chitosan patches that we reported previous[32,33,34] and also in a rat chronic TM perforation model of otitis media. Together, these observations provide the important insight that TM stem cells continuously act on the TM surface, especially during both acute and chronic TM regeneration, as a key regulator, and thus provide a novel target for regeneration of the TM without surgical intervention.

Cranial defect

A total of five patients (four females and one male) with an average age of 61.8 years (range, 54–75 years) underwent cranioplasty, and their bony cranial defects were filled with constructs consisting of granules of b-TCP seeded with autologous ASCs. None of the cranioplasty constructs contained rhBMP-2. All patients had bony cranial defects in the frontal, frontoparietal, or frontotemporal cranial regions. The

average defect size was 8.1 3 6.7 cm.

The indications for cranioplasties included cranial hemangi- oma (cranial patient 1), a previously resected meningioma with late loosening of the acrylic cranioplasty (cranial patient 2), an acute subdural hematoma and decompressive craniectomy with late bone flap infection (cranial patient 3), a previously resected meningioma with bone flap infection (cranial patient 4), and an unresected frontal bone meningioma (cranial patient 5). The hemangioma and meningioma tumors were removed with the outer and inner diploe¨ of bone, and, in the cases of failed acrylic cranioplasty or previously infected cranial bone flaps, the failing reconstructions were removed and the ASC-seeded b-TCP granular cranioplasty constructs were applied to the defects (Fig. 1A).

In cranial patient 1, an outer titanium mesh had been used to stabilize the granular cranioplasty reconstruction material and fixated with titanium screws. Cranial patient 2 had an outer resorbable mesh, RapidSorb (Synthes, Oberdorf, Switzerland, http://www.synthes.com), that is custom moldable in hot water in the operating room setting. This resorbable mesh was fixated with resorbable screws. Cranial patients 3 and 4 had the same resorbable mesh applied to both the inner and outer aspects of the cranial bone defects, which was also fixated with resorbable screws on the outer surface. Cranial patient 5 had a titanium mesh placed on the inner side of the cranial defect and the resorbable mesh placed and fixated with resorbable screws on the outside surface. The five patients were followed closely, and clinical evaluations were carried out at 1 week; 1, 6, and 12 months after surgery; and annually thereafter.

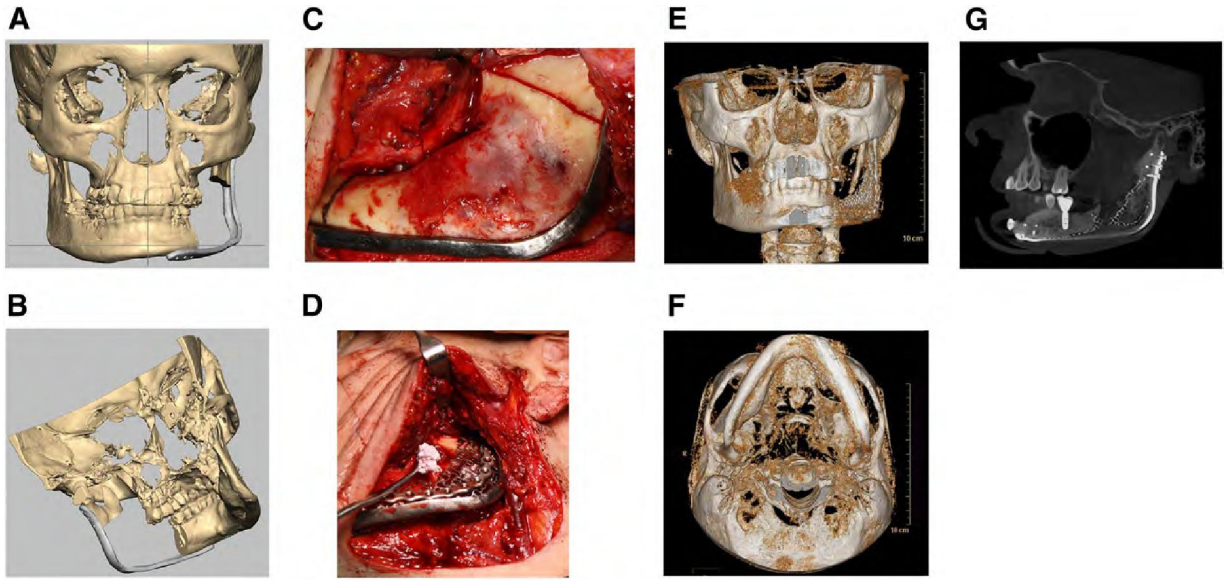

Fig. (1): Collage of mandibular reconstructions. (A): Virtual preoperative planning using Romexis software with computer-generated image of patient with large mandibular ameloblastoma showing the reconstruction plate over the area planned for resection from an anterior view. (B): Computer-generated image of patient with large mandibular ameloblastoma showing the reconstruction plate over the area planned for re- section from a medial view. (C): Intraoperative photograph showing reconstruction plate in position and resection lines on mandibular ramus posteriorly and body anteriorly. (D): Intraoperative photograph with adipose-derived stem cell-seeded b-tricalcium phosphate (b-TCP) granular construct with recombinant human bone morphogenetic protein-2 (rhBMP-2) being placed beneath titanium containment mesh at mandibular resection site. (E): Postoperative three-dimensional (3D) computed tomography (CT) scan anterior view of the regenerated left body and ramus of the mandible 12 months after reconstruction. (F): Postoperative 3D CT scan basilar view of the regenerated left body and ramus of the man- dible 12 months after reconstruction. (G): Cone beam CT scan showing mandibular reconstruction using autologous adipose-derived stem cell- seeded b-TCP granular construct with rhBMP-2 restored with successful functionally loaded dental implant in the regenerated bone.

Mandible

For mandible defects, rat, canine, goat, and monkey models have been used to evaluate the effect of bone regeneration based on stem cells and tissue engineering technology. Our group has successfully established and optimized the rat5 mm diameter circular defect model, canine border defects of 20 mm, 10 mm, and segmental defects of 30 mm length model[35]. These models are critical size defects (CSD), which means that complete calcification of the

defect will not occur during the lifetime of the animal[36]. More importantly, tissue-engineered bone constructed with BMSCs and scaffolds could achieve similar effect on bone regeneration in these models comparable to that of autogenous iliac bone graft, and the combination of growth factors could further enhance the effect.

Conclusion

Stem cell biology is now one of the most exciting and rapidly advancing areas of scientific effort. Replacement of damaged or malfunctioned tissues by using stem cells is on the horizon in clinical practice for the cure of a wide variety of diseases.

Stem cells are by definition a population of cells able to provide replacement cells for a specific differentiated cell type.

Stem cells are different from other cell types in their ability to divide and renew themselves over long periods of time and their ability to replicate or proliferate several times i.e. self-renewing. Stem cells are not specialized and can differentiate into specialized cells.

Stem cells may be defined as either totipotent, pluripotent, or multipotent whereby the stem cell is able to form all, most, or a small number of cells and/or tissues of an organism, respectively.

Pluripotent stem cells mainly come from embryos, hence the name embryonic stem cells. Multipotent (adult) stem cells have the plasticity to become all progenitor cells for a particular germ layer. These adult stem cells typically include hematopoietic stem cells, neural stem cells, bone marrow stromal cells, dermal stem cells, and fetal cord blood stem cells.

Pluripotent stem cells have not yet been used therapeutically in human beings while multipotent stem cells harvested from bone marrow have been used since the 1960s to treat leukemia, myeloma, and lymphoma.

The advantages and limitations of human embryonic and adult stem cells:

• *Human embryonic stem cells:*

Advantages: pluripotent.

Limitations: immune rejection, and it may generate tumors (teratomas).

• *Human adult stem cells:*

Advantages: no immune rejection.

Limitations: isolation, growth and differentiation are difficult, and it may contain more DNA abnormalities.

Human stem cells can be used:

- For the development of new methods to repair or replace tissues or cells damaged by injuries or diseases.
- For the generation of normal human cell lines to be used in drug development and in toxicology.
- In gene therapy: Stem cells could be used as vehicles for the therapeutic delivery of genes.
- For understanding of human development.
- For understanding of the basic mechanisms of cell differentiation and proliferation.

Applications of stem cell therapy in our research

Alveolar margine
Septal perforation
Tympanic membrane perforation
Cranial defect
Mandible defect

Recommendations

The promises of cures for human illness by stem cells have been much touted, but many obstacles must still be overcome.

First, more human pluripotent and multipotent cell research is needed since stem cell biology differs in mice and men.

Second, the common feature of unlimited cell division shared by cancer cells and pluripotent stem cells must be better understood to avoid cancer formation.

Third, the ability to acquire large numbers of the right cells at the right stage of differentiation must be mastered.

Fourth, specific protocols must be developed to enhance production, survival, and integration of transplanted cells. Finally, clinical trials must be completed to ensure safety and efficacy of the stem cell therapy. When it comes to stem cells, knowing they exist is a long way from using them therapeutically.

References

1. Bianchi B, Ferri A, Ferrari S, et al. (2013): Mandibular resection and reconstruction in the management of extensive ameloblastoma. J Oral Maxillofac Surg; 71: 528–537.
2. Ling XF, Peng X (2012): What is the price to pay for a free fibula flap? A systematic review of donor-site morbidity following free fibula flap surgery. Plast Reconstr Surg;129: 657–674.
3. Ling XF, Peng X, Samman N (2013): Donor-site morbidity of free fibula and DCIA flaps. J Oral Maxillofac Surg; 71: 1604–1612.
4. Gimbel M, Ashley RK, Sisodia M, Gabbay J, Wasson KL, Heller J, et al. (2007): Repair of alveolar cleft defects: reduced morbidity with bone marrow stem cells in a resorbable matrix. J Craniofac Surg; 18(4): 895-901.
5. Langer R, Vacanti JP (1993): Tissue engineering. Science; 260: 920–926.
6. Sàndor GK, Suuronen R (2008): Combining adipose-derived stem cells, resorbable scaffolds

and growth factors: An overview of tissue engineering. J Can Dent Assoc; 74: 167–170.
7. Cheng L, Hammond H, Ye Z, Zhan X, Dravid G (2003): Human adult marrow cells support prolonged expansion of human embryonic stem cells in culture. Stem Cells; 21: 131-142.
8. Clokie CM, Sa´ndor GK (2008): Reconstruction of 10 major mandibular defects using bioimplants containing BMP-7. J Can Dent Assoc; 74: 67–72.
9. Khojasteh A, Behnia H, Dashti SG, et al. (2012): Current trends in mesenchymal stem cell application in bone augmentation: A review of the literature. J Oral Maxillofac Surg; 70:972–982.
10. Wolff J, Sándor GK, Miettinen A, et al. (2013): GMP-level adipose stem cells combined with computer-aided manufacturing to reconstruct mandibular ameloblastoma resection defects: Experience with 3 cases. Ann Maxillofac Surg; 3: 114–125.
11. Clokie CM, Sa´ndor GK (2008): Reconstruction of 10 major mandibular defects using bioimplants containing BMP-7. J Can Dent Assoc; 74: 67–72
12. Behnia H, Khojasteh A, Soleimani M, et al. (2012): Repair of alveolar cleft defect with mesenchymal stem cells and platelet derived growth factors: A preliminary report. J Craniomaxillofac Surg; 40: 2–7.
13. Terheyden H, Menzel C, Wang H, et al. (2004): Prefabrication of vascularized bone grafts using recombinant human osteogenic protein-1-part 3: Dosage of rhOP-1, the use of external and internal scaffolds. Int J Oral Maxillofac Surg; 33: 164–172.
14. Clokie CM, Sa´ndor GK (2008): Reconstruction of 10 major mandibular defects using bioimplants containing BMP-7. J Can Dent Assoc; 74: 67–72.
15. Sàndor GK, Numminen JURA, Wolff J, Thesleff T, Miettinen A, et al. (2014): Adipose Stem Cells Used to Reconstruct 13 Cases With Cranio-Maxillofacial Hard-Tissue Defects. Stem Cells Translational Medicine; 3:1–11.
16. Knutsson J, Bagger-Sjöbäck D, von Unge M (2007): Distribution of different collagen types in the rat's tympanic membrane and its suspending structures. Otology & Neurotology; 28: 486–491.
17. Warnke PH, Springer IN, Wiltfang J, Acil Y, Eufinger H, Wehmöller M, Russo PA, Bolte H, Sherry E, Behrens E, Terheyden H (2004): Growth and transplantation of a custom vascularised bone graft in a man. Lancet; 364(9436): 766–770.
18. *J*afarian M, Eslaminejad MB, Khojasteh A, Mashhadi Abbas F, Dehghan MM, Hassanizadeh R, et al. (2008): Marrow-derived mesenchymal stem cells-directed bone regeneration in the dog mandible: a comparison between biphasic calcium phosphate and natural bone mineral. Oral Surg Oral Med Oral Pathol Oral Radiol Endod; 105(5): e14e24.
19. Khojasteh A, Eslaminejad MB, Nazarian H (2008): Mesenchymal stem cells enhance bone regeneration in rat calvarial critical size defects more than platelet-rich plasma. Oral Surg Oral Med Oral Pathol Oral Radiol Endod; 106(3): 356e362.
20. Pradel W, Tausche E, Gollogly J, Lauer G (2008): Spontaneous tooth eruption after alveolar cleft osteoplasty using tissue-engineered bone: a case report. Oral Surg Oral Med Oral Pathol Oral Radiol Endod; 105: 440e444.
21. Behnia H, Khojasteh A, Soleimani M, Tehranchi A, Khoshzaban A, Atashi A (2009): Secondary repair of alveolar clefts using human mesenchymal stem cells. Oral Surg Oral Med Oral Pathol Oral Radiol Endod; 108(2): 1e6.
22. *U*rist MR, Nilsson O, Rasmussen J (1978): Bone regeneration under the influence of the bone morphogenic protein (BMP) beta tricalcium phosphate (TCP) composite in skull trephine defect sin in dogs. Clin Orthop Relat Res; 21(14): 295e304.
23. Klongnoi B, Rupprecht S, Kessler P, Thorwarth M, Wiltfang J, Schlegel KA (2006a): Influence of platelet-rich plasma on bioglass and autogenous bone in sinus augmentation. An explorative study. Clin Oral Implants Res; 17: 312e320.
24. Klongnoi B, Rupprecht S, Kessler P, Zimmermann R, Thorwarth M, Pongsiri S, et al. (2006b): Lack of beneficial effects of platelet-rich plasma on sinus augmentation using a fluorohydroxyapatite or autogenous bone: an explorative study. J Clin Periodontol; 33: 500e509.
25. Lee C, Nishihara K, Okawachi T, Iwashita Y, Majima HJ, Nakamura N (2009): A quantitative radiological assessment of outcomes of autogenous bone graft combined with platelet-rich plasma in the alveolar cleft. Int J Oral Maxillofac Surg; 38: 117e125.
26. Lynch SE, Marx RE, Nevins M, Wisner-Lynch LA (2008): Tissue engineering; vol. 16; 2008, 234.
27. Herford AS, Boyne PJ, Rawson R, Williams RP (2007): Bone morphogenetic protein-induced repair of the premaxillary cleft. J Oral Maxillofac Surg; 65: 2136e2141.
28. *T*ai CC, Sutherland IS, McFadden L (2000):

Prospective analysis of secondary alveolar bone grafting using computed tomography. J Oral Maxillofac Surg; 58: 1241.
29. van der Meij AJW, Baart JA, Prahl-Andersen B (2003): Outcome of bone grafting in relation to cleft width in unilateral cleft lip and palate patients. Oral Surg Oral Med Oral Pathol Oral Radiol Endod; 96: 19.
30. Matsui Y, Ohta M, Ohno K, Nagumo M (2006): Alveolar bone graft for patients with cleft lip/palate using bone particles and titanium mesh: a quantitative study. J Oral Maxillofac Surg; 64: 1540e1545.
31. Knutsson J, von Unge M, Rask-Andersen H (2010): Localization of progenitor/stem cells in the human tympanic membrane. Audiology and Neurotology; 16: 263–269.
32. Kim J, et al. (2011): A healing method of tympanic membrane perforations using three-dimensional porous chitosan scaffolds. Tissue Engineering Part A; 17: 2763–2772.
33. Kim JH, et al. (2009): Development of water‐insoluble chitosan patch scaffold to repair traumatic tympanic membrane perforations. Journal of Biomedical Materials Research Part A; 90: 446–455.
34. Kim JH, et al. (2009): Tympanic membrane regeneration using a water-soluble chitosan patch. Tissue Engineering Part A; 16: 225–232.
35. Zhao J, Hu J, Wang SY, Sun X, Xia L, Zhang X, Zhang Z, Jiang X (2010): Combination of β-TCP and BMP-2 gene-modified bMSCs to heal critical size mandibular defects in rats. Oral Dis; 16(1): 46–54.
36. Vertenten G, Gasthuys F, Cornelissen M, Schacht E, Vlaminck L (2010): Enhancing bone healing and regeneration: present and future perspectives in veterinary orthopaedics. Vet Comp Orthop Traumatol; 23(3): 153–162.

4/10/2016