Original Article

Application of Autogenous Periosteum as a Membrane in Sinus Lifting

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Abstract

Aim: To evaluate the success level of autogenous periosteum in sinus lifting as a barrier membrane which contributes positively to wound healing and is effective in bone formation without the risk of tissue rejection. **Materials and Methods:** In this study, 32 male New Zealand rabbits were used and were divided into four groups, in which eight rabbits were placed randomly. Sinus lifting with lateral window technique was applied bilaterally to all rabbits. In the first group, the upper face of the graft materials applied was left open. In the second group, the removed bone walls were placed back over the graft materials. In the third group, synthetic membranes were placed over the graft materials. In the fourth group, the autogenous periosteums obtained from tibias of the rabbits were placed over the graft materials. After 6 weeks, the rabbits in all groups were sacrificed, and the operated regions were examined histologically, and stereological assessments were conducted regarding new bone formation, connective tissue, and osteoblasts. **Results:** After a 6-week recovery period, synthetic membrane showed the highest success rate regarding new bone formation. Autogenous periosteum, which achieved the second highest success rate regarding new bone formation, was the first in the number of osteoblasts. **Conclusion:** Autogenous periosteum was considered to have the potential to be an alternative to synthetic membranes.

Keywords: *Autogenous periosteum, barrier membrane, new bone formation, sinus lifting*

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Introduction

The primary requirement for implant success is to supply sufficient bone support that can provide primary implant stability. Following the loss of natural teeth, alveolar bone atrophy occurs both in buccolingual and apicocoronal directions.[1,2] The resorption in the maxilla is known to develop six times faster than the resorption in the mandible. Grafting the maxillary sinus floor to improve the inadequate vertical bone height and insertion of the implants at the resorbed maxillary posterior region is called "sinus lifting procedure." This method was first described by Boyne and James in the 1960s. Fifteen years later, Boyne and James reported on the elevation of the maxillary sinus floor in patients with large, pneumatized sinus cavities in preparation for the placement of blade implants.[3]

Periosteum is a specialized tissue that provides transverse bone growth, plays an active role in bone repair in cases of fracture and provides the nutritional needs of bone.[4] It has been shown by experimental studies that the periosteum contributes positively to osteogenesis and chondrogenesis as a free or vascularized graft in various environments.[5] Although there are several studies where periosteum was used as a free graft material and as a barrier membrane in guided tissue regeneration (GTR) operations in periodontal treatments, there is no study in which periosteum was used in sinus lifting as a barrier membrane.

The aim of this study is to evaluate the success level of autogenous periosteum in sinus lifting as a barrier membrane which contributes positively to wound

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healing and is effective in bone formation without the risk of tissue rejection and expense. To achieve this, three parameters were evaluated. These were new bone formation, connective tissue, and osteoblast numbers. The quantity of new bone formation was considered as a direct success indicator of new bone formation while the osteoblast numbers were assessed as the potential factor for new bone formation over a longer period of time. On the other hand, the volume of connective tissue is assumed as a failure regarding ossification.

Materials and Methods

This study was approved by Ondokuz Mayıs University Animal Experiments Local Ethics Committee with 2012/27 approval number in 26.03.2012 and funded by Ondokuz Mayıs University Commission of Scientific Research Projects with the project number of PYO. DIS.1904.12.013.

In this study, 32 male New Zealand rabbits were used with an average age of 3 months and 1.5–2 kg. The care of all rabbits was performed by Ondokuz Mayıs University Experimental Animal Research and Training Center. Thirty-two rabbits were used in this study and divided into four groups in which eight rabbits were randomly placed. Sinus lifting with lateral window technique was applied bilaterally to all rabbits.

All rabbits were put under general anesthesia via intramuscular injection using 50 mg/kg ketamine hydrochloride (Xylazinbio, Bioveta, Czech Republic) and 10 mg/kg xylazine (Ketasol, Richter Pharma Ag, Wels, Austria). In addition, all rabbits received 1 mL of articaine (Ultracain D-S Forte, Aventis, Istanbul, Turkey) with epinephrine (1/100.000) solution injected subcutaneously as a local anesthetic at the midline of the nasal dorsum.

Guidelines on the bone were achieved by creating a mark with a trephine bur 5 mm in diameter. A circular window (diameter, about 5 mm) was opened in the nasal bone with the usage of a rotating round bur. The window was located approximately 20 mm anterior to the nasofrontal suture line and 5 mm lateral to the midline. Mobilized bone walls over the windows were removed. Figure 1 shows the removal of the bone walls.

Sinus membrane was carefully raised from the floor, and lateral walls and a space-filling material were inserted into the created compartment. The volume of filling material was standardized to 0.1cc/sinus, synthetic graft material for the right sinus (Kasios TCP-ZI La Croix, Launaguet, France) and bovine-derived graft material (İntegros BonePlus-İntegros Health Products, Adana, Turkey) for the left.

In Group 1, no other substance was applied over the graft while removed bone wall was placed over the graft material in place of a barrier membrane in Group 2.

The bony windows were covered with 10 mm × 30 mm resorbable membrane (Cytoflex Resorb, Unicare Biomedical, USA) to prevent migration of soft tissue cells into the space-filled with graft material in Group 3. Figure 2 shows covering the inserted graft material with synthetic membrane.

In Group 4, periosteum obtained from the left tibias of the rabbits was used as barrier membranes. The medial portion of the left legs of the rabbits was shaved and disinfected with 10% of povidone-iodine solution (Batticon, Adeka, Turkey). Skin, subcutaneous tissue, and muscles were cut with a longitudinal incision at the medial part of the leg, and the tibia was revealed without damaging the periosteum. A 1 cm × 4 cm rectangular incision was performed with a scalpel on the uncovered tibia and periosteum was stripped out of the bone tissue. The extracted periosteal tissue was transferred into 0.9% of NaCl solution. The wound was washed, the subcutaneous tissue was sutured with 4/0 resorbable suture (PGLA Rapid, Ces, Turkey), and the skin was sutured with 3/0 silk suture (İpek, Ces, Turkey). Periosteal tissue obtained from the tibia and placed in the NaCl solution was fixed with mini pins as a barrier membrane over the bone window filled with graft material because of its slippery and mobile nature. Figure 3 shows covering the bone window with periosteum as a barrier membrane. Skin tissue in the sinus area was sutured with 3/0 silk suture. Because of the high metabolic rate of the rabbits, 6 weeks is considered to be sufficient for new bone formation.[6] Hence, all the rabbits were sacrificed after 6 weeks.

Due to its better results, stereological assessment was preferred instead of computed tomography imaging. Cavalieri principle was used to estimate the volume of new bone and connective tissue. According to the rules of systematic random sampling, the specimens were sliced coronally into serial sections (1/50), each about 5 μm thick. All the images of the serial sections of the region concerned were obtained at ×4 and ×20 magnification under a light microscope (Olympus BX50, Tokyo, Japan).

Statistical analysis

All statistical evaluations were performed using SPSS (SPSS version 21.0; SPSS Inc., Chicago, IL, USA) software. One-way ANOVA, Tukey's test was used in the comparison with each of the groups regarding new bone volume, new connective tissue volume, and osteoblast number. The level of statistical significance was defined as $P < 0.05$ in statistical evaluations made between

groups. The level of high significant was defined as $P < 0.01$.

Results

Histological findings

New bone formation, connective tissue, and osteoblast numbers were evaluated by detecting the histological images obtained with ×4 magnification in regions where graft materials were applied.

Figures 4-7 show histological images of the groups with ×4 magnification taken 6 weeks after surgery (YK: New bone; BD: New connective tissue; G: Graft).

New bone volume

New bone volumes formed after 6 weeks of the sinus lift operation were evaluated in each group. Table 1 shows the new bone volume values occurred in all groups. All groups were examined and compared in terms of new bone volume. No significant difference between Group 1–2 and Group 3–4 in terms of the volume of new bone formed in the right maxillary sinuses ($P \geq 0.05$) was determined; while the new bone formation in Group 3 and Group 4 was greater than Group 1 and Group 2 ($P < 0.05$).

Table 1: The new bone volume values occurred in all groups

Average new bone volume (mm^3)	Minimum value	Maximum value	SD
Group 1 - 7.45	6.72	8.01	0.34
Group 2 - 7.56	6.78	8.73	0.42
Group 3 - 8.19	7.69	8.85	0.39
Group 4 - 7.89	6.37	9.17	0.46

SD=Standard deviation

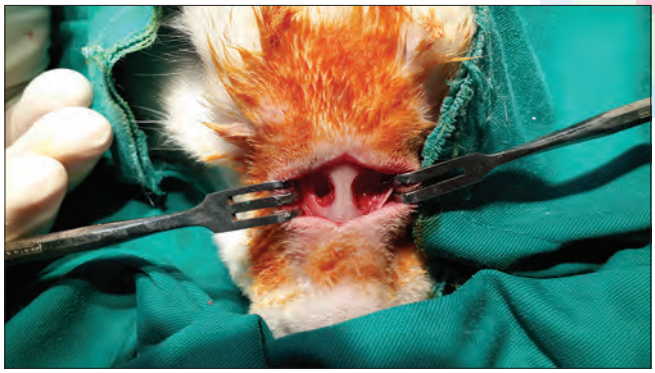

Figure 1: Removal of the bone walls

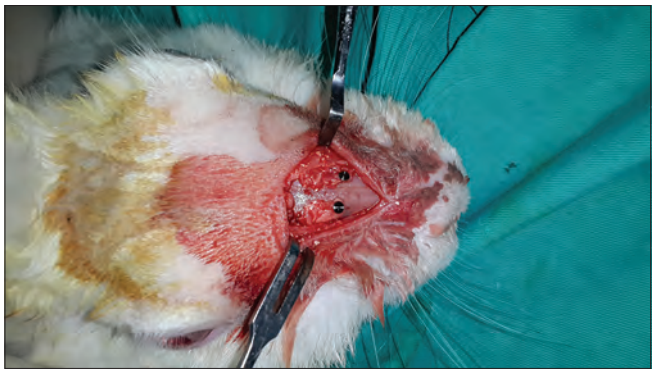

Figure 3: Covering the bone window with periosteum as a barrier membrane

Connective tissue volume

Connective tissue volumes formed after 6 weeks of sinus lift operation were evaluated in each group. Table 2 shows the connective tissue volume values occurred in all groups. All groups were examined and compared in terms of connective tissue volume. Connective tissue volume in Group 1 was significantly greater than the other groups, and no significant difference was seen between Group 1, Group 2 and Group 3 in terms of the volume of connective tissue ($P \geq 0.05$).

Osteoblast numbers

Osteoblast numbers formed after 6 weeks of sinus lift operation were evaluated in each group. Table 3

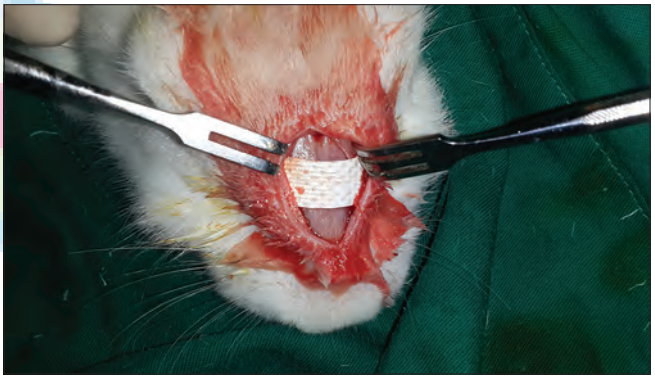

Figure 2: Covering the inserted graft material with synthetic membrane

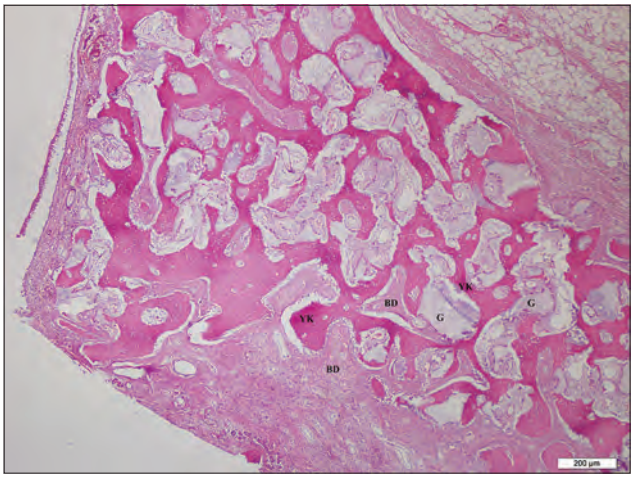

Figure 4: A histological image of Group 1 with ×4 magnification taken 6 weeks after surgery (YK: New bone; BD: New connective tissue; G: Graft)

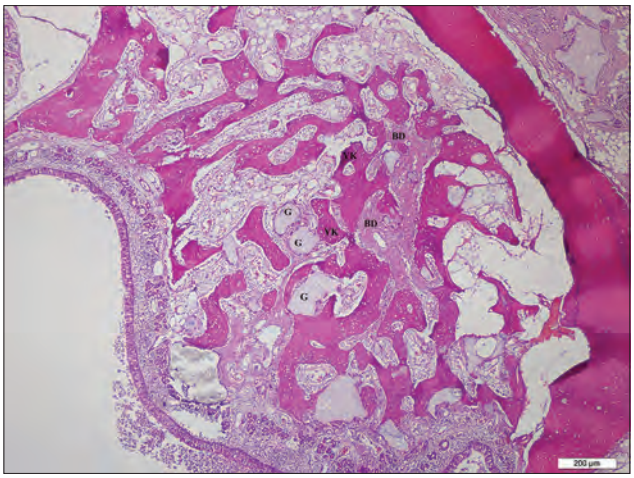

Figure 5: A histological image of Group 2 with ×4 magnification taken 6 weeks after surgery (YK: New bone; BD: New connective tissue; G: Graft)

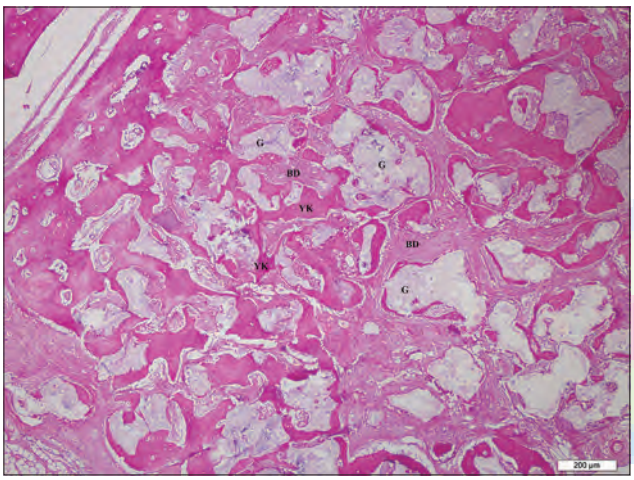

Figure 6: A histological image of Group 3 with ×4 magnification taken 6 weeks after surgery (YK: New bone; BD: New connective tissue; G: Graft)

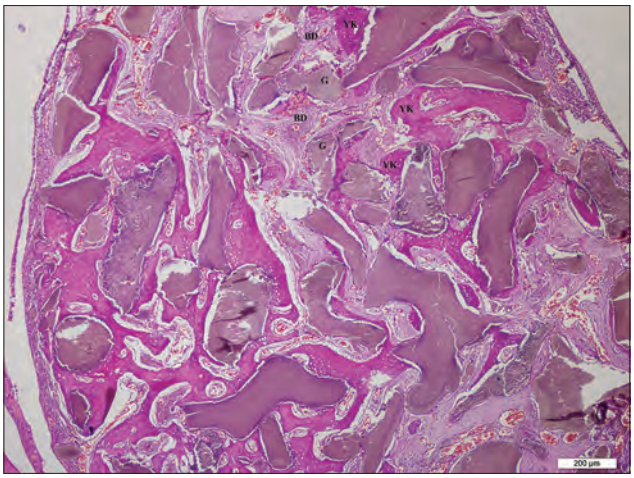

Figure 7: A histological image of Group 4 with ×4 magnification taken 6 weeks after surgery (YK: New bone; BD: New connective tissue; G: Graft)

shows the osteoblast numbers occurred in all groups. All groups were examined and compared in terms of

Table 2: The connective tissue volume values occurred in all groups

Average connective tissue volume (mm^3)	Minimum value	Maximum value	SD
Group 1 - 20.70	18.45	24.05	1.56
Group 2 - 19.08	17.65	21.80	1.16
Group 3 - 17.99	17.05	19.05	0.87
Group 4 - 18.26	17.35	19.65	0.79

SD=Standard deviation

Table 3: Osteoblast numbers occurred in all groups

Average osteoblast numbers	Minimum value	Maximum value	SD
Group 1 - 1,575,475	1,376,000	1,894,400	1.03
Group 2 - 1,630,720	1,376,000	1,945,600	1.19
Group 3 - 2,086,912	1,536,000	2,464,000	1.85
Group 4 - 2,095,360	1,728,000	2,476,800	1.58

SD=Standard deviation

osteoblast numbers. Osteoblasic activity in Group 3 and Group 4 was significantly greater than Group 1 and Group 2 ($P < 0.01$). No significant difference was seen between Group 1–2 and Group 3–4 in terms of the osteoblastic activity ($P \geq 0.05$).

Discussion

The use of barrier membranes to provide bone regeneration is preferred to prevent the development of undesired structures and to allow an increase in the population of desired bone cells on the grafted regions.[7] The clinical studies performed to compare the bone healing levels with or without barrier membrane show that barrier membranes increase the integration of bone grafts,[8] accelerate bone formation,[9] and increase bone quality.[10]

The most important obstacle for successful bone healing or new bone development is that the soft tissues grow faster than the bone tissue. Guided bone regeneration (GBR) provides a suitable gap in the defect region. With the help of the barrier membrane located between the bone defect and the surrounding soft tissues, fibroblasts are kept away from the defect region. Furthermore, this allows the osteoblasts to organize the bone healing in this region.[11]

If the studies are examined included in the clinical application of GBR, it can be seen that the researches advocate this technique[12] as well as the studies arguing that it is useless.[13]

In a study investigating the membrane activity level by performing sinus lift procedures over 113 cases, Froum *et al*.[14] found the average rate of vital bone in the areas treated with membrane as 27.6%, but this ratio decreased to 16% in the areas treated without the membrane.

As a result of a controlled trial managed by Tawil and Mawla,[15] higher success rates were observed with implant survival rates in the group treated with barrier membrane than the group treated without membrane (the overall survival rate was 78.1% for the membraneless sites and 93.1% for the membrane used sites). On the other hand, there was no statistically significant difference between the groups treated with/without the barrier membrane in a study conducted by De Souza Nunes *et al*.[13]

In our study, new bone formation was significantly higher in Group 3 (synthetic membrane group) and Group 4 (autogenous periosteum group) than Group 1 and Group 2 in which no membrane was applied. Group 1 was the least successful group regarding new bone formation in which the graft material was uncovered. These rewarding results support the effectiveness of barrier membranes in forming new bone in sinus lift operations.

The removal necessity of nonresorbable membranes with a second surgical intervention and infection risk when they are exposed has led the development of resorbable membranes. Some authors state a clinically significant difference between the resorbable and nonresorbable membranes[16] while others claim no difference.[17,18]

Resorbable and nonresorbable membranes were evaluated in a study conducted on 23 patients by Eickholz *et al*.[19] and no statistical difference was found between them.

GBR operation was applied to rabbits in another study by Ito *et al*.[20] to compare the resorbable and nonresorbable membranes. According to the findings, more new bone volume was formed by nonresorbable membranes than the resorbable ones thanks to their resistance and durability.

In a study performed by da Silva Pereira *et al*.,[17] the effectiveness of resorbable and nonresorbable membranes were compared and more new bone area was detected in the treated group with resorbable membrane.

Wallace *et al*.[16] investigated the same topic in another study and the membrane types were compared according to their activity on vital bone formation, but no statistically significant difference was detected between the resorbable and nonresorbable membranes. Both groups were more effective than the control group that was treated without membrane regarding vital bone development and implant survival rates.

Autogenous periosteum is widely used in general medical treatment and gives promising results.[21] On the contrary, its usage has always been limited in dentistry and its activity in regeneration has been quite overlooked.

Due to its high osteogenic potential, the usage of autogenous periosteum as an alternative barrier membrane in GTR and GBR operations have been proposed in several studies.[22-25] From this point of view, this study was designed to show the effectiveness of autogenous periosteum in sinus lifting operations. However, long-term results are waiting to prove its effectiveness.

Verma *et al*.[22] showed the effectiveness of periosteum as a barrier membrane in a study performed by GTR operations on 12 patients. In another study, Verdugo *et al*.[23] carried out GBR operations on 10 patients and periosteum was applied as a barrier membrane over the graft material by preserving the patients' own periosteum tissue. According to the results, it was concluded that preserving the periosteum to use as a barrier membrane is sufficient if a good primary closure is applied over the graft material. In this study, similar results achieved but autogenous periosteum was not as successful as synthetic barrier membrane regarding new bone formation.

Singhal *et al*.[24] used marginal pedicled periosteum as a barrier membrane in two-walled intrabony defects in their study and the effectiveness of periosteum has been reported to be successful.

Saimbi *et al*.[25] performed a GTR study on ten patients with bilateral intrabony defect. The periosteal membrane was applied on one side of the bony defect, and conventional open flap debridement procedure was completed on the contrary side. No statistical difference was observed between the two regions, so the periosteal barrier membrane used in intraosseous defects was concluded to be an alternative to prefabricated membranes. Although autogenous periosteum has the potential to be an alternative to synthetic membranes, our study does not support this view exactly. The results of our study show that autogenous periosteum may increase the effectiveness of the prefabricated membranes, but cannot replace the prefabricated membranes completely, probably due to its lack of durability. It may require an additional protection to the forces formed in that region. Further studies on this topic may be useful.

Conclusion

The results obtained within this study were as follows:

- When compared with synthetic membranes, autogenous periosteum was found to be less effective on new bone formation
- Autogenous periosteum was found to be more effective according to the assessment made regarding the number of osteoblasts
- Insufficient rigidity of autogenous periosteum was considered as the most important disadvantage in

the use as a barrier membrane. This disadvantage of autogenous periosteum group may be the cause of the lower quantity of new bone according to the synthetic membrane group

- Combined usage of synthetic membrane and autogenous periosteum may be studied in another study. Furthermore, long-term outcomes can reveal different results.

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Conflicts of interest

There are no conflicts of interest.

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