Are Mesenchymal Stem Cells Implanted in Lip Defect Capable of Returning Orbicularis Oris Muscle Function?

Baratali Ramazanzadeh, MD,* Arezoo Jahanbin, MD,* Neda Eslami, MD,† Mojgan Kazemian, MD,‡ Nasser Mahdavishahri, MD,§ and Samaneh Habibi, MD||

Introduction: This study aimed to evaluate the amount of orbicularis oris muscle formation and function after using scaffold-free cells and mesenchymal stem cells scaffold in repaired cleft lip of rats.

Methods: Forty male Wistar rats were used. A triangular cut ($7 \times 7 \times 4$ mm) was made on the upper lip of 30 rats and then divided into 3 equal groups. In the first group (control), the defected cuts were closed by routine surgical procedures. The second group consisted of cell scaffold and the third scaffold-free cells ($5 \times 5 \times 3$) in the area of the incision.Two months after restoration, muscle electromyography activity was examined alongside 10 normal rats and tissue samples were histologically examined. Data were analyzed statistically by SPSS software and ANOVA and the Kruskal–Wallis test. The significance level was considered at $P < 0.05$.

Results: Electromyography activity in second and third groups was not significantly different from each other and with normal muscle. The control group had, however, higher activity than normal muscle ($P < 0.001$). The amount of newly formed muscle tissue in the scaffold group had a clear statistical difference with the cell scaffold ($P = 0.018$) and control group ($P < 0.001$). Furthermore, the inflammatory reaction showed a significant statistical difference in the control and scaffold group ($P = 0.001$) and the scaffold and cell-scaffold groups ($P = 0.007$). There was no significant difference in the number of formed fibroblast cells and collagen tissue in the area between the 3 groups.

Conclusions: The results of this study show that extracellular matrix scaffold can reduce tension in the repaired cleft lip area by increasing muscle formation and decreasing scar tissue contraction, confirming the benefits of this type of scaffold.

Key Words: Cleft lip, electromyography, extracellular decellularized matrix, orbicularis oris muscle, stem cells

From the *Department of Orthodontics, Dental Research Center, Faculty of Dentistry, Mashhad University of Medical Sciences, Mashhad, Iran; †Dental Research Center, Department of Orthodontics, Dental Research Center, Faculty of Dentistry, Mashhad University of Medical Sciences, Mashhad, Iran; ‡Department of Oral and Maxillofacial Surgery, Dental Research Center, Faculty of Dentistry, Mashhad University of Medical Sciences, Mashhad, Iran; §Department of Biology, Ferdowsi University of Mashhad, Mashhad, Iran; and ||Department of Orthodontics, Birjand University of Medical Sciences, Birjand, Iran.

Received January 7, 2015.
Accepted for publication February 24, 2015.

Address correspondence and reprint requests to Arezoo Jahanbin, Department of Orthodontics, School of Dentistry, Park Square, Mashhad, Iran, 91735. E-mail: Jahanbina@mums.ac.ir; Dr_arezoo_jahanbin@yahoo.com

The authors report no conflicts of interest.

Copyright © 2015 by Mutaz B. Habal, MD

ISSN: 1049-2275
DOI: 10.1097/SCS.0000000000001837

(*J Craniofac Surg* 2015;00: 00–00)

Cleft lip ± palate is one of the most common congenital malformations and its prevalence varies from 1/500 to 1/2000 live births worldwide.[1] Chromosomal abnormalities, genetic factors, stress, folic acid deficiency, and some drugs have been proposed as etiologic factors.[2–11] Routine methods of surgical closure of cleft lip usually accompany common side effects including decreased forward growth of maxilla and lingual tipping of incisors. Contraction of scar tissue is suggested as a contributory factor for these side effects.[12] Strategies for closure of the bone defect include autologous bone grafts and soft tissue repair with local flaps.

Tissue engineering has recently introduced new strategies for reconstruction of cleft lip and palate defects. The principle of tissue engineering is based on selection and preparation of cells, designing scaffold matrix, and applying biologic signaling molecules. Behnia et al investigated secondary repair of alveolar cleft in 2 patients. The patients were treated with the composite scaffold of demineralized bone mineral and calcium sulphate loaded with mesenchymal stem cells. Computed tomograms showed only 34.5% formation of regenerated bone after 4 months in 1 case and 25.6% in the other. This implied that conventional bone substitute is not a suitable scaffold for mesenchymal stem cells induced bone regeneration.[13]

Bueno et al utilized stem cells derived from orbicularis oris muscle to enhance bone regeneration at the alveolar cleft area. These multipotent cells were able to differentiate into chondrogenic, adipogenic, osteogenic, and skeletal muscle cells, and showed the same phenotype and behavioral characteristics as other stem cells. They suggested that these cells are a potential source of stem cells for reconstruction of alveolar clefts, especially in young patients with cleft lip and palate.[14]

Although there is some literature on the reconstruction of bone defects in cleft palate patients using stem cells, there are limited data available on the reconstruction of orbicularis oris muscle utilizing stem cells. The aim of the present study was to evaluate the orbicularis oris muscle function and structure after implantation of mesenchymal stem cells to repair the surgically induced lip defect in rats.

METHODS

Forty male Wistar rats, weighing 320 to 420 g, were studied. All animals were kept individually in 22°C temperature, and in a light and humidity-controlled environment.

Preparation of Scaffold

In this study, extracellular matrix derived from the rats' orbicularis oris muscle was used for preparation of scaffold. Initially, 5 adult Wistar rats were sacrificed and muscles of their upper lip were dissected.

To make the samples free of cells physical and chemical methods including incubation of samples in 1% sodium dodecyl sulfate for 24 hours were applied. Then, they were placed in phosphate buffer saline for 2 hours, before being immersed in 75% ethanol. This procedure was repeated 8 times. After complete washing, the samples

Copyright © 2015 Mutaz B. Habal, MD. Unauthorized reproduction of this article is prohibited.

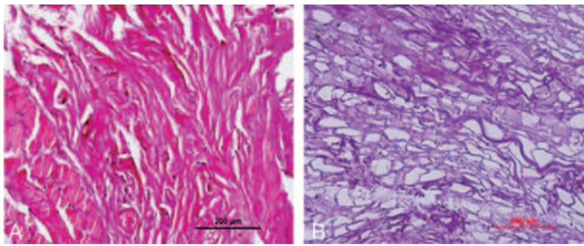

FIGURE 1. H&E staining of the orbicularis oris muscle of the rat. A, Before cell removal, B, after cell removal. H&E = hematoxylin–eosin.

were placed in 70% ethanol for 30 minutes to sterilize the acellular scaffold. After washing with sterile water, they were immersed in phosphate buffer saline for 1 hour (53). The scaffolds were then placed in Dulbecco's Modified Eagle's Medium (DMEM) and incubated at 37°C and 5% CO_2. (Fig. 1)

Mesenchymal Stem-Cell Culture with Rat Muscle Scaffolds

Stem cells were provided by the Cell Bank of the Biotechnology Research Institute of Ferdowsi University of Medical Sciences, Mashhad, Iran. These undifferentiated mesenchymal stem cells were derived from human adipose tissue and were labeled with green fluorescent protein. To cultivate the cells on the prepared scaffolds, they were smeared with trypsin enzyme to be separated from the flask. Then, the cells were counted, centrifuged, and extracted from the culture medium. Finally, adding DMEM to cellular sediment, cellular suspension of 5×10^5 cells in 50 μL was obtained.

Two types of scaffolds (cellular and acellular) were prepared (n = 12 each). After removal of scaffolds from the culture media, 5×10^5 cells were cultured on each scaffold in the cellular group. In the acellular group, only 50 μL DMEM was added to culture media.

After 1 hour, 1 mL of DMEM was added and the scaffolds were then incubated at 37°C and 5% Co2. Culture medium was refreshed every 2 to 3 days.

Histologic Evaluation of Cellular Scaffolds Before Graft

Four prepared scaffolds were histologically studied at 7, 14, 21, and 28 days to evaluate the interaction of mesenchymal stem cells with the scaffolds. Paraformaldehyde was used for 24 hours to stabilize the samples. Then, they were dehydrated using 70% ethanol and paraffin blocks were prepared. Sections of 5 to 7 μm thickness were prepared and studied under a microscope. Samples were stained either with hematoxylin–eosin (H&E) or DAPI (4–6 Diamidino-2-Phenylindole).

Figure 2 shows the behavior of mesenchymal stem cells in prepared scaffolds at day 7 using a fluorescent microscope and DAPI staining.

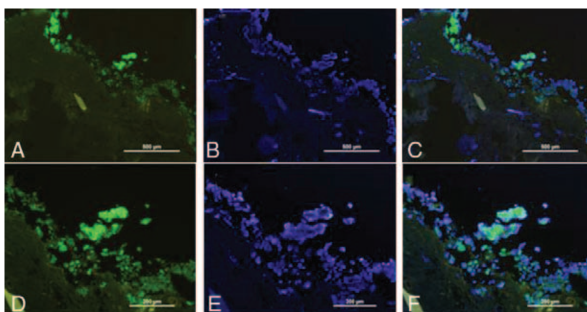

FIGURE 2. Behavior of mesenchymal stem cells in the prepared scaffolds at day 7. A, D, Green cells in the scaffold observed with a fluorescent microscope. B, E, After DAPI staining. F, C, Combination of blue and green colors.

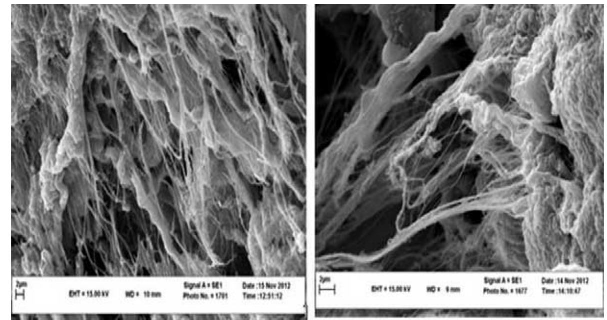

FIGURE 3. Scaffold of the orbicularis oris muscle of the rat after cell removal. Note that the overal structure of muscle tissue is maintained.

SEM Evaluation of Acellular and Cellular Scaffolds

Four samples were studied under a scanning electron microscope (SEM) to evaluate the penetration of stem cells into the scaffolds. Figure 3 shows the maintenance of muscle tissue structure after cell removal. Also, Figure 4 shows the penetration of mesenchymal stem cells into the surface of the scaffold after 7 days.

Surgical Procedure

Animals were anesthetized with an intraperitoneal injection of 5% ketamine hydrochlorides (80 mg/kg) and 2% Xylazine (5 mg/kg). In 30 rats, a triangular incision (7 × 7 × 4 mm) was removed in the left side of the upper lip. Surgical scissors and a blade were used to create the upper lip defect. Then, the rats were divided into 3 groups: In group I, the lip defect was sutured according to routine surgical methods. In groups II and III, an acellular scaffold and a scaffold loaded by cells (5 × 5 × 3 mm) were placed in the area of the lip defect, respectively. The scaffolds were fixed with two 0.5 chromic resorbable sutures to the muscular layer, and then mucosal and dermal layers were sutured. Intramuscular injection of penicillin G (Jaber Hayan, Tehran, Iran) was prescribed postoperatively to prevent infection.

Electromyographic Evaluation

Electromyographic signals of the orbicularis oris muscle at the defect area were evaluated using Powerlab 4.30 (AD Instruments, Bella Vista, Australia). Ten normal rats served as the control group. All 3 experimental groups and control rats were anesthetized similar to the previous stage. Electromyographic recording (EMG) was performed by placing 2 recording electrodes on the orbicularis oris muscle with a 2 mm distance from each other. Also, 2 stimulating electrodes were placed on the area of the buccal nerve, a ground electrode was attached to the leg (Fig. 5) and a 0.5 V voltage was used for nerve stimulation, which lasted for 20 ms per stimulation. The peak EMG activity was measured and the mean value based on the 5 stimulatory pulses was calculated.

FIGURE 4. Surgical procedure to create the defect at the left side of the upper lip of the rats.

© 2015 Mutaz B. Habal, MD

Copyright © 2015 Mutaz B. Habal, MD. Unauthorized reproduction of this article is prohibited.

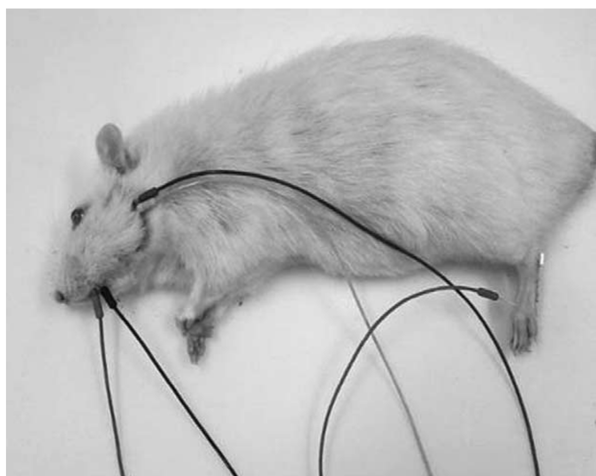

FIGURE 5. Two stimulating electrodes (blue and yellow) at the region of the buccal nerve, 2 recording electrodes placed in the orbicularis oris muscle, and a ground electrode attached to the leg.

Postoperative Histological Evaluation

The rats were sacrificed 2 months after the operation. After which 5 to 7 mm sections of the repaired area were removed. Tissue samples were fixed in paraformaldehyde and Bouin's fluid for 24 hours, dehydrated, and paraffin embedded. They were stained with H&E, and picrofuchsin for observation of connective tissue.

For quantitative histologic evaluation (histomorphometric analysis), microscopic pictures with 40× magnification were taken, and areas where muscular fibrils could be observed were measured. For qualitative analysis, density of inflammatory cells was considered an indicator of inflammation. Also, formation of new capillaries, proliferation of fibroblasts, and fibrous tissue formation (collagen accumulation) were measured as the criteria of tissue repair (56).

Each criteria was assigned a number of 0 (normal), 1 (mild), 2 (moderate), or 3 (severe). Moreover, density of inflammatory cells, fibroblasts, and new capillaries were classified into 3 consecutive groups: mild,[3–10] moderate,[11–30] and severe (≥31).

Statistical analysis was performed using SPSS (Version 16.0, Chicago, IL). One-way ANOVA and Tukey tests were used for comparison of groups. To compare the quantitative data between groups, the Kruskal–Wallis and Mann–Whitney U test were used. $P < 0.05$ was set as significant.

RESULTS

Mean EMG activity of the orbicularis oris muscle of the normal group is shown in Table 1. Also, mean EMG recordings at the repaired area of the cleft lip in the control, scaffold, and cellular scaffold groups are shown in Table 1.

ANOVA analysis showed a significant difference between the 4 groups ($P < 0.001$). The Tukey test showed a significant difference between the control and normal group ($P < 0.001$). Moreover, there was a significant difference between the control and scaffold ($P = 0.04$) and cellular scaffold ($P = 0.022$) groups.

Table 2 shows the distribution of samples in regard to intensity of inflammatory cellular response, formation of new capillaries, number of fibroblastic cells, and formation of collagen fibers in the control, scaffold, and cellular scaffold groups. According to this table, there was a significant difference between the 3 groups regarding intensity of inflammation and new capillary formation. Mann–Whitney analysis revealed a significant difference between the control and scaffold groups ($P = 0.001$) and scaffold and cellular scaffold groups ($P = 0.007$).

Similarly, a significant difference in new capillary formation was observed between the control and scaffold ($P = 0.007$) and scaffold and cellular scaffold groups ($P = 0.04$). No significant difference in other variables was, however, found between the other groups.

Microscopic view of the repaired cleft area stained with picrofuchsin in the 3 groups is shown in Figure 3.

DISCUSSION

Cleft lip ± palate is a very common congenital craniofacial anomaly.[15] Previous studies have shown that routine surgical procedures of lip repair usually result in deficient maxillary growth.[16–19] Contraction of postoperative scar tissue at the cleft area has been proposed as a contributory factor for maxillary deficiency.[16,20,21] Introducing tissue engineering methods and recent progress in deriving multipotent stem cells have suggested alternative regenerative techniques to repair the cleft area.[22,23]

The present study aimed to regenerate orbicularis oris muscle at the cleft lip using stem cells and scaffolds. Electromyographic activity of the repaired area was also recorded to confirm the histological findings.

In this study, natural heterogeneous extracellular matrix derived from the rat's orbicularis oris muscle was used as scaffold. Recent studies have confirmed the nonantigenic potential of extracellular matrix. Therefore, it can be transplanted from an animal to another without being rejected. Also, undifferentiated human mesenchymal stem cells derived from adipose tissue were used in this study. Considering the increased frequency of liposuction surgeries, ease of access to these types of cells justifies their use. Ability of allogeneic transplantation of adipose-derived stem cells allows the surgeons to use them in emergency cases.[24]

Stem cells derived from human orbicularis oris muscle in conjunction with collagenase membrane have been shown to regenerate cranial defects in rats.[14] Costa et al showed new bone formation at the site of cranial defect using stem cells derived from the pulp of primary teeth.[25]

We found statistically higher electromyographic activity of the upper lip in rats from the control group compared with normal, scaffold, and cellular scaffold groups. This implies the contraction of scar tissue in rats with routine surgical lip closure and thus increased electromyographic activity records, which is in line with similar studies.[26,27] On the other hand, there was not a significant difference in the muscular activity of scaffold and cellular scaffold groups in comparison to the normal rats. This can be the result of decreased contraction of scar tissue due to the placement of the scaffold. Also, histologic findings showed less formation of muscle

TABLE 1. Mean and SD of EMG Activity of Orbicularis Oris Muscle in the Normal Rats and the 3 Experimental Groups

Group	Normal Mean ± SD	Control Mean ± SD	Scaffold Mean ± SD	Acellular Scaffold Mean ± SD
EMG activity (mv)	0.806 ± 0.18	1.94 ± 0.87	1.06 ± 0.38	1.21 ± 0.46
ANOVA		$F = 8.215$ $P < 0.001$		

EMG = electromyography; SD = standard deviation.

Copyright © 2015 Mutaz B. Habal, MD. Unauthorized reproduction of this article is prohibited.

TABLE 2. Distribution of Samples in Regard to the Studied Variables in 3 Experimental Groups

		Intensity of Inflammatory Response N (%)	New Capillaries Formation N (%)	Number of Fibroblasts N (%)	Formation of Collagen Fibers N (%)
Control	Normal	0 (0)	0 (0)	0 (0)	0 (0)
	Mild	0 (0)	3 (42.9)	1 (14.2)	2 (28.6)
	Moderate	3 (42.9)	4 (57.1)	3 (42.9)	3 (42.8)
	Severe	4 (57.1)	0 (0)	3 (42.9)	2 (28.6)
	Total	7 (100.0)	7 (100.0)	7 (100.0)	7 (100.0)
Scaffold	Normal	3 (42.9)	4 (57.1)	0 (0)	0 (0)
	Mild	3 (42.9)	3 (42.9)	4 (57.1)	3 (42.9)
	Moderate	1 (14.2)	0 (0)	3 (42.9)	4 (57.1)
	Severe	0 (0)	0 (0)	0 (0)	0 (0)
	Total	7 (100.0)	7 (100.0)	7 (100.0)	7 (100.0)
Cellular scaffold	Normal	0 (0)	1 (14.2)	0 (0)	0 (0)
	Mild	1 (14.2)	3 (42.9)	3 (42.9)	3 (42.9)
	Moderate	3 (42.9)	3 (42.9)	3 (42.9)	3 (42.9)
	Severe	3 (42.9)	0 (0)	1 (14.2)	1 (14.2)
	Total	7 (100)0)	7 (100.0)	7 (100.0)	7 (100.0)
Kruskal–Wallis		$P = 0.003$	$P = 0.016$	$P = 0.10$	$P = 0.56$

tissue at the repaired cleft area in the control group, whereas the scaffold group showed the most frequent number of muscle fibers. These findings confirm the advantages of implantation of scaffolds (cellular or acellular) in the cleft area. Similarly, Chaturvedi et al[28] observed regeneration of muscle tissue after placement of bioactive scaffolds.

It seems that in the present study, the scaffolds played a similar role as extracellular matrix and stimulated the migration, proliferation, and differentiation of peripheral cells to regenerate new functional tissue.

In a similar study, Buneo et al[14] used orbicularis oris muscle as a new source of stem cells with potential for alveolar bone reconstruction in cleft lip/palate patients. Thus, it is probable that muscle formation in the acellular scaffold in this study is induced by stem cells of the peripheral tissues. On the contrary, some studies have reported the potential of activation, proliferation, and differentiation of satellite cells after minor damage to muscle tissue throughout life. In case of severe damage and formation of fibrous tissue, these cells are, however, not able to repair the muscle tissue. Therefore, they can be known as stem cells in the muscles.[30] It is probable that these cells have played a role in the induction of muscle fibers before fibrous tissue formation in the acellular scaffold group in the present study.

In this study, we found less number of inflammatory cells in the acellular group compared with the other 2 groups (Table 2). Ophof et al[31] observed less formation of scar tissue and inflammatory reactions in skin-derived substrates implanted in palatal wounds compared with the control group, which is in agreement with our findings.

In the present study, more severe inflammatory reaction was found in the control group, which could be the result of wound strain at the repaired area.[32]

On the contrary, more severe inflammation was observed in the cellular scaffold group when compared with acellular scaffolds. Reaction of the host immunity system to the stem cells can be a contributory factor for this finding. It should be remembered that labeled stem cells could not be seen at the repaired cleft area after 2 months. This could be the result of either an immunity defence response and thus rejection of the labeled stem cells or the accumulation of inflammatory cells at the cleft area.

Studies on transplantation of myoblasts have shown different results. Rapid rejections of transplanted myoblasts have been observed in rats, which can be the result of activation of the cellular and humeral immunity system.[33] Most of the successful myoblasts transplantations have been reported in immunodeficient rats.[34]

In the present study, antigenic difference between human stem cells and host tissue might be an etiologic factor for rejection of stem cells and severe inflammatory response.

Better results were found in terms of muscle tissue formation and functional activity of the muscle in the cellular scaffold group compared with control rats. Although labeled stem cells were not observed in the cellular scaffold group in the peripheral muscle, migration of other stem cells in the peripheral muscle might induce muscle regeneration of the cleft area.

It seems that more a severe inflammatory reaction in the cellular scaffold group is the main reason for decreased formation of muscular fibers in the cellular scaffold group in comparison with the scaffold group (Table 2).

In this present study, significantly less number of capillaries was found at the repaired cleft area in the scaffold group in comparison with the other 2 groups (Table 2). It should be remembered that formation of new capillaries increases during the fibroblastic phase of wound repair, whereas it usually decreases during the reconstructive phase. Therefore, rapid maturation of the wound healing phases might be the reason for less observation of new capillaries in the scaffold group, which could be due to the mild inflammation observed in this group compared with the other groups.

ACKNOWLEDGMENT

This study was supported financially by Vice Chancellor for Research of Mashhad University of Medical Sciences. This study was derived from student thesis.

REFERENCES

1. Eslami N, Jahanbin A, Ezzati A. Palm and finger print characteristics in nonfamilial cleft lip and palate patients and their parents. *J Craniofac Surg* 2013;24:769–772
2. Vieira AR, Karras JC, Orioli IM, et al. Genetic origins in a South American clefting population. *Clin Genet* 2002;62:458–463
3. Carinci F, Scapoli L, Palmieri A, et al. Human genetic factors in nonsyndromic cleft lip and palate: an update. *Int J Pediatr Otorhinolaryngol* 2007;71:1509–1519
4. Stoll C, Mengsteab S, Stoll D, et al. Analysis of polymorphic TGFB1 codons 10, 25, and 263 in a German patient group with non-syndromic cleft lip, alveolus, and palate compared with healthy adults. *BMC Med Genet* 2004;5:15

© 2015 Mutaz B. Habal, MD

Copyright © 2015 Mutaz B. Habal, MD. Unauthorized reproduction of this article is prohibited.

5. Kohli SS, Kohli VS. A comprehensive review of the genetic basis of cleft lip and palate. *J Oral Maxillofac Pathol* 2012;16:64–72
6. Deroo LA, Wilcox AJ. First-trimester maternal alcohol consumption and the risk of infant oral clefts in Norway: a population-based case-control study. *Am I Epidemiol* 2008;168:638–646
7. Wallace GH, Arellano JM, Gruner TM. Non-syndromic cleft lip and palate: could stress be a causal factor? *Women Birth* 2011;24:40–46
8. Tolarova M, Harris J. Reduced recurrence of orofacial clefts after periconceptional supplementation with high-dose folic acid and multivitamins. *Teratology* 1995;51:71–78
9. Johansen AM, Lie RT, Wilcox AJ. Maternal dietary intake of vitamin A and risk of orofacial clefts: a population-based case-control study in Norway. *Am J Epidemiol* 2008;167:1164–1170
10. Hozyasz K, Chełchowska M, Surowiec Z. Plasma vitamin A in mothers of children with orofacial clefts. *Ginekol Pol* 2004;75:139–144
11. Munger RG, Sauberlich HE, Corcoran C, et al. Maternal vitamin B-6 and folate status and risk of oral cleft birth defects in the Philippines. *Birth Defects Res A Clin Mol Teratol* 2004;70:464–471
12. Priya VK, Reddy JS, Ramakrishna Y, et al. Post-surgical dentofacial deformities and dental treatment needs in cleft-lip-palate children: a clinical study. *Plast Reconstr Surg* 2010;125:1573–1574
13. Behnia H, Khojasteh A, Soleimani M, et al. Secondary repair of alveolar clefts using human mesenchymal stem cells. *Oral Surg Oral Radiol Endod* 2009;108:1–6
14. Bueno DF, Kerkis I, Costa AM, et al. New source of muscle-derived stem cells with potential for alveolar bone reconstruction in cleft lip and/or palate patients. *Tissue Eng Part A* 2009;15:427–435
15. Supit L, Prasetyono T. Cleft lip and palate review: epidemiology, risk factors, quality of life, and importance of classifications. *Med J Indones* 2008;17:226–239
16. Padwa BL, Mulliken JB. Complications associated with cleft lip and palate repair. *Oral Maxillofacial Surg Clin N Am* 2003;15:285–296
17. Bardach J, Mooney MP. The relationship between lip pressure following lip repair and craniofacial growth: an experimental study in beagles. *Plast Reconstr Surg* 1984;73:544–555
18. Bardach] J, Kelly KM, Jakobsen JR. Simultaneous cleft lip and palate repair: an experimental study in beagles. *Plast Reconstr Surg* 1988;82:31–41
19. Filho LC, Normando ADC, da Silva Filho OG. Isolated influences of lip and palate surgery on facial growth: comparison of operated and unoperated male adults with UCLP. *Cleft Palate Craniofac J* 1996;33:51–56
20. Zhang L, Yang L, Li X. The influence of pharmacological manipulation on postsurgical labial scar contraction and craniofacial growth in rabbits [in Chinese]. *Zhonghua Zheng Xing Shao Shang Wai Ke Za Zhi* 1996;12:101–103
21. Hosokawa R, Nonaka K, Morifuji M, et al. TGF-beta 3 decreases type I collagen and scarring after labioplasty. *J Dent Res* 2003;82:558–564
22. Gimbel M, Ashley RK, Sisodia M, et al. Repair of alveolar cleft defects: reduced morbidity with bone marrow stem cells in a resorbable matrix. *J Craniofac Surg* 2007;18:895–901
23. Stern-Straeter J, Riedel F, Bran G, et al. Advances in skeletal muscle tissue engineering. *In Vivo* 2007;21:435–444
24. Gimble J, Katz A, Bunnell B. Adipose-derived stem cells for regenerative medicine. *Circ Res* 2007;100:1249–1260
25. Costa A, Bueno DF, Martins MT, et al. Reconstruction of large cranial defects in nonimmunosuppressed experimental design with human dental pulp stem cells. *J Craniofac Surg* 2008;19:204–210
26. Li WR, Lin JX, Fu MK. Electromyography of perioral muscles in the cleft lip and palate patients with crossbite [in Chinese]. *Zhonghua Kou Qiang Yi Xue Za Zhi* 1994;29:342–345
27. Genaro KF, Trindade AS, Trindade IE. Electromyographic analysis of lip muscle function in operated cleft subjects. *Cleft Palate Craniofac J* 1994;31:56–60
28. Chaturvedi V, Dye D, Coombe D, et al. Bioactive Scaffolds in skeletal muscle regeneration and tissue engineering. *Aust Biochem* 2011;42: 8–10
29. Schuler F, Sorokin LM. Expression of laminin isoforms in mouse myogenic cells in vitro and in vivo. *J Cell Sci* 1995;108:3795–3805
30. Vachon P, Loechel F, Xu H, et al. Merosin and laminin in myogenesis; specific requirement for merosin in myotube stability and survival. *J Cell Biol* 1996;134:1483–1497
31. Ophof R, Maltha JC, Von den Hoff JW, et al. Histologic evaluation of skin-derived and collagen-based substrates implanted in palatal wounds. *Wound Repair Regen* 2004;12:528–538
32. Hupp J, Ellis E, Tucker M. *Oral and Maxillofacial Surgery*. 5th ed. St. Louis: Mosby Elsevier; 2008:47–69.
33. Labrecque C, Roy R, Tremblay JP. Immune reactions after myoblast transplantation in mouse muscles. *Transplant Proc* 1992;24:2889–2892
34. Huard J, Roy R, Guerette B, et al. Human myoblast transplantation in immunodeficient and immunosuppressed mice: evidence of rejection. *Muscle Nerve* 1994;17:224–234

© *2015 Mutaz B. Habal, MD*

Copyright © 2015 Mutaz B. Habal, MD. Unauthorized reproduction of this article is prohibited.