Influence of a collagen membrane positioned subjacent the sinus mucosa following the elevation of the maxillary sinus. A histomorphometric study in rabbits

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University of Bern, Bern, Switzerland

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Abstract
Objective: To evaluate the healing after elevation of the sinus mucosa when a collagen membrane was placed between the sinus mucosa and a xenograft used as filler.

Materials and Methods: Eighteen rabbits were used. Sinus mucosa elevation was performed bilaterally, and a collagen membrane was applied subjacent to the sinus mucosa only at a randomly selected test site. At both sites, a collagenated corticocancellous porcine bone was placed within the elevated space and the access window was covered with a collagen membrane. The animals were sacrificed after 2, 4, and 8 weeks of healing, six animals for group. Ground sections were prepared.

Results: At the histomorphometric evaluation, the elevated area after 2 and 8 weeks was 11.8 and 8.8 mm² at the test, and 10.0 and 5.3 mm² at the control sites, respectively. The available area was obtained subtracting the remaining area occupied by the membrane from the elevated area and, after 8 weeks, was 6.7 ± 0.9 mm². After 8 weeks of healing, the mineralized new bone within the elevated space was 18.2 ± 5.5% at the test and 26.7 ± 7.7% at the control sites. Within the available space at the test site, the percentage was 27.3 ± 7.0% after 8 weeks of healing. At 2 and 8 weeks of healing, within the elevated space, the xenograft proportion was 30.9 ± 4.4% and 6.9 ± 2.8% at the test, and 35.2 ± 7.3% and 9.6 ± 4.9% at the control sites, respectively.

Conclusions: The placement of a collagen membrane subjacent the sinus mucosa did not reveal any major morphometric difference. The collagen membrane was not completely resorbed after 8 weeks.

Sinus floor elevation is widely applied to increase bone volume in the posterior maxillary regions to allow implant installment. In case of a perforation of the sinus mucosa, it has been suggested to apply resorbable collagen membranes in order to protect the perforation and, subsequently, to fill the elevated space with the selected biomaterials [Pikos 1999; Proussaeufs et al. 2003; Testori et al. 2008; Kim et al. 2014].

In experiments in sheep, the influence on healing of the placement of a collagen membrane subjacent the sinus mucosa was evaluated. The outcomes were studied at sites where a perforation of the sinus mucosa was created bilaterally [Favero et al. 2016] or at sites with no perforations of the mucosa [Scala et al. 2016]. In both experiments, a collagen membrane was placed only at the test sites, while control sites were left without membranes. No statistically significant differences in bone formation between test and control sites could be disclosed in both experiments. It was concluded that the placement of a collagen membrane subjacent the sinus mucosa did not influence the healing at elevated sinus.

Various biomaterials have been recommended to fill the elevated space in sinus floor elevation. In a recent review [Corbella et al. 2016], it was shown that autogenous bone alone produced a statistically significant higher amount of bone formation compared...
to bovine bone while, when bovine bone was added to the autogenous bone, the comparison with bovine bone alone did not yield significant differences. Bovine bone presented better results compared to hydroxyapatite, but worse outcomes when compared to a mixture of hydroxyapatite and tricalcium phosphate.

However, it is important to highlight the fact that autogenous bone undergoes resorption as demonstrated in animal experiments (Scala et al. 2015; De Santis et al. 2017). Deproteinized bovine bone mineral (DBBM) has also been widely used in a clinical setting as well as in animal experiments. In rabbits, DBBM was used for sinus mucosa elevation (e.g., Xu et al. 2004; Caneva et al. 2016; De Santis et al. 2017). It was shown that this biomaterial was very stable over time and underwent a very slow resorption.

A collagenated corticocancellous porcine bone (CCPB), prepared in such a way to preserve tissue collagen, was tested in self-contained defect in the maxilla of rabbits (e.g., Nanmark & Sennerby 2008). After 8 weeks of healing, new bone formation reached percentages of 43–44%, while the graft material was found at percentages of about 4–9%. A morphometric analysis performed in human biopsies collected 6 months after sinus floor elevation using again a collagenated corticocancellous porcine bone (Barone et al. 2012), and new bone was found at a percentage of about 44% and residual graft material at about 14%. The studies cited above reported a higher resorption rate of CCPB compared to the data reported on DBBM.

From the evidence provided here, it seems that there is the need for further information on the fate of a collagen membrane placed subjacent the sinus mucosa and the influence of this procedure on the healing after sinus lifting performed with a collagenated corticocancellous porcine bone. Moreover, a description of tissue reaction to a resorbable material may be also needed.

Hence, the aim of this study was to evaluate the healing after elevation of the sinus mucosa when a collagen membrane was placed between the sinus mucosa and a xenograft used as filler.

To describe histologically the healing events, it seemed appropriate from an ethical point of view using an animal model.

Material and methods

The experimental protocol was submitted to and approved by the local Ethics Committee of the Faculty of Dentistry of Ribeirão Preto, University of São Paulo, Brazil (2015.1.834.58.7). All experimental procedures were performed according the regulations for animal care in Brazil. The checklist of ARRIVE was compiled for the present article (Appendix S1).

Sample

Eighteen male New Zealand white rabbits, 3.5–4.0 kg of weight and 5–6 months old, were used and divided into three groups composed of six animals each, and sacrificed after 2, 4, and 8 weeks, respectively.

The present study was a split mouth and intervention study designed to be able to describe the healing process at three periods of observation.

Randomization and allocation concealment

The randomization for the selection of test/control sites was performed electronically (randomization.com) by an author neither involved in the selection of the rabbits nor in the surgical procedures (DB). The surgeon received the information about test/control sites after the elevation of the flaps and the opening of the lateral window access bilaterally.

Surgical procedures

An experienced surgeon (ECMN) performed all surgeries. A general anesthesia was carried out using acepromazine [1.0 mg/kg, Acepran®, Vetnil, Louveira, São Paulo, Brazil], administered subcutaneously before surgery. Subsequently, a mix of xylazine [3.0 mg/kg, Dpers®, Hertape Calier, Juatuba, Minas Gerais, Brazil] and 60 mg/kg ketamine [50.0 mg/kg, União Química Farmacêutica Nacional S/A, Embuaguará, São Paulo, Brazil] was injected i.m. Local anesthesia was also added in the experimental region.

The experimental area was shaved and disinfected, and an incision ~3.5 cm long was performed along the midline of the nasal dorsum. The flaps were elevated to expose the nasal bone, bilaterally of the nasal-incisal suture (Fig. 1a). Trephines were used to prepare an osteotomy of about 3.5 mm of diameter, both sides of the nasal dorsum, paying attention to locate the center of the osteotomy at about 1 cm in front of the apex of the nasal-frontal suture and about 0.4–0.5 cm laterally of the nasal dorsum suture (Fig. 1b). After elevation of the sinus mucosa, a small piece of equine collagen membrane (Evolution, 0.3 mm, OsteoBiol®, Tecnoss®, Giaveno, Italy), of dimensions exceeding those of

Fig. 1. Clinical views of the surgical procedures. [a] The flaps were elevated to expose the nasal bone, bilaterally of the nasal-incisal suture. [b] Trephines were used to prepare the osteotomies. [c] At the test site, a small piece of equine collagen membrane was placed subjacent the sinus mucosa. [d] A collagenated corticocancellous porcine bone was placed within the elevated space.
the access osteotomy window of about 1 mm each side, was placed subjacent the sinus mucosa at one site (Fig. 1c), according to the randomization [test site], while no membranes were placed within the vicinity at the control sites. With the use of an amalgam carrier [Amalgam gun curve 941, KERR GMBH, Rastatt, Germany], a similar amount of a collagenated corticocancellous porcine bone [Gen-Os®, 250–1000 µm, OsteoBiol®, Tecnoss®, Giaveno, Italy] was positioned within the elevated space [Fig. 1d]. As reference for the histological processing, a small screw was positioned in the naso–conchal suture at the level of the center of the two osteotomies and an equine collagen membrane [Evolution, 0.3 mm, OsteoBiol®, Tecnoss®] was placed bilaterally of the osteotomies. Resorbable sutures were provided to the peristomal layer [Poliglactyn 910 5-0, Vicryl®; Ethicon, Johnson & Johnson, São José dos Campos, Brazil], while for the skin nylon sutures were used [Ethilon 4-0®, Ethicon, Johnson & Johnson].

Oxytetracycline [Biovet, Vargem Grande Paulista, São Paulo, Brazil] 0.2 ml/kg per day was injected intraperitoneally for 4 days. Buprenorphine [0.02 mg/kg, Bupaq®, Richter Pharma AG, Wels, Austria] and profenid® (3.0 mg/kg, Ketojet, Agener Uniao, São Paulo, Brazil) were administrated i.m.

**Maintenance**

All rabbits were kept for the whole period of the experiment at the animal facilities of university in individual cages and in acclimatized room. A postoperative monitoring of the wound conditions and of the basic biological functions, such as feeding and excretion, was carried out.

**Sacrifice**

An overdose of sodium thiopental [1.0 g, 2 ml, Thiopentax, Cristália Produtos Químicos Farmacêuticos, Itapira, São Paulo, Brazil] was administered intravenously at the sacrifice. Biopsies representing the healing after 2, 4, and 8 weeks were obtained (n = 6 per period).

**Histological preparation**

The experimental region was dissected and fixed. The biopsies were first dehydrated and then imbedded in resin [LR White™ hard grid, London Resin Co Ltd, Berkshire, UK]. After polymerization, each biopsy was cut following a transverse plane in the center of the elevated zone, guided by the screw placed in the naso–incisal suture. Two central sections of about 100–150 µm were prepared using a precision slicing equipment [Exakt®, Apparatebau, Norderstedt, Germany], and then ground to a thickness of about 50–60 µm using a cutting-grinding machine [Exakt®, Apparatebau]. The sections were stained with either toluidine blue or Steve-nel’s blue and alizarin red.

**Histological evaluations**

The linear measurements were taken in three different locations, and a mean value was used. Area measurements were taken twice and a mean value was used. Before making the morphometric measurements, the examiner [KAAA] was calibrated by an expert [DB] in the recognition of the histological tissues. After training, the intra-examiner agreement according to the kappa statistic was substantial (about 0.89 varying from 0.83 to 0.96).

All measurements were performed with the software NIS-Elements D [v 4.0, Laboratory Imaging, Nikon Corporation]. For this purpose, an Eclipse Ci microscope [Nikon Corporation, Tokyo, Japan], equipped with a digital video camera [Digital Sight DS-2Mv, Nikon Corporation] connected to a computer was used.

The elevated area was defined as the area of the elevated region delimited by the inner outline of the sinus mucosa [epithelial layer and lamina propria], the original sinus bone walls and the inner outline of the osteotomy. The available area was defined as the elevated region without the region occupied by the membrane. Hence, the available area was obtained subtracting the area occupied by the collagen membrane [Membrane area] from the elevated area. At the control sites, the available area coincided with the elevated area.

The areas of the elevated space and that occupied by the membrane were measured at a magnification of ×20. The width of the membrane subjacent to the sinus mucosa and that of the membrane placed on the osteotomy was measured at three different positions, equidistant between them and the peripheral edges of the membrane, at a magnification of ×40. A mean value of the three measurements was calculated for each membrane.

The area occupied by biomaterial outside the osteotomy zone was also measured both at test and control sites at a magnification of ×40. The width of the sinus mucosa [epithelium and lamina propria with mixed glands; submucosal layer and periosteum were excluded] was measured at three sites in pristine areas of the sinus [pristine mucosa] and at three different positions, about equidistant between them and the peripheral edges of the elevated space, in the elevated regions [elevated mucosa] at a magnification of ×200. A mean value of the three measurements was obtained for both pristine and elevated mucosa.

The histomorphometric evaluation was performed in the various zones of the elevated sinus, that is, close to the medial and lateral bone walls, subjacent to the sinus mucosa and in the middle regions. Mean values were obtained for both the elevated area and the available area, for both test and control sites.

A point counting procedure was used to determine the tissue composition within each region [Schroeder & Münnzel-Pedrazzoli 1973]. For this aim, a lattice, with squares of 75 µm in dimensions, was superposed over the tissues at a magnification of ×100. Several lattices were used to examine all regions.

The direct connection of the computer to the microscope allowed to examine the regions at a higher magnification whenever was necessary. The percentages of new mineralized bone, marrow spaces [in various periods of maturation], dense matrix tissue, loose matrix tissue, connective tissue, inflammatory cell infiltrate, vessels, areas containing multinucleated units, biomaterial [Gen-Os], and collagen membrane were evaluated in each of the four different zones.

**Data analysis**

Mean values and 95% confidence interval [CI] were calculated for each outcome variable. The main outcome variables for histomorphometric measurements were the elevated and available areas while, for morphometric measurements, it was the percentage of new mineralized bone.

The software IBM SPSS Statistics [IBM Inc., Chicago, IL, USA] was used for testing analyses. The parametric t-test was performed to evaluate differences between the membrane sites [test] and the non-membrane sites [control]. The level of significance was set at 5%.

**Results**

During the surgical procedures, small perforations (<1 mm) occurred at one test site in the 8-week group, and one test and one control sites in the 4-week group. The perforation at
the control site was left untreated. None of the animal showed complications during the healing period. All histological slides were available for histological examination, and hence, an n = 6 was obtained for all periods of examination, that is, 2, 4, and 8 weeks of healing.

Mean values ± standard deviation are reported in the text. In the Tables 1-3, mean values, mean differences, confidence intervals and P values are reported.

### Histometric evaluation

The elevated area decreased progressively during healing both at test and control sites. At the test sites, this area was 11.8 ± 2.2, 12.3 ± 1.5, and 8.8 ± 1.5 mm² after 2, 4, and 8 weeks, respectively [Figs 2a-c and 3a-c]. The shrinkage between 2 and 8 weeks was about 25%. At the control sites, the respective values were 10.0 ± 1.4, 7.0 ± 1.0, and 5.3 ± 1.3 mm². The shrinkage between 2 and 8 weeks was about 47% [Fig. 4]. The differences between test and control sites were statistically significant at 4- and 8-week healing periods.

At the test sites, the collagen membrane subjacent to the sinus mucosa appeared in many instances wavy or partly folded at the 2-week observation [Fig. 2a], occupying an area of 3.3 ± 1.4 mm² and representing about 28% of the total area. At the 4-week period, the area increased compared to the 2-week period of healing, being 3.9 ± 1.0 mm² and representing about 32% of the total area. In some cases, the collagen membrane appeared as a uniform mass, with some inflammatory cell infiltrate and signs of degradation while, in other specimens, it was folded and displacing the biomaterial [Fig 2b]. After 8 weeks of healing, resorptive processes were evident, resulting in a shrinkage of the volume of the membrane to 2.0 ± 1.7 mm² only. This represented about 22% of the total area [Fig. 2c].

The available area at the test sites was 8.5 ± 2.1, 8.4 ± 1.6, and 6.7 ± 0.9 mm², after the 2-, 4-, and 8-week period of healing. The shrinkage between 2 and 8 weeks was about 21%. The available area at the test sites after 2 weeks of healing was lower compared to the total area at the control sites, and the difference was statistically significant. However, at the 4- and 8-week periods, the available area was statistically significantly higher at the test sites compared with the control sites.

### Table 1. Tissue components after 2 weeks of healing

<table>
<thead>
<tr>
<th>Variable</th>
<th>Area</th>
<th>Test site</th>
<th>Control site</th>
<th>Mean difference</th>
<th>Lower 95%</th>
<th>Upper 95%</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mineralized bone</td>
<td>Available</td>
<td>8.90</td>
<td>6.17</td>
<td>−2.73</td>
<td>−6.42</td>
<td>0.95</td>
<td>0.1147</td>
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<td>Elevated</td>
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<td>6.17</td>
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<td>−4.48</td>
<td>2.71</td>
<td>0.5551</td>
</tr>
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<td>0.85</td>
<td>0.43</td>
<td>−0.42</td>
<td>−1.10</td>
<td>0.27</td>
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</tr>
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<td>Elevated</td>
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<td>−0.48</td>
<td>0.35</td>
<td>0.6952</td>
</tr>
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<td>−14.99</td>
<td>5.43</td>
<td>0.2823</td>
</tr>
<tr>
<td></td>
<td>Elevated</td>
<td>20.67</td>
<td>19.53</td>
<td>−1.13</td>
<td>−9.21</td>
<td>6.93</td>
<td>0.7330</td>
</tr>
<tr>
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<td></td>
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<td>3.08</td>
<td>4.92</td>
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<td>4.44</td>
<td>0.1309</td>
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Bold value indicates P <0.05.

### Table 2. Tissue components after 4 weeks of healing

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<th>Variable</th>
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<th>Test site</th>
<th>Control site</th>
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<th>Lower 95%</th>
<th>Upper 95%</th>
<th>P-value</th>
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<td>−23.57</td>
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</table>

Bold value indicates P <0.05.
The original width of the collagen membrane used in the present study was 0.3 mm, as declared by the manufacturer. The width of the membrane placed below the sinus mucosa was thicker compared to the original dimensions, being 0.6 ± 0.1, 0.8 ± 0.2, and 0.5 ± 0.2 mm after 2, 4, and 8 weeks, respectively. A similar trend was observed for the membranes placed on the osteotomy window of the test (0.5 ± 0.1, 0.7 ± 0.1, and 0.3 ± 0.1 mm) and of the control sites (0.5 ± 0.1, 0.7 ± 0.2, and 0.3 ± 0.1 mm) after 2, 4, and 8 weeks of healing.

Table 3. Tissue components after 8 weeks of healing

<table>
<thead>
<tr>
<th>Variable</th>
<th>Area</th>
<th>Test site Mean difference</th>
<th>Lower 95%</th>
<th>Upper 95%</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mineralized bone</td>
<td>Available</td>
<td>27.28</td>
<td>26.72</td>
<td>–0.57</td>
<td>–11.19</td>
</tr>
<tr>
<td></td>
<td>Elevated</td>
<td>18.22</td>
<td>26.72</td>
<td>8.50</td>
<td>–1.49</td>
</tr>
<tr>
<td>Marrow spaces</td>
<td>Available</td>
<td>19.43</td>
<td>28.42</td>
<td>8.98</td>
<td>–9.48</td>
</tr>
<tr>
<td></td>
<td>Elevated</td>
<td>11.67</td>
<td>28.42</td>
<td>16.75</td>
<td>–1.95</td>
</tr>
<tr>
<td></td>
<td>Elevated</td>
<td>15.65</td>
<td>14.97</td>
<td>–0.68</td>
<td>–18.54</td>
</tr>
<tr>
<td>Dense matrix</td>
<td>Available</td>
<td>16.33</td>
<td>15.12</td>
<td>–1.22</td>
<td>–7.61</td>
</tr>
<tr>
<td></td>
<td>Elevated</td>
<td>13.98</td>
<td>15.12</td>
<td>1.13</td>
<td>–7.01</td>
</tr>
<tr>
<td>Gen-Os</td>
<td>Available</td>
<td>7.85</td>
<td>9.62</td>
<td>1.77</td>
<td>–2.55</td>
</tr>
<tr>
<td></td>
<td>Elevated</td>
<td>6.88</td>
<td>9.62</td>
<td>2.73</td>
<td>–2.02</td>
</tr>
<tr>
<td>Membrane</td>
<td>Available</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Elevated</td>
<td>24.52</td>
<td>0.00</td>
<td>–24.52</td>
<td>–36.74</td>
</tr>
<tr>
<td>PMN</td>
<td>Available</td>
<td>2.18</td>
<td>0.00</td>
<td>–2.18</td>
<td>–5.75</td>
</tr>
<tr>
<td></td>
<td>Elevated</td>
<td>4.82</td>
<td>0.00</td>
<td>–4.82</td>
<td>–10.32</td>
</tr>
<tr>
<td>Vessels</td>
<td>Available</td>
<td>5.05</td>
<td>4.33</td>
<td>–0.72</td>
<td>–4.89</td>
</tr>
<tr>
<td></td>
<td>Elevated</td>
<td>3.75</td>
<td>4.33</td>
<td>0.58</td>
<td>–2.77</td>
</tr>
<tr>
<td>Multinucleated cell zone</td>
<td>Available</td>
<td>0.70</td>
<td>0.83</td>
<td>0.13</td>
<td>–0.15</td>
</tr>
<tr>
<td></td>
<td>Elevated</td>
<td>0.48</td>
<td>0.83</td>
<td>0.35</td>
<td>–0.22</td>
</tr>
</tbody>
</table>

Bold value indicates P < 0.05.

Fig. 2. Photomicrographs of ground sections of the test sites after [a] 2, [b] 4, and [c] 8 weeks of healing. Bone formation appeared to start from the bone walls of the sinus. The membranes were often found folded. A shrinkage of the available area was noted. Stevenel’s blue and alizarin red stain. Original magnification ×20.

Fig. 3. Photomicrographs of ground sections of the control sites after [a] 2, [b] 4, and [c] 8 weeks of healing. New bone was found forming from the bone walls of the sinus. A shrinkage of the elevated area was observed. Stevenel’s blue and alizarin red stain. Original magnification ×20.
8 weeks, respectively. No statistically significant differences were found between test and control sites.

The areas occupied by the biomaterial outside the osteotomy region were 1.0 \( / \) \( C_6 \), 0.8 \( / \) \( C_6 \), 0.6 \( / \) \( C_6 \), and 0.0 \( / \) \( C_6 \) after 2, 4, and 8 weeks of healing. The respective values at the control sites were 0.6 \( / \) \( C_6 \), 0.5 \( / \) \( C_6 \), 0.4 \( / \) \( C_6 \), and 0.3 \( / \) \( C_6 \) mm\(^2\). None of the differences between test and control sites reached statistical significance.

The width of the pristine sinus mucosa at the test sites was 71 \( / \) \( C_6 \), 89 \( / \) \( C_6 \), 84 \( / \) \( C_6 \) \( \mu \)m at 2, 4, and 8 weeks, respectively, while at the control sites, it was 74 \( / \) \( C_6 \), 76 \( / \) \( C_6 \), and 79 \( / \) \( C_6 \) \( \mu \)m, respectively. None of the differences between test and control sites was statistically significant.

The mean values of the width of the pristine sinus mucosa among the various measurements were 81 \( / \) \( C_6 \) and 77 \( / \) \( C_6 \) \( \mu \)m at the test and control sites, respectively.

The width of the elevated mucosa was 91 \( / \) \( C_6 \), 114 \( / \) \( C_6 \), 182 \( / \) \( C_6 \) \( \mu \)m at the test and 99 \( / \) \( C_6 \), 116 \( / \) \( C_6 \), and 176 \( / \) \( C_6 \) \( \mu \)m at the control sites after 2, 4, and 8 weeks of healing. The increased dimensions were ascribed to the lamina propria that presented a clear proliferation of the glands compared to the pristine mucosa [Fig. 5a,b]. The differences between test and control sites did not reach statistical significance in any of the periods evaluated. However, the differences between the width of the pristine sinus mucosa and that of the elevated sinus mucosa were statistically significant for all periods of healing for both test and control sites.

Morphometric evaluation

2-week healing
After 2 weeks of healing, little new mineralized bone was found in the elevated region at both the test (7.0 \( \pm \) 2.7\%) and the control sites (6.2 \( \pm \) 2.7\%). Within the available region at the test site, mineralized bone reached 8.9 \( \pm \) 3.2\% [Fig. 6]. The zone with the higher bone formation was that close to bone walls [Fig. 7a]. Small percentages of marrow spaces (<1.0\%) were seen at this stage of healing at both sites. In the elevated region, the grafting material was present at percentages of 30.9 \( \pm \) 4.4\% at the test and 35.2 \( \pm \) 7.3\% at the control sites. The biomaterial was mainly embedded into a matrix rich in fibroblast-like cells that presented two tissues of different density in cells and fibers. The dense matrix tissue was surrounding the xenograft particles and it was in close contact with them, while the loose matrix tissue presented a stromal-like aspect and it was interposed among the particles of the biomaterial [Fig. 7b]. The percentage of the dense matrix tissue in the elevated region was 23.3 \( \pm \) 3.3\% and 29.0 \( \pm \) 4.6\% and that of the loose matrix tissue was 20.7 \( \pm \) 5.3\% and 19.5 \( \pm \) 5.0\% at the test and control sites, respectively. Mineralized bone was found in contact with the surface of some particles close to the bone walls [Fig. 7c]. Several
Multinucleated cells were observed encompassing the particles of the graft at percentages of 3.1 ± 1.7% and 4.9 ± 3.0% at the test and control sites, respectively (Fig. 7d). Vessels were present at percentage of ~4% at both groups, while inflammatory cell infiltrate was rarely found (<0.5%). The collagen membrane occupied about 10.0 ± 3.5% of the elevated region and it was occupying mainly the zone subjacent the sinus mucosa. Considering the whole elevated area, only the differences of dense matrix and collagen membrane were statistically significant between test and control sites.

4-week healing

After 4 weeks of healing, in the elevated region, new mineralized bone and total new bone increased in percentages to 11.6 ± 11.3% and 15.8 ± 15.5% at the test, and 11.7 ± 6.4% and 16.6 ± 12.7% at the control sites, respectively. Within the available region at the test site, the respective values were 19.8 ± 19.0% and 27.6 ± 26.7% (Fig. 6). New bone and marrow spaces were found especially close to the bone walls zones, both at the test and at the control sites, often in contact with graft particles (Fig. 8a). Lower amounts of new bone were seen in the regions located in the center of the elevated region, while very little bone was found underneath the sinus mucosa region at both test and control sites. None of the differences was statistically significant. The xenograft could be often found in contact with newly formed bone (Fig. 8b) and it decreased to about half of the percentage found in the 2-week period, the percentage reaching within the elevated region 14.6 ± 7.0% and 18.2 ± 4.9% at the test and control sites, respectively. Particles were still surrounded by a dense matrix tissue, separated by a loose matrix tissue that was interposed among the particles (Fig. 8c). The respective percentages were 16.1 ± 4.4% and 15.1 ± 5.6% at the test, and 27.0 ± 6.0% and 28.2 ± 6.7% at the control sites.

Zones containing multinucleated cells were still found around the xenograft material at percentages of about 2–4% at both groups (Fig. 8c). Inflammatory cell infiltrates were absent at the control sites while, at the test sites, it was found to be 8.5%, located predominantly in the submucosa zones. Vessels were present with approximately 3% at the test and 6% at the control sites. The collagen membrane presented zones of degradation and was occupying 24.2 ± 10.7% of the elevated area in close vicinity of the sinus mucosa (Fig. 8d). Considering the whole elevated region, statistically significant differences were found for dense and loose matrix and for membrane and inflammatory cell infiltrate while no statistically significant differences were found within the available region.
Vessels were represented at percentages of about 4% at both test and control sites. The collagen membrane at the test sites was still present at percentage of 24.5 ± 11.6%, located mainly in the submucosa region and presenting clear signs of degradation (Fig. 9c,d).

Discussion

The aim of the present analysis was to evaluate the tissue composition during healing at sinus mucosa elevation sites using a collagenated corticocancellous porcine bone and an internally placed collagen membrane subjacent to the sinus mucosa. For this purpose, at the test sites, a collagen membrane was placed below the sinus mucosa after elevation while the other sites were left without a membrane.

The elevated area was reduced between 2 and 8 weeks of healing by about 25% at the test and 47% at the control sites. A large number of studies have shown that, after elevation of the sinus mucosa, the gain in volume within the sinus will be lost if no procedures will be applied to maintain the elevated space. For instance, in a study in rabbits (Caneva et al. 2016), the area at the DBBM sites was maintained throughout the entire duration of the experiment while, at the sites filled with a collagen sponge, the area found after 7 days was reduced to 25% of its original dimensions after 21 days.

In another experiment of sinus floor elevation in minipigs (Scala et al. 2015), the space filled with autogenous bone lost height from 8.9 to 3.9 mm between 15 and 180 days of healing. It was concluded that the shrinkage of the elevated space was a consequence of the resorption of the autogenous bone. In the present study, the biomaterial used underwent resorption as well and this resulted in a partial re-pneumatization of the sinus cavity, especially at the control sites. At the test sites, the shrinkage was reduced by the presence of the collagen membrane that was not yet resorbed completely at the 8-week period, even though its degradation had been initiated. However, the available area was also slightly higher compared to the control sites. This, in turn, means that the collagen membrane contributed to maintain the available area as well.

The membrane subjacent to the sinus mucosa appeared to be wavy or partly folded already after 2 weeks of healing. Similar patterns were also seen in the subsequent periods of healing. This aspect may be partly owing to the larger dimensions of the collagen membrane in respect of the elevated mucosa that may have occurred when it was trimmed during surgical procedures. However, the reason may be also related to the faster resorption of the bone substitute compared to that of the collagen membrane and the subsequent collapse of the area.

The original thickness of the collagen membrane reported in the present study was 0.3 mm. However, the thickness increased in dimensions almost to the double after 2 weeks and reached 0.7-0.8 mm after 4 weeks. Finally, it was reduced after 8 weeks due to the degradation process of the membrane.

In the present study, biomaterial was found outside the osteotomy at both test and control sites, ranging between 1.0 and 0.6 mm² after 2 weeks. This may be due to an overfilling of the sinus performed by the surgeon or to a migration of the biomaterial through the window due to the internal pressure within the sinus. The migration of the material through the osteotomy after sinus floor elevation has been recently disclosed in a human study using CBCT methodology (Nosaka et al. 2015).

The width of the pristine sinus mucosa ranged between 71 and 89 μm. The width
was found thicker at the elevated mucosa where it reached at both groups 91–99 µm after 2 weeks, and 176–182 µm after 8 weeks. The differences in dimensions between the pristine and the elevated mucosa were statistically significant. It was observed that the increase in the width was in the lamina propria in which a proliferation of the glandular structures had occurred.

Morphometric analyses were performed to analyze the proportional impact of the various tissue components in different locations of the elevated area. From the results of the present study, new bone appeared to form from the native bone of the sinus walls and then propagated toward the middle and the submucosa regions. When new bone was seen below the sinus mucosa, it appeared to be mainly in continuity with bone formed from the sinus bone walls. The fact that the sinus mucosa did not clearly actively participate in bone formation, at least in the early phases of healing, is in agreement with another animal study (Scala et al. 2010, 2012). In that experiment in monkeys, it was shown that the space created after the elevation of the mucosa shrank progressively during 30 days of healing so that the mucosa collapsed onto the implant body. Bone was found to form from the parent bone while the sinus mucosa did not show evidence of bone induction in this early phase of healing. It was previously demonstrated that the sinus mucosa may have the potential to generate new bone (Gruber et al. 2004; Srouji et al. 2009, 2010, 2013). In contrast, the results from the present in vivo study appear not to support this notion.

Comparing the data obtained from the whole elevated region, it appeared that bone formation proceeded faster at the control compared to the test sites and that the application of an internally placed collagenous membrane impinging on the healing process of the space created by the sinus elevation, with the exception of the area close to the lateral bony walls. However, it has to be considered that a large portion of the elevated region was still occupied by the collagen membrane placed subjacent the sinus mucosa, especially in the submucosa area. Conversely, comparing the data obtained from the available region, that is the area that excluded the membrane, and in which new bone had chances to be formed, similar values and patterns of healing were seen for both new mineralized and total bone. This, in turn, means that the collagen membrane placed below the sinus mucosa did not jeopardize the healing within the elevated area.

This outcome from the present study is in agreement with that of experimental studies previously mentioned, in which membranes were placed subjacent to the sinus mucosa in sheep [Favero et al. 2016; Scala et al. 2016]. In both studies, it was concluded that no clear evidence could be unveiled for the participation of the sinus mucosa in bone formation.

The biomaterial used in the present study at the 2-week period ranged between 31% and 35%, and decreased to about 7–10% at the 8-week period in the elevated region. The resorptive processes occurring during the healing were substantiated by the presence of a high number of zones occupied by multinucleated cells located in Howship’s lacunae that were surrounding the particles of biomaterial, especially at the 2- and 4-week periods. The resorptive process encountered in the present study had similar aspects to that described for autogenous bone particles used for sinus floor elevation in minipigs [Scala et al. 2015]. In that experiment, after 15 days of healing, very little amount of bone particles were found (>10%). The particles, characterized by empty lacunae, were partly integrated into new bone or surrounded by several osteoclasts, denoting the presence of active resorptive processes. Bone particles almost disappeared in the following periods of healing.

In the present study, particles of biomaterial were surrounded by a dense matrix tissue, while a loose matrix was found interspersed among the particles. This finding was previously described in another study in rabbits [Caneva et al. 2016] in which a dense matrix was found surrounding the DBBM particles and embedded into a loose matrix. It was further shown that during healing, the dense matrix became progressively mineralized so that the DBBM material could be integrated into new bone. Conversely, the loose matrix was transformed progressively into marrow spaces. This, in turn, means that the DBBM material ensured an osteoconductive activity that allowed over time bone apposition around the biomaterial within the elevated space. Nevertheless, after 8 weeks of healing, some areas were still devoid of newly formed bone, especially within the medial zone and underneath the sinus mucosa. This means that a longer period of healing may be necessary when DBBM is used for bone augmentation, as postulated in previous studies [Stavropoulos et al. 2001; Donos et al. 2004; Calciolari et al. 2016].

However, in the present study, the particles were progressively resorbed by osteoclasts, so that the biomaterial could express its osteoconductivity only in the early phases of healing.

Small areas of degradation of collagen membranes were seen after 4 weeks even though this process became more evident after 8 weeks of healing. However, after 8 weeks of healing, the degradation was more evident even though still a large amount of membrane residues was still present. Hence, it may be speculated that the observation period of 8 weeks was too short to complete the resorptive processes of the internally placed collagenous membranes in the present study.

In the present study, a high content of vessels was found. This is in agreement with an in vitro study that showed high rates of endothelial cell proliferation and capillary-like structures formation in cultures with Gen-Os [Rombouts et al. 2016].

In conclusion, the morphometric analyses of the healing in the elevated region after sinus membrane elevation were very similar when an internal collagenous membrane was placed as without the placement of such a device. Likewise, the healing process in the elevated region appeared to be largely unaffected by the application of an internal collagenous membrane.

From a clinical point of view, even though the placement of a collagen membrane below the sinus mucosa does not create negative effect on healing, the use of this material in absence of perforations after sinus elevation should be considered a useless extra-cost procedure.

Moreover, it has to be realized that the time for the resorption of such a membrane will require a prolonged time. Furthermore, the inference of the results from the present animal study to similar clinical situation in human has to be carefully applied given that it is known that the healing in phylogenetically lower animals is faster than in human. Similar studies in human should be performed to confirm the hypothesis claimed in the present animal model.

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References


Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. The ARRIVE Guidelines Checklist.