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Original Article

Amniotic membrane as an accelator in mandibular bone defects repair

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ABSTRACT

Background: The fetal amniotic membrane is a biological graft with unique qualities which all lead to wound protection, reducing discomfort, and achieving adequate epithelialization.

Materials and Methods: In this animal study, the second and third premolars of the mandible of 4 dogs were extracted. After 4 weeks, 20 mm of mandibular premolar site area were resected on both sides. The created defects on both sides were filled with xenograft. On one side, an amniotic membrane was placed over the graft particles and the reflected flap was sutured. The amount of bone formation in the defects was measured after 4 weeks for two of the dogs and after 8 weeks for the other two, using a caliper. Three histopathological samples from both sides were taken. The collected data were subjected to statistical analysis (Wilcoxon signed-rank and paired sample *t*-test) using SPSS software at a significant $P = 0.05$.

Results: In the test group, the quantity of bone was 56.81, whereas in the control group bone quantity was 37.38 with statistically significant differences ($P = 0.025$). In the amniotic membrane group, the inflammation intensity after the graft procedure was moderate (50%) in comparison to the control group where the inflammation was severe (62.5%) ($P = 0.041$).

Conclusion: The amniotic membrane can induce positive osteoinduction effects and be helpful in repairmen of bone defects such as the natural periosteum.

Key Words: Amniotic membrane, graft, osteoinduction

INTRODUCTION

Bone defects in the jaw region can be categorized into two groups: alveolar defects, and those with larger dimensions that may cause the discontinuity of bone. The important points to reconstruct these defects with grafts are: preventing infections, the possibility of graft rejection, and maximum reduction of graft resorption.[1,2] However in most defects with discontinuity, the lack of periosteum may increase the risks of graft failure.[2,3] Therefore, researchers have been working on methods to increase the success rate of grafts and find a suitable substitute for the lost periosteum.

The human amnion membrane is developed from the growth and culture of the fetus' extra-embryonic

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tissues.[2] This membrane includes a layer of epithelium containing human amniotic epithelial cells in a uniform and orderly fashion with underlying thick basal lamina and a layer of nonvascular stroma.[4,5] The human amniotic membrane has some specific qualities such as preventing bacterial growth, preventing inflammatory reactions of the immune system, preventing ulcers, enhancing wound repair, and accelerating epithelialization.[4,6] Amnion is a tissue without vessels and nerves. The basal lamina of this membrane includes type 4 and 6 collagens, type 1 and 5 laminin, fibronectin, and 6FGF (fibroblast growth factor) which all have positive effects on bone growth.[6,7] Therefore, for this research human-derived amniotic membrane has been selected compared to an animal-derived one.

Fetal membranes, especially the amniotic membrane were used for the first time in 1910 by Davis[8] in skin lesions, and by using them, the repair process was accelerated and there was less scar tissue left behind. These features have caused the increased use of amniotic membranes in medicine.[2] Various studies have used the amniotic membrane as a biologic dressing or a biodegradable graft in surgeries, skin burns, surgeries in the peritoneum and hip region, eye defects (cornea and conjunctiva), spinal cord injuries, and Ear, nose and throat (ENT) injuries.[7,9-11] One of the advantages of using amniotic membrane is the acceleration of the repairing process that may eliminate the common complications than could happen from lack of necessary natural tissues.[12] Further studies on the amniotic membrane's epithelial cells have shown that these cells are multi-potential and can differentiate into all three types of mesodermal, ectodermal, and endodermal cells. The ability of these cells to differentiate into mesoderm line cells such as myocytes, cardiomyocytes, osteocytes and adipocytes, endoderm line cells such as hepatocyte and pancreatic cells and also ectoderm line cells such as neurons.[4-7,9]

In the bone repair process, the periosteum is necessary due to its osteogenic properties. The lack of periosteum causes the repairman of bone tissues to be compromised.[2,3] Therefore, in many defects which lead to discontinuity of bone and periosteum removal in the mandible, application of amniotic membrane was considered, based on previous studies and its multi-potential properties.[12] Hence, this study was carried out to analyze the osteoinduction, resorption prevention, and other effects of this membrane in repairing large mandibular bone defects (with discontinuity and lack of periosteum) in Iranian mixed dogs.

MATERIALS AND METHODS

In this animal study was approve in research and ethics committee of Isfahan (NO:393323), four Iranian mixed dogs with an average age of 1.5 years and an average weight of 20 kg were included into this study. They were kept under controlled conditions in the animal shelter at Torabinejad Research Center, School of Dentistry and all the procedures were under the supervision of a veterinarian with all animal protections rights being preserved.

The dogs were anesthetized using intramuscular ketamine (20 mg/kg) and were intubated. Their mouths were rinsed using betadine solution and the second and third premolar teeth on each side were extracted to provide adequate space for creating defects. After 4 weeks, the dogs were anesthetized with the previously mentioned procedure. Oral incisions were performed on each side to expose the body of the mandible. An en bloc defect with a width of 20 mm was created in each side using a saw, then margins of the defect were dyed grossly (with Hematoxylin and Eosin) for future identification of the margins.

The periosteum surrounding the defect was then removed on both sides and in all dogs (in each dog one side was randomly considered as the case and the other side as control). To maintain the size of the defect and stabilize both sides, an 8 holed stainless steel construction plate was used and screwed to the jaw by 6 cortical screws. In the repair process the created defects on both sides were filled with human bone xenograft containing 2–10 mm bone particles that had no antigenicity (Tissue regeneration corp. Kish free zone, Iran). Then on one side an amniotic membrane (Amni patch, tissue regeneration corp. Kish free zone, Iran) was used to cover the xenograft and the defect instead of the periosteum and it was sutured to the surrounding tissue. Then, the bone was fixed using a reconstruction plate to supply more rigidity and gently covered with soft tissue so that the membrane would not be displaced. The other side of the defect was filled using the xenograft and the bone was fixed using a steel reconstruction plate then the soft tissue (without periosteum) was sutured back in place.

After the surgical procedure, all the dogs were kept in separate cages in equal living conditions in terms of food, location, air conditioning, water. The veterinarian visited the dogs every day. To prevent infection after the surgery, the dogs were given 1 gr intramuscular ceftriaxone daily for 7 days and 1 mg/kg tramadol intramuscularly for pain control for 7 days. They also had soft food diet for 3 weeks after the surgery.

After 4 weeks half of the dogs and after 8 weeks the other half were put under general anesthesia and the soft tissue was incised and retracted to gain access to the defect area. Then the dimensions of the bone produced were macroscopically measured using a caliper (the stained margins were considered as a marker for measuring the width of the defect).

Then using a 9 mm trephine bur, a sample was taken from the margin of the defect which included both the intact and the newly developed tissue for histomorphometric analysis (analyzing the type and density of the bone). Each sample was first fixed in 10% formalin solution and sent to the laboratory. At the laboratory after demineralizing the sample in 5% acid phosphoric, the samples were sectioned and placed on slides and then stained with H and E dye.

After preparing the histologic samples, each slide was coded and the samples were given to pathologists blindly so that the pathologist did not know which the control or test groups were. In the histologic examination, the samples were examined using a light microscope (Olympus, CXIFS, and Tokyo, Japan) with ×100 and ×400 magnifications. A scaled lens was used and the presence of newly formed bone in each sample was examined and the average amount of bone in the area was calculated. The calculations were repeated using Adobe photoshop. 7 (San Jose, CA, USA) with the help of sectional images of the samples to confirm the results. Furthermore, the amount and type of bone formed in each sample (lamellar, woven) was calculated by the pathologist based on the available histologic criteria, and the lamellar to woven bone percentage was recorded. The inflammation severity in the samples was also examined and recorded based on this 4 scale histologic index:

1. None or very few inflammatory cells present
2. Mild reaction (<25 inflammatory cells)
3. Moderate reaction (25–125 inflammatory cells)
4. Severe reaction (more than 125 inflammatory cells).

Finally, after recording the data each time, the data were collected and analyzed using the SPSS Statistics for Windows, version 18. 0 (SPSS Inc., Chicago, Ill., USA) and Wilcoxon test and paired *t*-test with a significance level of $\alpha = 0.05$.

RESULTS

This experimental clinical trial was carried out on 4 dogs. The quantity of bone was equal to 56.81 in the test group and 37.38 in the control group. The average amount of lamellar bone formation in the test and control groups was 41.25 and 23.01, respectively. The average amount of woven bone formation for the test and control groups was 15.56 and 14.37, respectively. The lamellar to woven bone percentage in the test and control groups were 70.84 and 60.59 respectively [Table 1].

The paired *t*-test showed a statistically significant difference between the average quantity of bone formed in the group with amniotic membranes (test group) and the group without them (control group) ($P = 0.025$) [Table 1].

The intensity of local inflammation in the control group was higher [Figure 1], such that in the group in which the amniotic membrane was used the postoperative inflammation was mostly moderate (50%) [Figure 2], whereas in the group

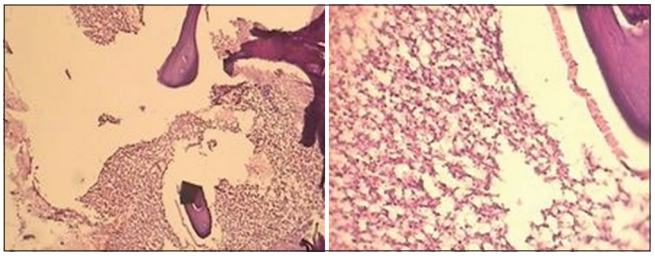

Figure 1: Histopathological view of control group in magnification of ×100 (right) and ×400 (left).

Table 1: Average quantity of lamellar, woven bone formation and proportion of lamellar to woven bone formation

Group	Application of amniotic membrane	No application of amniotic membrane	*P*
Lamellar bone percentage	41.25±17.45%	23.01±6.40%	0.055
Woven bone percentage	15.56±8.85%	14.37±2.71%	0.754
Bone quantity (lamellar and wowen)	56.81±14.45%	38.37±6.38%	0.025
Proportion of lamellar to woven bone (%)	70.84±15.66	60.59±9.020	0.227

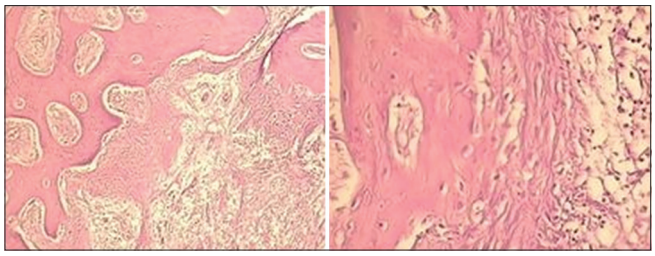

Figure 2: Histopathological view of test group in magnification of ×100 (right) and ×400 (left).

Table 2: Inflammation percentage in two groups

Group	Application of amniotic membrane	No application of amniotic membrane	*t*
Without inflammation	12.5%	0	6.3
Mild inflammation	37.5	12.5	25
Moderate inflammation	50	25	37.5
Severe inflammation	0	62.5	31.3
Total	100	100	100

without the membrane the inflammation was mostly severe (62.5%) [Table 2]. Furthermore, the inflammation in the dogs examined after 4 weeks was more intense than the group examined after 8 weeks. The Wilcoxon test was used to compare the severity of the inflammation between the test and control groups. The results of this test showed a statistically significant difference ($P = 0.041$) [Table 2].

During the clinical analysis, it was noted that the graft in the control side had a particulate state and no continuity could be seen between the particles of the graft whereas the test side had a consistent state and the particles had continuity.

DISCUSSION

The fetal amniotic membrane is a biological graft with unique qualities such as being nonadhesive, bacteriostatic, and more importantly lack of immunogenicity which all lead to wound protection, reducing discomfort, and achieving adequate epithelialization.[13]

The current study has analyzed the use of amniotic membranes as a substitute to periosteum in repairing bone defects.

The results of this study were analyzed in two aspects, a clinical (presence of any kind of local inflammation or obvious infection with pus discharge and lack of consistency in the graft) and a para clinical aspect (histomorphometry, bone quality and quantity, and the presence of inflammatory cells).

The results of the present study showed that better repair took place in the test group which used amniotic membranes compared to the control group which had no coverage on the bony defect. Furthermore, the intensity of inflammation was less in the test group compared to the control group.

The necessary resources to repair bone defects are supplied through several ways such as periosteum, bone defect margins, and osteogenic potential of grafts.

Various materials are used in maxillofacial surgeries to restore bone defects which include: Autogenous bone grafts, xenogeneic bone grafts, allogeneic bone grafts, and alloplasts.[14,15] From these grafts, only autogenous grafts are both osteoinductive and osteoconductive and also in terms of adaptability, function, and immunogenicity they are superior compared to the rest. Nevertheless, this kind of graft needs a donor site and it is limited in terms of size.[15]

In this study, a xenogeneic graft was used with only an osteo-conductive property that only provides a scaffold for bone formation and lacks the ability to induce osteoblast differentiation. Considering the created defect size was 20 mm and that the critical size of dog's mandibular defects with the presence of periosteum is 5 cm and without it is 1.5 cm[16] Therefore, the size of the defect which had no periosteum was too large for the cells that surround the defect to act as a source of osteoblasts and repair the defect, therefore, the effect of defect margins in bone repair were minimized. In such situations, osteoblast induction is performed by the periosteum.[15,16]

In the current study, the periosteum was replaced with an amniotic membrane in the test group and considering the lack of resources for bone repair, the ossification process can be considered the result of the amniotic membrane's abilities in bone repair. The results of this study show desirable bone repair in the test group. Therefore we can conclude that the amniotic membrane alike bone periosteum has osteoinductive abilities.

In an experimental clinical trial, Samandari *et al.*[17] analyzed the effect of using the human fetal amniotic membrane as an osteoinductive factor in vestibuloplasty

surgery and in wound repair in 10 dogs. The results of the study were analyzed clinically and histologically after 2, 8, and 12 weeks. According to these results using human fetal amniotic membrane has obvious osteoinductive effects, wound repair effects, and inflammation sign reductions such as fibrino leukocytic exudate discharge.[17] The results of the present study also show the membrane's ability in osteoinduction.

In similar studies Kothari *et al*.[13] and Sharma *et al*.[18] analyzed the use of the amniotic membrane as coating for exposed bone and graft material in vestibuloplasty surgery of the mandible using the Clark technique. The results showed the suitability and effectiveness of this membrane as a substitute for periosteum in vestibuloplasty. The results of the current study confirm the results of these two clinical studies.

Kesting *et al*.[19] investigated the use of a human fetal amniotic membrane in closing induced oronasal fistulas in laboratory pigs during an experimental clinical trial. Seven pigs were used in this study. In 3 pigs the amniotic membrane was used, in 3 other pigs a collagen membrane was used and in 1 pig nothing was used. After 40 days, the pigs were put down and the oronasal fistula was examined, in 2 out of 3 pigs in which the amniotic membrane was used the fistula had been closed and in one of them it had become smaller; in all 3 of the pigs with the collagen membrane the size of the fistula had reduced but none had closed completely and the control pig showed no difference in fistula size. The results of the study which showed that the amniotic membrane is suitable for closing orinasal fistula and osteoinduction are confirmed in the current study.

Koushaei *et al*.[20] have recently investigated ossification as a result of amniotic and absorbable collagen membrane grafts in dog's tibia. This study directly analyzed the ability of the amniotic membrane in osteoinduction compared to the collagen membrane in repairing osseous defects.[20] Researchers in this study suggested that considering the osteo-inductive abilities of the amniotic membrane, later studies should concentrate on using the membrane in jaw bones to test its effectiveness in repairing bone defects in that region.[20]

The current study has been based on the findings of the previous authors and its results confirm those of the previous study. The results show that the amniotic membrane can act as a suitable replacement for the periosteum in repairing bone defects with are damaged or have no periosteum. It is suggested that future studies to be carried out on human subjects and to compare the amniotic membrane with other membranes such as collagen membranes. It is also suggested to compare the effect of the periosteum and the amniotic membrane in future studies.

CONCLUSION

The amniotic membrane can induce positive and desirable osteoinduction in bone defects of the mandible.

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Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or nonfinancial in this article.

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