A 6-month histological analysis on maxillary sinus augmentation with and without use of collagen membranes over the osteotomy window: randomized clinical trial

**Key words:** biomaterial, collagen membrane, sinus augmentation

**Abstract**

**Introduction:** Over the years, several modifications have been made to the sinus augmentation technique and to the materials used. However, there is still controversy about the need for using a barrier concurrently with a graft in sinus augmentation procedures. On this basis, the aim of this randomized clinical study was to investigate the effect of resorbable collagen membrane over the osteotomy window on maxillary sinus augmentation healing.

**Materials and methods:** Patients who required maxillary sinus augmentation were evaluated and selected to enter the study. After maxillary sinus grafting, each patient was randomly assigned to control (membrane over the osteotomy window) or test (no membrane) group. After 6 months, one bone biopsy was harvested from the lateral window and sent to the histology laboratory. The Mann-Whitney nonparametric test was used for comparing the two groups.

**Results:** Eighteen patients entered the study and were randomly allocated in control (nine patients) or test group (nine patients). The histomorphometric measurements revealed that newly formed bone was 30.7% ± 15.5% of the total volume from the membrane group (control). The average percentage of connective tissue was 50.6% ± 18.7% and residual graft percentage was 18.4% ± 20.3%. On the other hand, data regarding the nonmembrane group (test) showed that the percentage of newly formed bone was 28.1% ± 19.4%. The mean percentage of connective tissues was 59.3% ± 15.4% and 12.6% ± 12.4% for the residual graft particles. No significant difference was detected in the histomorphometrical evaluation between the two groups.

**Discussion:** Our results showed that, compared with sites not covered, the use of the membrane did not substantially increase the amount of vital bone over a period of 6 months. On the other hand, the use of membrane seems to reduce the proliferation of the connective tissue and the graft re-absorption rate. It is plausible that blood supply of maxillary sinus can play a role in such a result. Further studies are needed to explore whether the use of membrane could really be advantageous for the sinus augmentation procedure and to evaluate what influence this method can have on the amount and quality of reconstructed bone.
substitutes such as deprotenized bovine bone and collagenated porcine bone have recently obtained the clinicians’ attention because they seem to be just as effective as autogenous grafts for sinus floor augmentation, in patients with severely re-absorbed alveolar ridges in the posterior maxilla [Maiorana et al. 2006; Esposito et al. 2008]. In the scientific literature, there is still controversy over the need to use a barrier concurrently with a graft both in guided bone regeneration and sinus augmentation procedures. It is well known that guided bone regeneration procedures, which are largely used in periodontics and oral surgery, often involve the use of membranes. These seem to have certain benefits by generating the highest percentages of trabecular bone volume [Wallace et al. 1996] and promoting bone growth without soft tissue interference. Yet, their positive benefits should be carefully balanced by the significant risk of infection, added cost and time, and the potential necessity for return visits from patients who, as a result, develop complications.

Some authors have reported advantages in using Gore-Tex membranes combined with mineralized cancellous grafts, such as a more favorable healing response and the prevention of soft tissue eneletation [Jensen & Greer 1995]. Other studies have indicated that the placement of membranes tends to increase vital bone formation and, as a consequence, it has a positive effect on implant survival [Tarnow et al. 2000]. On the other hand, there is some evidence that, although placement of a membrane after sinus floor augmentation induces bone formation more quickly, these procedures may also be successful and predictable without membrane barriers [Wagner 1991; Wallace et al. 1996]. Although the use of membranes could have some advantages such as preventing connective invasion of the graft, possible increased bone formation, and containment of particulate graft material, a criticism that has arisen is that the exclusion of the buccal flap could decrease vascular supply to the graft, compromising the formation of vital bone.

To our knowledge there are no randomized clinical trials where clinically relevant instruments with proven reliability and sensitivity have been used to assess the effectiveness of membrane placement. It is well known that randomized clinical trials [and meta-analyses of multiple trials] provide the highest level of evidence because randomization limits confounding and prevents bias of treatment assignment. In addition, randomized trials include the standardization of interventions, prospective data collection, and masked outcome measures.

The aim of this study is to investigate the effect of resorbable collagen membrane over the osteotomy window on maxillary sinus augmentation healing: maxillary sinus grafts covered by collagen resorbable membranes were compared to maxillary sinuses grafts not covered by membranes. This study is a preliminary report focusing on histological and histomorphometrical outcomes that occurred up to 6 months after grafting. Furthermore, to evaluate the success of the procedures over time, the patients were to receive a follow-up until the fifth year. The present study was reported according to the CONSORT guidelines [Schulz et al. 2010].

Material and methods

Patients who could benefit from a maxillary sinus augmentation procedure, who had a residual bone height under the maxillary sinus lower than 5 mm and who were 18 years or older and able to sign an informed consent form were eligible for inclusion in the trial. Patients were not included in the study if any of the following exclusion criteria were present: [1] systemic medical contraindications to implant surgery; [2] history of irradiation in the head and neck area; [3] poor oral hygiene and motivation; [4] uncontrolled diabetes; [5] current pregnancy and lactation; [6] acute or chronic pathologies of the maxil- lary sinuses; [7] smoking more than 20 cigarettes per day.

Ethical committee of Versilia Hospital, Lido di Camaiore, Italy approved the study. Patients who were referred to the Versilia Hospital from December 2008 to April 2010, were asked to participate in the present study. All patients received thorough explanations and had to complete a written informed consent form prior to being enrolled in the trial. After the informed consent was signed, all patients underwent at least one session of oral hygiene prior to the augmentation procedures to provide an oral environment more favorable to wound healing. Each case was accurately evaluated examining diagnostic casts to assess the inter-arch relationship, moreover, panoramic radiographs and computed tomography were taken.

Maxillary sinuses were allocated to either a control [membrane] or test [nonmembrane] group using a computerized random allocation process. A computer generated restricted randomization list was created. Only one of the investigators, not involved in the selection and treatment of the patients, was aware of the randomization sequence and could have access to the randomization list. The randomized codes were enclosed in sequentially numbered, identical, opaque, and sealed envelopes.

All patients received prophylactic antibiotic therapy of 2 g of amoxicillin [or clindamycin 600 mg if allergic to penicillins] and 4 mg dexamethasone 1 h before the augmentation procedure and continued to take the antibiotic postoperatively, 1 g amoxicillin [or 300 mg clindamycin] twice a day for 7 days. All patients rinsed for 1 min with chlorhexidine mouthwash 0.2% prior to the surgery (and twice a day for the following 3 weeks), and were treated under local anesthesia using lidocaine with adrenaline 1 : 50,000.

All surgeries were undertaken by the two surgeons [A. B. and U. C.] and their surgical teams. All the patients were treated with the same surgical technique consisting of sinus floor augmentation via a lateral approach [Barone et al. ]. Briefly, a mucoperiosteal flap was elevated exposing the lateral bone wall of the maxillary sinus, a modification of the conventional lateral wall approach was used to perform the osteotomy to access the sinus membrane [Galindo-Moreno et al. 2007]. A bone scraper [Safe scraper®; Meta corp. Remigia, Italy] was used to harvest autologous cortical bone and to reduce the lateral bone thickness, allowing an easy access to the sinus membrane with ultrasound [Piezosurgery, Mectron, Genova, Italy]. Subsequently, large flat curettes were used to raise the sinus membrane exposing the sinus bone wall up to the medial wall. Once the sinus membranes were elevated, all the sinususes were grafted with a mixture of autogenous bone harvested form the lateral bone wall and collagenated cortico-cancellous porcine bone [MP3®, Osteobiol-Tecnoss, Coazzz, Italy] in a 1 : 1 ratio. After maxillary sinus grafting, the randomization envelope was opened and indicated to the blindfolded surgeons to include the sinus as a test or a control site according to the randomization list. As a result, the treatment allocation was concealed to the investigators who were involved in enrolling and treating the patients. Sinuses in the control group were covered with a reabsorbable collagen membrane (Fig. 1a and b) [Evolution®, Osteobiol-Tecnoss], while sinuses in the test group (Fig. 2a and b) did not receive any membranes over the osteotomy window. The mucoperiosteal flaps were sutured with 3-0 reabsorbable sutures.

Patients were instructed to continue with prophylactic antibiotic therapy, and naproxen
sodium 550 mg tablets were prescribed as an anti-inflammator to be taken two times a day as long as required. Removable prosthesis, if present, was not permitted for use until they had been adjusted and refitted no sooner than 3 weeks after surgery. Patients were instructed to avoid blowing their nose and advised to administer corticosteroids nasal drops three times a day in both nasal cavities over a period of 4 weeks. Patients were seen 1 week after surgery for suture removal and thereafter for regular follow-up visits.

After 6 months of graft healing, radiographic examinations (orthopantomography and CT scan) were taken to evaluate the outcome of the surgical procedure. Immediately prior to the implant placement, at least one bone biopsy from each augmented maxillary sinus was harvested from the lateral window, using a trephine burr with an inner diameter of 2 mm and an outer diameter of 3 mm. Lateral window was identified by the surgeon observing the healed area in comparison with the surrounding bone. After fixation, the bone samples were forwarded to the Institute of Biomedicine, the Sahlgrenska Academy Gothenburg University, Sweden for histological examination.

After the retrieval, the functional implants were inserted in the augmented maxillary sinuses (Fig. 3).

The following outcome evaluations were considered in this study:

1. Surgical complications during maxillary sinus augmentation procedures; in particular, hemorrhage during lateral bone wall osteotomy or perforations of the sinus membrane;
2. Dimensions of osteotomy windows to access the sinuses were evaluated such as bony window length (L), bony window height (H), and lateral bone wall thickness (T);
3. Early or late postoperative complications such as wound dehiscence and acute/chronic sinusitis;
4. Histomorphometric parameters such as trabecular bone volume, soft tissues, and residual graft particles percentages.

**Specimen processing and analysis**

Specimens were decalcified in ethylenediaminetetraacetic acid (15%) for a period of 2 weeks. Specimens were again X-rayed to verify the decalcification procedure. After dehydration in graded series of ethanol, the specimens were embedded in paraffin, sectioned (3–5 mm sections), and stained with hematoxyline and eosine and modified Mallory aniline blue. Examinations were performed in a Nikon Eclipse 80i microscope (Teknooptik AB, Huddinge, Sweden) equipped with an easy image 2000 system (Teknooptik AB) using X1.0 to X40 objectives for descriptive evaluation and morphometric measurements. Histomorphometric measurements were performed in order to calculate the percentages (i.e., area fraction) of mineralized bone, residual graft materials, and soft tissue components (i.e., connective tissue and/or bone marrow) 6 months after the sinus augmentation procedure. All measurements were determined by point counting directly in the light microscope, using an optically superimposed eyepiece test square grid (distance between 6 ¥ 6 test lines 1/4 255 mm) at a magnification of 160-fold. The number of points of intersection between the test lines and the outlines of mineralized bone, bone substitute particles, and nonmineralized tissue were recorded.

**Statistical analysis**

The Mann–Whitney nonparametric test was used for comparing the differences between the two groups. Statistical significance was set at 5%.

**Results**

A flow diagram showing the several phases of the trial was reported in Fig. 4.

Twenty-one patients were screened for eligibility, but three patients could not be enrolled in the trial for the following reasons: one patient was being treated with oral bisphosphonates, one patient showed signs of chronic maxillary sinusitis and one patient, during surgical procedure, showed a thin lateral bone wall, which did not allow an adequate harvest of autogenous bone. As a result, 18 patients were enrolled and randomized: nine patients in the membrane group and nine patients in the nonmembrane group. Patients recruited in the study (7 men, 12 women) had a mean age of 59.4 years (an range of 49–64 years old). Only one sinus was elevated for each patient. All patients were treated according to the allocated intervention, no dropouts or exclusion occurred up to the 6 months follow-up, all data were used for statistical analysis. Patients underwent maxillary sinus augmentation between April 2009 and April 2010.
The follow-up presented in this study is referred to the period between sinus augmentation procedure and implant placement. No significant differences were present at the baseline between the two groups in terms of the residual ridge height.

All the bone osteotomy to access the sinuses was performed without any hemorrhagic complications. Sinus membrane perforations occurred in three clinical cases, all the perforations were smaller than 2 mm, even though the perforations were present the augmentation procedures were completed raising the sinus membrane and protecting the perforations with reabsorbable collagen barriers. One perforation occurred in the membrane group and two perforations in the nonmembrane group.

Dimensions of the osteotomy window showed no significant differences between test and control groups. The mean length of the osteotomy window (L) was $17.5 \pm 1.5$ mm in the membrane group (control) and $18.3 \pm 1.7$ mm in the nonmembrane group (test). The lateral bone wall thickness was $0.7 \pm 0.3$ mm in the membrane group (control) and $0.8 \pm 0.3$ mm in the nonmembrane group (test) (Table 1).

Four patients developed a mild hematoma which resolved gradually, two patients each group. A small area of soft tissue wound dehiscence occurred in two patients (one in each group) who did not need intervention and healed spontaneously by secondary intention.

The histological examination showed newly formed bone in all specimens. Residual graft particles were detected in all samples and were easily identified by their typical structure and by the presence of empty lacunae and separation lines. Where residual graft material was present, new bone and connective tissue were in direct contact with particles (Figs 5 and 6). Moreover, osteoblasts were found around particles in close contact with newly formed bone. Multicellular units with osteoclasts were present; no foreign body reaction and necrosis were observed (Fig. 7).

The histomorphometric measurements (Table 2) revealed that newly formed bone occupied a mean percentage of $30.7 \pm 15.5$% (median 30.4%) of the total volume.

Fig. 4. Flow diagram of a trial comparing membrane on the osteotomy window with no-membrane on the osteotomy window for maxillary sinus augmentation.

Fig. 5. Histologic section of a biopsy taken from the control group 6 months after augmentation. Corticocancellous porcine bone particles are surrounded by woven bone. The marrow spaces are rich in cells and blood vessels. Magnification: bar = 100 μm.

Fig. 6. Histologic section of a biopsy taken from the test group 6 months after augmentation. Residual porcine bone particles were covered with new bone, showing ongoing bone formation, i.e., osteoblastic seams. The soft tissues were without signs of inflammation and showing a high density of newly formed vessels. Magnification: bar = 100 μm.

Fig. 7. Light micrograph of a biopsy taken from the control group 6 months after augmentation. The interface between biomaterial granules and autologous bone was tight and interconnected. The section showed healthy maturing bone that is well vascularized. Magnification: bar = 20 μm.

**Table 1. Clinical parameters (mean and standard deviation) in the membrane (control) and nonmembrane group (test)**

<table>
<thead>
<tr>
<th>Clinical parameters</th>
<th>Test group (no membrane) (mean ± SD)</th>
<th>Control group (membrane) (mean ± SD)</th>
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<tbody>
<tr>
<td>Length of the osteotomy window (L)</td>
<td>$18.3 \pm 1.7$ mm</td>
<td>$17.5 \pm 1.5$ mm</td>
</tr>
<tr>
<td>Height of osteotomy window (H)</td>
<td>$9.9 \pm 1.3$ mm</td>
<td>$10.1 \pm 1$ mm</td>
</tr>
<tr>
<td>Lateral bone wall thickness</td>
<td>$0.8 \pm 0.3$ mm</td>
<td>$0.7 \pm 0.3$ mm</td>
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Assessed for eligibility (n = 21)

Excluded (n = 3); not meeting inclusion criteria (n = 2)

Other reasons (n = 1, impossibility to obtain adequate autologous bone)

Randomised (n = 18)

Allocated to the intervention (n = 9)

Received allocated intervention (n = 9)

Lost to follow up (n = 0)

Discontinued intervention (n = 0)

Analysed (n = 9)

Excluded from the analysis (n = 0)
in bone biopsies from the membrane group [control]. The average percentage of connective tissue was 50.6% ± 18.7% [59.2%] median, and residual graft percentage was 18.4% ± 20.3% [median 10.3%]. On the other hand, data regarding the nonmembrane group [test] showed that the percentage of newly formed bone was 28.1% ± 19.4% [median 19.2%]. The mean percentage of connective tissues was 59.3% ± 15.4% [median 55.7%] and 12.6% ± 12.4% [median 15.9%] for the residual graft particles.

No significant difference was detected in the histomorphometrical evaluation between the two groups (χ²: 3; n: 3; U: 5; P: 0.82726; Z = 0.218218).

### Discussion

A large number of systematic reviews have indicated that sinus floor augmentation is one of the most reliable procedures in preprosthetic surgery [Chiapas et al. 2006; Aghaloo & Moy 2007; Del Fabbro et al. 2008; Pjetursson et al. 2008; Tan et al. 2008] and, nowadays, this method is routinely used in the treatment of patients with severe atrophic posterior maxilla although there is a lack on of long term outcomes reports on this surgical procedures.

More and more studies have evaluated the source of osteogenesis in the grafted maxillary sinus. Boyne and Kruger first indicated the potential of the floor of the maxillary sinus to induce bone formation [Boyne & Kruger 1962]. Later, Boyne and James confirmed that elevation of the Schneiderian membrane could induce bone formation directly from the sinus floor [Boyne & James 1980]. Moreover, Misch first observed that the lateral wall of the sinus tend to induce bone formation when the membrane is reflected [Misch & Dietsh 1991]. Other studies validate such conclusions, showing the greatest bone formation in the lateral areas of the sinus with the least near the elevated Schneiderian membrane.

Finally, there is some evidence of bone formation from the sinus floor and lateral walls inwards, both in primates [Margolin et al. 1998; Scala et al. 2010] and humans [Boyne et al. 1997; Nevins & Fiorellini 1998].

Some authors have evaluated the outcome of sinus floor elevation not involving the use of barrier membranes and concluded that unfavorable healing in the lateral wall area was obtained [McAllister et al. 1998]. These studies reported that the main complications of the nonuse of a membrane were the graft particle displacement and/or the proliferation of connective tissue into the sinus cavity (encleflation). The presence of soft tissue displaces graft material thus preventing the formation of bone in this area. The absence of vital bone in the area may, in turn, have an adverse effect on implant survival due to the amount of bone available for osteointegration.

In this randomized clinical trial, histological and histomorphometrical analysis were used to assess the effectiveness of the use of membrane in lateral sinus augmentation procedures. Our results indicated that the percentage of vital bone in patients where a membrane was used was 30.7% ± 15.5%, while in the absence of membrane the amount of vital bone was 28.1% ± 19.4%. Moreover, sites without membrane presented 59.3% ± 15.4% of soft tissues and 12.6% ± 12.4% of the residual graft material, whereas sinuses covered by membrane showed 50.6% ± 18.7% of soft tissues and 18.4% ± 20.3% of residual graft material.

Although this data lacks statistical significance, it leads us to conclude that the use of a membrane to cover the lateral windows does not seem to greatly influence the increase of vital bone. These results suggest that the placement of membrane may reduce the formation of connective tissues and slow down the substitution of graft material. However, it should be stressed that many variables such as patient age, volume of the grafted area, height of the residual ridge, postoperative healing time, and, finally, type of biomaterial used, and harvesting of the biopsy may also influence such results.

Regarding the re-absorption of the biomaterial, several studies have reported that a large fraction of residual graft may remain in the area for years. Hallman et al. (2005) observed that on average 14.1% and 12.4% of residual graft can be retrieved in the same patients after 6 months and 3 years respectively [ref].

Mordenfeld et al. [2010] revealed that up to 17.3% of bovine bone can be detected in augmented sinuses after 11 years [ref]. Our study confirms previous papers which demonstrated the high capacity of re-absorption of collagenated porcine bone [Boyne et al. 2010]. Moreover, the present study research have showed that the re-absorption rate in sites without membrane is greater (2.2) than in sites with membrane (1.6), although test sites presented a percentage of vital bone tissue slightly higher. It is plausible that blood supply of maxillary sinus can play a role. In fact vascularization is derived primarily from the maxillary artery and to a lesser degree, from the anterior ethmoidal and superior labial arteries. As a consequence, the reduction of blood supply due to the membrane’s area may explain such a delay in the re-absorption of the biomaterial. Regarding vital bone formation, this study shows only slight differences between test and control sites. This data disagrees with the studies of Tarnow et al. [2000] who demonstrated a significant increase in the volume of vital bone in sites with membrane (25.5% on average of vital bone) in comparison with nonmembrane sites (11.9% on average of vital bone) using various biomaterials as grafts. Similar conclusions were drawn by Tarnow et al., who evaluated the implant failure in covered vs. noncovered augmented sinuses. They observed that the highest failure rate was found when implants were loaded 6–9 months postoperatively in sites without membrane if compared to sites with membrane. This suggests that healing may be more advanced in the deeper part of the graft as compared to the more superficial part when a membrane is used.

There are several strengths to our study. First of all, the randomization was carried out rigorously and, as a consequence, there were no baseline differences between the groups. In both intervention arms, identical surgical procedures, biomaterials and similar follow-up recalling were achieved. Finally, extreme care in biopsy harvesting was taken. It is well recognized that good biopsy technique is of great importance because the mineralization and degree of bone formation seem to depend on tissue depth and location, at least in the early phase of graft healing [Margolin et al. 1998; Artzi et al. 2005]. Nevertheless, our study also has its limitations: the sample size was not adequate and this fact may have jeopardized the statistical analysis; moreover, the heterogeneity of patients [large differences in age and residual bone volume] could have influenced the outcome of the interventions.

### Table 2. Histomorphometric measurements showing the mean values and variations in the membrane group and the nonmembrane group

<table>
<thead>
<tr>
<th>Histomorphometric measurements</th>
<th>Test group (no membrane)</th>
<th>Control group (membrane)</th>
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<tr>
<td></td>
<td>(mean ± SD, %)</td>
<td>(mean ± SD, %)</td>
</tr>
<tr>
<td></td>
<td>(median, %)</td>
<td>(median, %)</td>
</tr>
<tr>
<td>Newly formed bone</td>
<td>28.1 ± 19.4 (19.2)</td>
<td>30.7 ± 15.5 (30.4)</td>
</tr>
<tr>
<td>Connective tissue</td>
<td>59.3 ± 15.4 (55.7)</td>
<td>50.6 ± 18.7 (59.2)</td>
</tr>
<tr>
<td>Residual graft material</td>
<td>12.6 ± 12.4 (15.9)</td>
<td>18.4 ± 20.3 (10.3)</td>
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</table>
To our knowledge, this is the first pilot study based on a randomized trial which has analyzed the effectiveness of the placement of membrane on the lateral wall in sinus augmentation procedures. Our results showed that compared with sites which were not covered, the use of the membrane may increase the amount of vital bone slightly over a period of 6 months. On the other hand, the use of membrane seems to reduce the proliferation of the connective tissue and the graft re-absorption rate. Further studies are needed to explore whether the use of membrane could really be advantageous for the sinus augmentation procedure and to evaluate what influence this method can have on the amount and quality of reconstructed bone.

References


