# Histological Outcomes on the Development of New Space-making Devices for Maxillary Sinus Floor Augmentation

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## ABSTRACT

*Background:* Previous studies have pointed out that the mere elevation of the maxillary sinus membrane promotes bone formation without the use of augmentation materials.

*Purpose:* This experimental study aimed at evaluating if the two-stage procedure for sinus floor augmentation could benefit from the use of a space-making device in order to increase the bone volume to enable later implant installation with good primary stability.

*Materials and Methods:* Six male tufted capuchin primates (*Cebus apella*) were subjected to extraction of the three premolars and the first molar on both sides of the maxilla to create an edentulous area. The sinuses were opened using the lateral bone-wall window technique, and the membrane was elevated. One resorbable space-making device was inserted in each maxillary sinus, and the bone window was returned in place. The animals were euthanatized after 6 months, and biopsy blocks containing the whole maxillary sinus and surrounding soft tissues were prepared for ground sections.

*Results:* The histological examination of the specimens showed bone formation in contact with both the schneiderian membrane and the device in most cases even when the device was displaced. The process of bone formation indicates that this technique is potentially useful for two-stage sinus floor augmentation. The lack of stabilization of the device within the sinus demands further improvement of space-makers for predictable bone augmentation.

*Conclusions:* It is concluded that (1) the device used in this study did not trigger any important inflammatory reaction; (2) when the sinus membrane was elevated, bone formation was a constant finding; and (3) an ideal space-making device should be stable and elevate the membrane to ensure a maintained connection between the membrane and the secluded space.

KEY WORDS: augmentation, bone formation, endosseous implants, maxillary sinus, sinus membrane elevation

## INTRODUCTION

Several studies have indicated that the mere lifting of the membrane may induce bone formation at the maxillary sinus floor. A previous experimental study in primates[1] showed that (1) membrane elevation with or without bone grafts and insertion of implants resulted in a similar amount of bone after 6 months, (2) oxidized titanium implants showed a stronger tissue response than machined-surfaced implants, and (3) the membrane followed the contour of the implants. Clinical studies have confirmed that simultaneous placement of dental implants resulted in bone formation without the use of adjunctive grafting materials.[2–6]

The principle governing bone formation by simply elevating the sinus membrane could also be applied in

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cases where insufficient bone anchorage hinders simultaneous implant placement. Recently, this group tested in primates a resorbable dome-shaped device aiming at creating bone formation for delayed implant placement.[7] The histological outcomes showed that there were only minor or no signs of bone formation in the sites with a space-making device only. Sites with simultaneous implant placement showed bone formation along the implant surface. Sites with delayed implant placement showed minor or no bone formation and/or formation of a dense fibrous tissue. The displacement of the device because of lack of stabilization, together with too much direct contact between the device's surface and the sinus membrane, thus causing a reduction in the exchange of cells and fluid between the inner compartment of the sinus and the membrane, was probably the main reason for this.

Based on these observations, modified devices were developed with the intention to increase the stability and the biological properties of space-making device. This new experimental study was designed to give some answers to the following questions: can a resorbable dome be used to elevate the sinus membrane in order to make bone prior to implant placement? can the material properties of the dome influence the outcome?

## MATERIALS AND METHODS

This animal study was carried out in accordance with the rules by the Brazilian Institute for Protection of the Environment and approved by the Animal Ethics Committee at the Faculty of Dentistry of the University of the State of Sao Paulo – UNESP, Aracatuba, Brazil.

A total of six young adult male tufted capuchin primates (*Cebus apella*), 8 to 12 years old, and weighing between 2 and 3.0 kg were included in this study. Before surgery, the animals were maintained in individual cages at the Primate Procreation Nucleus Faculty of Dentistry, UNESP, Aracatuba, Brazil, with water and food *ad libitum*. For all procedures involved in the study, the primates were first sedated with ketamine hydrochloride (Ketamin™, Cristalia Produtos Químicos Farmacêuticos Ltd., Campinas, Brazil) 10 mg/kg body weight administered intramuscularly. Prior to surgery or any animal manipulation, general anesthesia was obtained with pentobarbital sodium (Abbott Laboratories North Chicago, Chicago, IL, USA) in the dosage of 30 mg/kg. The anesthesia was supplemented by local administration of 2% mepivacaine HCI with 1:100.000 epinephrine (DFL Ltd., Rio de Janeiro, Brazil). Previously to surgeries, the animals received dental prophylaxis, and all the surgical sites were washed with 0.12% chlorhexidine gluconate solution (Periogard™, Colgate-Palmolive Ltd., Sao Paulo, Brazil). The surgeries were performed under sterile conditions.

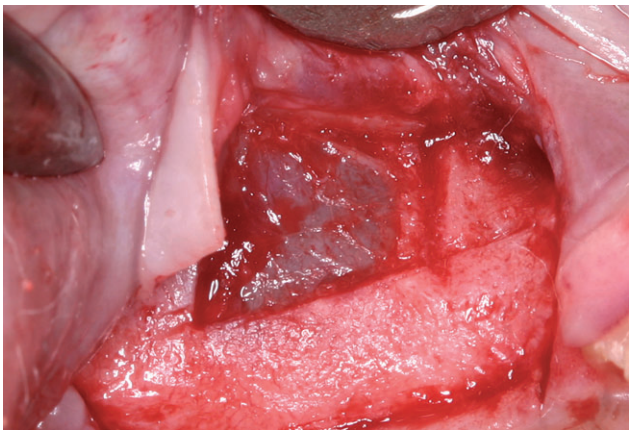
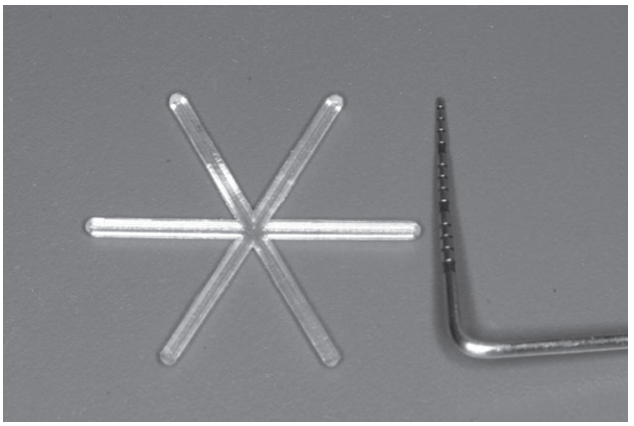


**Figure 1** Showing the lateral aspect of the sinus after the bone window removal and before the sinus membrane elevation.

### Surgeries

The first, second, and third upper premolars and the first molar were extracted bilaterally 3 to 4 months prior to the start of the experiment. Extractions were performed under general anesthesia, according to the technique described earlier. All animals underwent bilateral maxillary sinus surgery. After a mid-crestal incision and vertical releasing incisions, mucoperiosteal flaps were raised and reflected at the edentulous posterior maxilla on both sides in order to access the alveolar bone. The lateral aspect of the maxillary sinus was fully exposed using a reciprocating saw to create a 0.8 cm × 0.6 cm ± 0.2 cm window under continuous saline irrigation (Figure 1). The osseous window was freed by fracturing along the osteotomy lines, removed and kept in saline solution. The sinus membrane was then carefully elevated with specially designed elevators (Friatec™, Friedrichsfeld AG, Mannheim, Germany). All six animals received either H-shaped or star-shaped space-making devices (polylactide 70/30, Radi Medical System AB, Uppsala, Sweden) (Figure 2, A and B). The device was warmed up to 50°C in saline solution to shape it to the sinus floor topography and introduced into the maxillary sinus cavity in order to maintain the sinus membrane elevated (Figure 3). The bone windows were then repositioned and stabilized with n-butyl-2-cyanoacrylate tissue glue (Indermil™, Henkel

A



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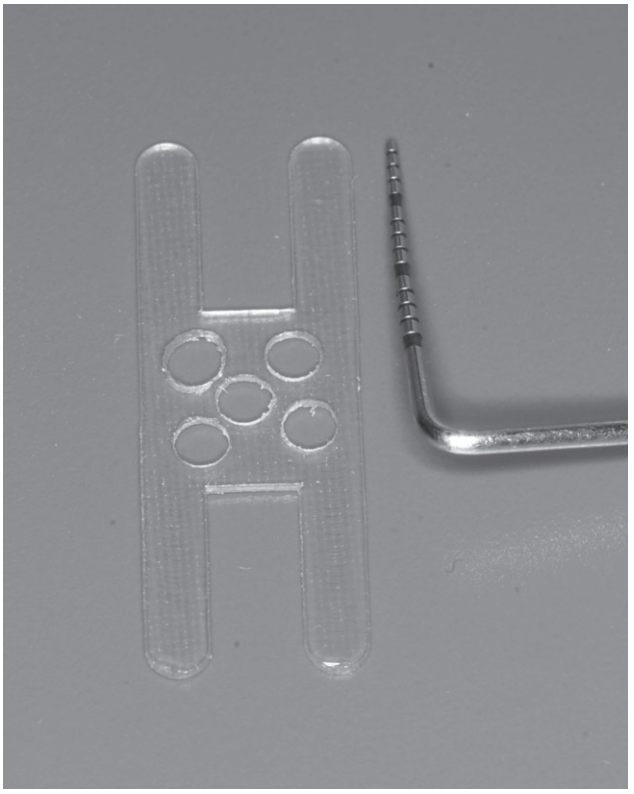

**Figure 2** *A*, Showing the star-shaped space-making device (polylactide 70/30) used in the present study. *B*, Showing the H-shaped space-making device (polylactide 70/30) used in the present study.

Loctite Ltd., Whitestown, Republic of Ireland) (Figure 4). The mucoperiosteal flap was sutured with Vicryl 5-0 (Ethicon™, Johnson & Johnson, Sao Jose dos Campos, Brazil). The wound was finally rinsed with 0.12% chlorhexidine gluconate solution.

## Postoperative Follow-Up

The animals were fed with a soft diet (Sustagen™, Nestlé, Sao Paulo, Brazil) during the first 15 days, and

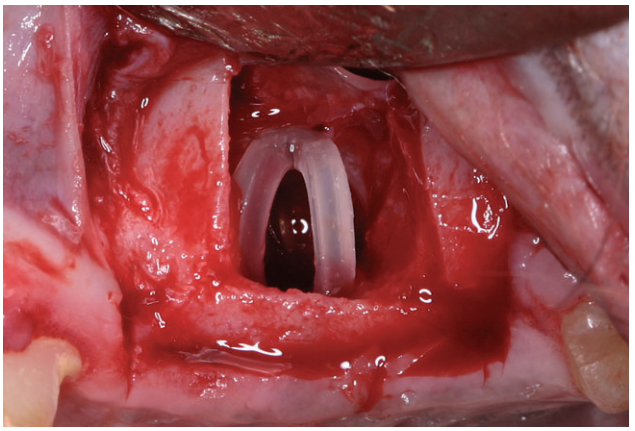

**Figure 3** Showing the space-making device positioned into maxillary sinus after bone window removal and sinus membrane elevation.

with fruits and cooked vegetables thereafter. Three times daily, the animals were given an oral dose of Cefalotina™ (20 mg/kg, Stiefel, Guarulhos, Brazil) mixed with fruits shakes during 7 days and Tylenol™ (30 mg/kg, Janssen-Cilag, Sao Jose dos Campos, Brazil) mixed with fruits shakes during 2 days and water *ad libitum*. The animals were inspected after the first, third, and fifth postoperative months for signs of wound and general health complications. During this period, a systematic periodontal care was carried out, as well as local applications of 0.12% chlorhexidine gluconate solution.

## Sacrifice and Specimens Postprocessing

All six animals were sacrificed after 6 months of the initial maxillary sinus surgery. The animals were anesthetized with pentobarbital sodium associated with analgesics to undertake vascular perfusion with

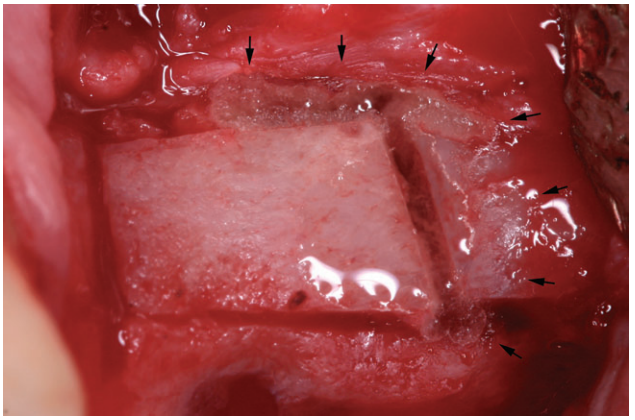

**Figure 4** Showing the repositioned bone window, which has been stabilized with cyanoacrylate glue (*arrows*).

paraformaldehyde. The maxilla was retrieved *en bloc*, and the surrounding soft tissues were detached. The specimens were trimmed and immersed in 4% paraformaldehyde in 0.1 M in sodium phosphate buffer (pH 7.4).

### Histological Preparation and Assessments

The specimens were dehydrated in a series of ethanol embedded in hard-grade acrylic resin (LR White™, London Resin Company Ltd., Berkshire, England) and polymerized in dry heat oven at 60°C under vacuum environment. The plastic blocks were mounted on glass slides, and three buccal-palatine sections (anterior, central, and posterior) were taken from each sinus (Microslice 2™, Ultratec Inc., Santa Ana, CA, USA) and stained with toluidine blue/pyronin-Y method. Only the central ground sections were examined under a Leica DMLB™ microscope (Leica Microsystems Wetzlar GmbH, Wetzlar, Germany), equipped with a Leica Digital Camera DFC 300FX (Leica Microsystems Wetzlar GmbH).

## RESULTS

### Surgery

Sinus membrane perforations occurred in 5 out of 12 treated sinuses. All of them could be considered minor perforations (less than 2 mm) and were treated by folding the membrane in, as a consequence of elevation, before the device was definitely installed.

### Clinical and Anatomical Examination

The postoperative period occurred uneventful, and the animals were healthy throughout the follow-up time. The anatomical examination at termination revealed that the space-making devices were not found in their original positions in the sinuses (Figure 5).

### Histological Examination

Invariably, the schneiderian membrane was found in intimate contact with mineralized tissue (Figure 6). New bone formation was a common finding in all sinuses irrespective of the type of device. As a general rule, trabecular bone originating from the sinus periphery was projected into the center (Figure 7) in the vast majority of the cases. The trabeculae exhibited different stages of bone deposition, typically a mature pattern outlined by newly formed bone, or newly formed bone surrounding

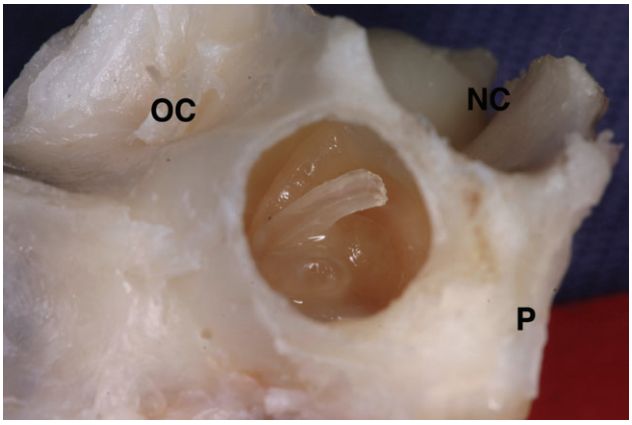


**Figure 5** Showing a retrieved maxillary sinus cavity observed from the posterior aspect (access from tuber). NC = nasal cavity; OC = orbital cavity; P = palatal.

fat cells (Figure 8). Even in regions where the soft tissue predominated, mineralized islands (Figure 9) could be captured, indicating an ongoing and diffuse process of bone formation within the sinus cavity.

An important histological finding in many specimens was the presence of marrow-like tissue in the center of the sinus, characterized by a loose connective tissue and the presence of vessels, fat cells, and hemopoietic cells close to forming trabeculae (Figures 10). The device's surface was frequently found separated from the bone tissue by a thin layer of soft tissue featuring foreign-body giant cells and macrophages (Figure 11) in close proximity with the device. No signs of resorption of the material could be identified.

## DISCUSSION

The results from the present experimental study corroborates with previous reports suggesting

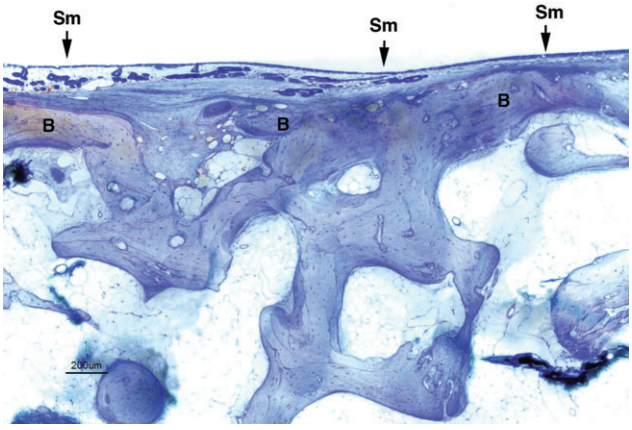


**Figure 6** Light micrograph featuring the schneiderian membrane (Sm) with blood vessels in intimate contact with new bone tissue (B).

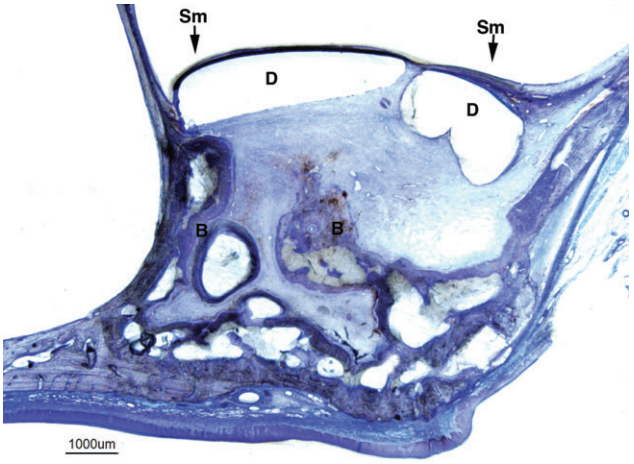


**Figure 7** Light micrograph featuring new bone (B) formation below the elevated schneiderian membrane (Sm) by the device (D) 6 months after healing.

osteoinductive properties of the schneiderian membrane.[1,8,9] In the present study, the sinus membrane elevation surgery triggered bone formation between the membrane and the secluded space in the sinus cavity.

The outcomes comparison between our latest study on space-making device development and the present one revealed that the biomechanical modifications of the device resulted in improved biological performance as bone formation was evident.

The lack of stability of the space-making device in the sinus is rather intriguing and deserves further analysis, as it may be the key factor for more predictable bone formation. The ideal space-making device may need

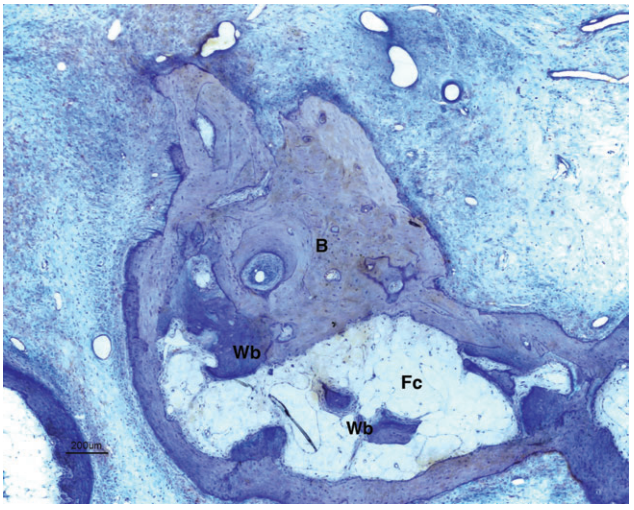


**Figure 8** Light micrograph featuring different stages of bone deposition: mineralized bone (B), woven-bone (Wb), and fat cells (Fc).

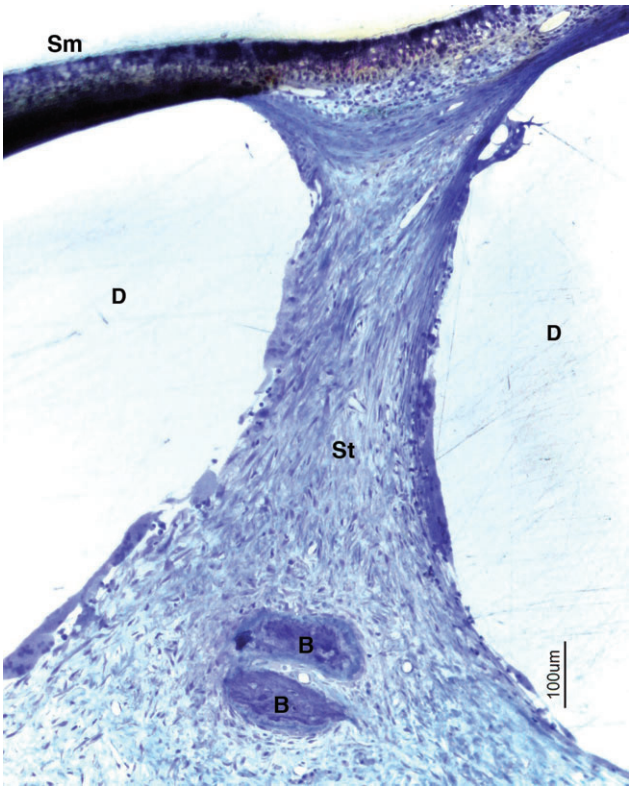


**Figure 9** Light micrograph depicting the device (D), the schneiderian membrane (Sm), and small bone (B) islands surrounded by soft tissue (St).

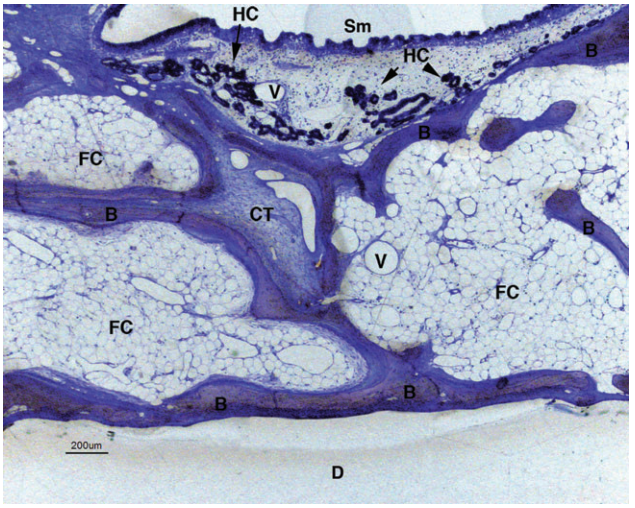


**Figure 10** Light micrograph depicting marrow-like tissue in the center of the augmented area, exhibiting a loose connective tissue (CT), vessels (V), fat cells (FC), and hemopoietic cells (HC), close to trabecular bone (B). The photomicrography also shows newly formed bone between the device (D) and the schneiderian membrane (Sm) because of displacement of the device.

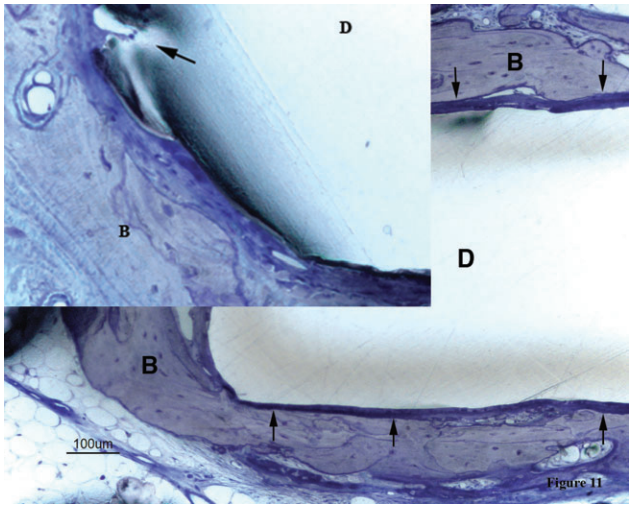


**Figure 11** Light micrograph featuring a layer of soft tissue (*arrows*) separating the device's (D) surface from bone tissue (B). The upper left corner shows macrophages (*arrows*) in close proximity with D.

anchorage to the neighboring bone walls to remain stable. Moreover, the size of the sinus cavity in primates such as tufted capuchin primates is rather small. This may have cooperated for an increased risk of membrane perforation during the insertion of the space-making device into the sinus. Indeed, the perforation of the membrane was obvious in some cases, but it was treated by folding the membrane inward, before the device was installed. Even in the cases of membrane perforation, bone formation could be seen within the sinus if a device's leg was sustaining the membrane elevated. The capacity of the sinus membrane for self-repair was shown by Forsgren and colleagues.[10]

A cyanoacrylate glue was used in the present study to stabilize the replaced bone window after the insertion of the space-making device. Previous experimental studies have shown the possibility to use cyanoacrylate for oral hemostasis, fixation of soft and hard tissues, and closing sinus membrane perforation with low toxicity.[11–17] Good clinical outcomes, with cyanoacrylate in osteosynthesis fixation of mandibular fractures, were reported.[18] An intact lateral sinus wall was found in the retrieved specimens of the present study, which indicates that the cyanoacrylate glue did not interfere with the healing process. However, further experimental studies are needed to evaluate the bone tissue responses to cyanoacrylate glue.

The results of the present study indicate that the concept of creating new bone with a space-making device to maintain the sinus membrane elevated can be explained from the principles of guided-bone regeneration.[19,20] However, further studies are required in order to establish a protocol with a space-making device with improved stability inside the maxillary sinus.

## CONCLUSION

It is concluded that (1) the device used in this study did not trigger any important inflammatory reaction; (2) when the sinus membrane was elevated, bone formation was a constant finding; and (3) an ideal space-making device should be stable and elevate the membrane to ensure a maintained connection between the membrane and the secluded space.

## ACKNOWLEDGMENT

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