Radiographic comparison of the repaired bone in maxillary alveolar cleft of dog by tissue engineering and autogenous bone grafting techniques

Sheikhi, M.[1], Karbasi Kheir, M.[2*], Dakhilalian, A.[3]

[1] *Torabinejad Research Center, Faculty of Dentistry, Isfahan University of Medical sciences, Isfahan, Iran*

[2] *Department of Oral and Maxillofacial Radiology, Faculty of Dentistry, Islamic Azad University of Isfahan (khorasgan branch), Isfahan, Iran*

[3] *Faculty of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*

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Correspondence
Karbasi Kheir, M.
Department of Oral and Maxillofacial Radiology, Faculty of Dentistry, Islamic Azad University of Isfahan (khorasgan branch), Isfahan, Iran
Tel: +98(31) 35354001
Fax: +98(31) 35354060
Email: mastoor28@yahoo.com

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Abstract:

BACKGROUND: Dental disease, trauma and maxillofacial surgeries can cause alveolar bone defects. Among different kinds of treatment, autogenous bone graft is accepted as a golden standard. On the other hand, because of limitations of treatment with autogenous bone grafts, osteogenic cells derived from stem cells are suggested. **OBJECTIVES:** The aim of this study was to compare the mean density of the repaired bone in maxillary alveolar cleft of dog by tissue engineering and autogenous bone grafting techniques using digital radiography. **METHODS:** Two, 15*15mm, defects were made in the maxillary alveoli of 4 dogs. A stent was placed in the defect to prevent the effects of soft tissue. After 60 days, the defects were filled with tibial auto graft in one side and mesenchymal stem cells were isolated from the neck adipose tissue in the other side. The density of the regenerated bone was evaluated 15, 30, 45, 60, 75 and 90 days after graft implantation by direct digital radiography, Digora windows software (70 KVP, 10 mA and 0.40 seconds). **RESULTS:** The findings of one-way ANOVA test showed no significant difference between tissue engineering and autogenous bone grafting methods (p-value = 0.94). Visually, the repaired bone pattern was homogenized in autogenous bone graft method but it was cotton-wool in tissue engineering bone graft method. **CONCLUSIONS:** Digital radiography software provides the possibility of quantification of bone repair by densitometry. When autogenous bone graft is not available, tissue engineering bone graft can be considered an acceptable alternative to repair the bone defects.

Introduction

Dental disease, trauma and maxillofacial surgeries can cause alveolar bone defects. Complete repair of alveolar bone with or without grafts is an ideal goal in regenerative treatments (Lynch and Marx, 2008). Among different kinds of treatment, autogenous bone grafts are accepted as a golden standard because of their high osteogenic potential. The grafts can be obtained from various extraoral donor sites like iliac crest, calvarium, tibia and rib, or intra-oral donor sites like mandibular symphysis, retromolar area and maxillary tuberosity. On the other hand, because of limitation of treatment with autogenous bone grafts, osteogenic cells derived from stem cells are suggested. Stem cells taken from adipose tissue (lipid cells) have osteogenic capability (Albrektsson, 1980; Lauric and Kaban, 1984; Dolanmaz et al., 2015; Ochs, 1996; Gładysz and Hozyasz, 2015; Janssen et al., 2014). Histological examination is the gold standard for bone repair, which can show the type and exact amount of bone. But in clinical cases, other alternative nonaggressive techniques are required. Radiography is suggested as a non-aggressive method which can show bone formation over time (Genant and Jiang, 2006; Chiristagau et al., 1998). The density of radiographic film is a criterion for the amount of calcification. In digital radiography, density is shown by the average pixel number. Conventional radiographs have high special and contrast resolution, but some artifacts may be created during film handling and processing and there is no possibility of image enhancement after film processing. Despite lower special and contrast resolution in digital radiography, additional techniques like image enhancement, image storage, densitometry, etc. are available in digital radiography (White and Pharoah, 2014). This study was conducted to compare the mean density of the repaired alveolar bone in maxillary alveolar cleft of dogs by tissue engineering and autogenous bone grafting methods using digital radiography.

Materials and Methods

In this study, four 12-24 month-old mongrel dogs were selected as the study sample. The dogs were systematically healthy and did not have any lesions in their mouth. They were anesthetized with ketamine (20mg/kg) and Rompun (2mg/kg), followed by intubation and administration of Halotane and N2O. Both their maxillary incisors were extracted, but their molars and premolars were kept to be able to eat (Figure 1). In each dog after teeth extraction, a defect with 15 mm width was made from alveolar crest to nasal floor, and nasal mucosa was sutured to the oral mucosa. Endotracheal tube number 8 was placed on both sides of the defect and fixed with wire to canine teeth. The tube was filled with self-cure acryl and used as a tolerable stent for the dogs. It remained there for two months until the oral-nasal fistula was formed. Subcutaneous adipose tissue (20 gram) was taken from each dog's neck. Mesenchymal stem cells were isolated from this adipose tissue. Undifferentiated cells were incubated with a 3mm×3mm×3mm hydroxyapatite/beta-tricalcium phosphate scaffold in a specific osteogenic medium for 21 days in the cell culture lab of Torabinejad Research Center. After two months, the dogs were prepared for the next surgery. They were anesthetized the same way as the first surgery. In this stage, the defect of one side was filled with 10cc corticocancellous tibial auto graft and the defect on other side was filled with tissue engineered bone graft from adipose-derived stem cells. The density of the regenerated bone was evaluated 15, 30, 45, 60, 75 and 90 days after the second surgery by direct digital radiography (Figure 2). The radiographs were taken with parallel method using a size 2 (27.38mm) CCD sensor (Imaging Company USA Signus) and Trophy X- ray tube (106 rule de Ia, Paric, France). The exposure factors were

Table 1. Mean density of repaired bones by tissue engineering and autogenous bone grafting methods.

Variables / Days		0	15	30	45	60	75	90
Autogenous bone grafts	Mean	0	110.72	82.70	75.76	93.57	100.22	100.32
	SD	0	1.80	12.75	16.20	19.86	38.16	41.17
Tissue engineering	Mean	0	120.75	87.62	83.72	92.02	92.30	93.77
	SD	0	7.73	25.58	27.01	35.20	34.43	29.73
Total	Mean	0	115.73	85.16	79.74	92.80	96.26	97.05
	SD	0	7.46	18.89	21.05	26.47	33.91	33.43

70 KVP, 10 mA and 0.40 seconds. Dr. Suni software was used for interpretation of data on a LG LED computer viewer (E2042C, Korea). The density of regenerated bone was measured by the densitometer software, Digora for windows, density measurement part. The region of interest was selected by Mark area option, and the mean density was measured afterwards. The data were analyzed by the Statistical Package for Social Sciences (SPSS) (version 22, SPSS Inc., Chicago, IL).

Ethical considerations: In this animal study, the principles of sterile and painless surgery were respected and suitable antibiotic and analgesic drugs were prescribed. The living conditions of animals (nutrition, light, temperature, etc.) were controlled. This research was done under the "PROTECTION CODE OF ANIMAL SUBJECT IN MEDICAL RESEARCH", defined by Ethics Committee of Medical Sciences Researches of Isfahan University of Medical Sciences (Research project and ethical code number: 388489)

Results

In this study, seven time intervals were considered to observe the bone repair. The bone density in tissue engineering bone grafting decreased until day 45 because of resorption of scaffold and death of some graft cells under the influence of inappropriate blood supply. The decrease of bone density was less in autogenous bone grafts during the 45 days. After 45 days, the bone density increased. Further, 60 days after bone graft implantation, the bone densities of two methods became similar. The mean densities of the repaired bones of dogs measured by both methods are shown in Table 1. The findings of one-way ANOVA test showed no significant difference between tissue engineering and autogenous bone grafting methods (P-value = 0.94). Visually, the repaired bone was homogenized in autogenous bone graft method, but it was cotton-wool in tissue engineering bone grafting method.

Discussion

This study used dog as the animal model. It has been found that among different species (human, dog, sheep, pig, cow and chicken), there are similarities between dogs and humans in bone composition (ash weight, hydroxyproline, extractable proteins and IGF-1 content), bone density, water fraction, organic fraction, volatile inorganic fraction and ash fraction (Gimble et al., 2007; Pearce et al.,2007; Aerssens et al., 1997; Gong et al., 1964; Kamal et al., 2017).

Bone grafting is fully revascularized between 14 and 21 days, and new bone is formed in approximately six weeks and matures after six months (Marx, 2007). This study showed that the pattern of repaired bone by autogenous bone grafting was more homogenized. However, there was no statistically significant difference between the bone densities of tissue engineering and autogenous bone grafting methods. This means that tissue engineering bone grafts can be considered acceptable for repairing bone defects, especially when autogenous bone grafts are not available.

In this study, digital intra-oral radiography was used. Although there were some superimpositions of adjacent structures in intra-oral radiographs, the high resolution of these radiographs helped to evaluate the small changes of bone structure.

Mikołajczak et al assessed the mineralization of the grafted alveolar bone of patient using digital radiographs. They concluded that digital radiography with tomosynthesis option is very useful for assessment of bone graft mineralization. Furthermore, they concluded that the patient's age affected the relative density of bone grafts. Younger patients had better bone mineralization (Mikołajczak et al., 2007). This study did not assess the effect of age on the bone mineralization of grafts and the sample animals had a similar age range.

CBCT has been used to volumetrically evaluate bone graft in alveolar cleft defects (Zhou et al., 2015). Both intra-oral digital radiograph and CBCT can measure the bone graft density, and they can be used in the follow-up evaluations after surgery. The advantages of CBCT are the ability of calculating the volume of alveolar defect and determining the amount of bone graft needed to adequately fill the bone defects which are not evident in digital intraoral radiograph. But these advantages should be considered along with higher patient radiation doses in CBCT.

Iino et al compared the bone repair after grafting in CT and intra-oral radiography and claimed that CT performed a better assessment than intraoral radiographs. However, their method was different from that of the present study; they compared bone height while the current study assessed bone density (Iino et al., 2005).

Ihan-Hern and Miljavec evaluated the spontaneous bone healing of patients using bone densitometry method in digital radiographs. They concluded that the final bone density increased over time, being found to be higher in small defects than in large ones (Ihan-Hern and Miljavec, 2008). Although the present study was carried out on animals, the bone densities were higher than that of the above study because of using bone grafts.

Conclusion: According to the present study, the digital radiography software provides the possibility of quantification of bone repair by densitometry. When autogenous bone graft is not available, tissue engineering bone graft can be regarded an acceptable alternative to repair the bone defects.

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References

Albrektsson, T. (1980) Repair of bone grafts. A vital microscopic and histological investigation in the rabbit. Scand J Plast Reconstr Surg. 14: 1-12.

Aerssens, J., Boonen, S., Joly, J., Dequeker, J.

(1997)Variations in trabecular bone composition with anatomical site and age: potential implications for bone quality assessment. J Endocrinol. 155: 411-421.

Chiristagau, M., Hiller, KA., Schemalz, G., Kolbek, C., Wenzel, A. (1998) Quantitative digital subtraction radiography for the determination of small changes in bone thickness: an in vitro study. Oral surg oral med oral pathol oral radiol endod. 85: 462-472.

Dolanmaz, D., Esen, A., Yıldırım, G., İnan, Ö. (2015) The use of autogeneous mandibular bone block grafts for reconstruction of alveolar defects. Ann Maxillofac Surg. 5: 71-76.

Genant, HK., Jiang, Y. (2006) Advanced imaging assessment of bone quality. Ann N Y Acad sci. 1068: 410-428.

Gładysz, D., Hozyasz, KK. (2015) Stem cell regenerative therapy in alveolar cleft reconstruction. Arch Oral Biol. 60: 1517-32.

Gimble, M., Ashley, R., Sisodia, M., Gobbay, J. (2007) Repair of alveolar cleft defects: reduced morbidity with bone marrow stem cell in a resorbable matrix. J Craniofacial Surgery. 18: 895-901.

Gong, JK., Arnold, JS., Cohn, SH. (1964) Composition of trabecular and cortical bone. Anat Rec. 149: 325-332.

Ihan-Hern, N., Miljavec, M. (2008) Spontanous bone healing of the large bone defects in the mandible. Int J Oral maxillofac Surg. 37: 1111-1116.

Iino, M., Ishii, H., Matsushima, R., Fukuda, M., Hamada, Y., Kondoh, T., Seto, K. (2005) Comparison of intraoral radiography and computed tomography in evaluation of formation of bone after grafting for repair of residual alveolar defects in patients with cleft lip and palate. Scand J Plast Reconstr Surg Hand Surg. 39: 15-21.

Janssen, NG., Weijs, WLJ., Koole, R., Rosenberg, AJWP., Meijer, GJ. (2014) Tissue engineering strategies for alveolar cleft reconstruction: a systematic review of the literature. Clin Oral Investig. 18: 219-26.

Kamal, M., Andersson, L., Tolba, R., Bartella, A., Gremse, F., Hölzle, F., Kessler, P., Lethaus, B. (2017) A rabbit model for experimental alveolar cleft grafting. Journal of Translational Medicine. 15: 50.

Lauric, S., Kaban, B. (1984) Donor site morbidity after harvesting rib and iliac bone. Plast Reconstr Surg. 73: 33-80.

Lynch, SE., Marx, RE. (2008) Wisner-lynch LA. Tissue engineering: Application in maxillofacial surgery and periodontitis. (2nd ed.) Hanover Park: Quintessence book. Chicago, USA. p. 3-47.

Marx, RE. (2007) Bone and bone grafting healing. Oral Maxillofac Surg Clin North Am. 19: 455-466.

Mikołajczak, T., Wilk, G., Gawrych, E. (2007) Assessment of bone grafting mineralization in patients with alveolar clefts using digital radiography. Ann Acad Med Stetin. 53: 66-73.

Ochs MW. Alveolar cleft bone grafting (part I): Secondary bone grafting. (1996) J Oral Maxillofac Surg. 54: 83-88.

Pearce, AI., Richards, RG., Milz, S., Schneider, E., Pearce, SG. (2007) Animal models for implant biomaterial research in bone: A Review. Eur Cell Mater. 13: 1-10.

White, SC., Pharoh, MJ. (2014) Oral radiology: principle and interpretation. Sth ed. Philadelphia: Mosby, USA.

Zhou, WN., Xu, YB., Jiang, HB., Wan, L., Du, YF. (2015) Accurate evaluation of cone-beam computed tomography to volumetrically assess bone grafting in alveolar cleft patients. J Craniofac Surg. 26: e535-539.