Comparison of two different absorbable membranes for the coverage of lateral osteotomy sites in maxillary sinus augmentation: A preliminary study

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Abstract
Introduction: Barrier membranes, both absorbable and non-absorbable, have been used in sinus augmentation for many years. Some years ago, a new autologous blood substrate called Platelet-Rich-Fibrin (PRF) was introduced, and to date, the supporting effect on bone regeneration has been controversial. This study aimed to evaluate the effect of PRF on bone regeneration when used as a barrier membrane at the lateral osteotomy site in sinus augmentation.

Material and methods: Twelve sinuses from six patients requiring bilateral sinus floor augmentation were treated with a two-stage surgical technique using sinus augmentation and implant placement after 5 months. The sinuses were grafted with autologous bone and bone-substitute material (Bio-Oss®/C210) mixed in a 1:1 ratio and were covered in a randomized split-mouth design with a PRF or a conventional collagen membrane (Bio-Gide®/C210), respectively. Five months later threaded titanium dental implants were inserted and bone specimens harvested with a trephine burr were evaluated histomorphometrically.

Results: Bone quality seemed to be equal at both sites of the grafted sinuses. Mean vital bone formation after 5 months was 17.0% and 17.2%, for the PRF and collagen sites, respectively. The mean of residual bone-substitute was 15.9% and 17.3% for PRF and collagen, respectively. No local complications, such as dehiscences or membrane exposures, were detected at either site in any of the treated patients. After 12 months all implants reached primary stability in the augmented maxillary sinus floor without any peri-implant tissue inflammation.

Conclusions: Within the limits of the study the coverage of the lateral sinus window with two different absorbable membranes has been shown to result in a similar amount of vital bone formation and residual bone-substitute.

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1. Introduction

Resorption of the upper jaw bone after tooth loss or trauma frequently leads to problems in dental implant placement caused by a lack of sufficient bone mass. Thus, maxillary sinus floor augmentation, first described by Tatum (1977), and first published by Boyne and James (1980), is often necessary to achieve implant placement in an appropriate prosthetic position, especially in the edentulous posterior maxilla. For a long time, the gold standard for sinus floor augmentation procedures has been autologous bone transplants due to their osteoinductive and osteoconductive properties (Cordaro, 2003). The main disadvantages are donor site morbidity, size restriction, and the resorption of bone transplants (van den Bergh et al., 1998). To overcome these drawbacks, currently bone-substitute materials are widely accepted as additional or replacement materials in bone augmentation procedures.

The most important criterion for the success of sinus floor augmentation is the extent of mineralization which supports the stable insertion of dental implants in the case of undisturbed bone graft healing (Lioubavina-Hack et al., 2006). To ensure graft stabilization and to prevent the invasion of soft tissue, barrier membranes, both absorbable and non-absorbable, are normally used to cover the buccal fenestration at the osteotomy site (Avera et al., 1997). In recent years, absorbable membranes have been

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preferred since non-absorbable membranes have to be removed and often lead to complications such as dehiscences of the wound-edges. An in vitro comparison of bioabsorbable and non-absorbable membranes has shown that collagen membranes particularly promote bone regeneration through their activity on osteoblasts (Marinucci et al., 2001).

The first autologous bioabsorbable membrane is Platelet-Rich-Fibrin (PRF), a second generation platelet concentrate first described by Choukroun et al. (2001). Its essence is a fibrin matrix in which the platelets, cytokines and cells are trapped and may be released after a certain time (Mossesson, 2005). These cytokines have been shown to stimulate the mitogenic response of the periosteum during the early stage of bone repair (Gruber et al., 2003) and in general are strongly associated with the bone healing process (Lind, 1998; Metzler et al., 2012). The use of PRF in sinus floor procedures as an addition to bone graft materials has also been successfully proven (Zhang et al., 2012). It is known that the healing of bone autografts is supported by the periosteum and especially by the differentiation of mesenchymal stem cells (MSC) from the inner cambium layer into bone cells (Kostopoulos and Karring, 1995; Stevenson, 1999; Stockmann et al., 2012). In a previous in vitro study we investigated two different membrane types (PRF and collagen) as a matrix for periosteal tissue engineering and found that PRF leads to a significantly higher amount of periosteal cell proliferation (Gassling et al., 2010).

The aim of this study was to evaluate the effect of two different absorbable membranes, PRF and Bio-Gide®, on the vital bone formation of bone autografts in sinus augmentation when covering the lateral osteotomy site.

2. Material and methods

2.1. Patients

Six healthy patients requiring bilateral maxillary sinus floor augmentation were included in this controlled study. The mean age of the patients at primary surgery was 61 years (range 54–69 years). Patients were enrolled between 2010 and 2011 from the outpatient department of our clinic. To participate in this study, each subject had to give written informed consent. All patients were treated using a two-stage surgical technique with bilateral maxillary sinus floor augmentation and implant placement after 5 months due to a residual bone height of less than 5 mm. The sinuses were grafted with a mixture of autologous bone and bone-substitute material mixed in a 1:1 ratio. The buccal window was covered by a randomized split-mouth design with an absorbable single button sutures (4/0) were placed. The clinical photographs illustrating the application of collagen and PRF membrane are shown in Figs. 2 and 3. Penicillin (Unacid PD®, 3 × 375 mg/day, Pfizer GmbH, Berlin, Germany) was prescribed for 1 week and analgesics (Ibuprofen 400 mg, Pfizer GmbH, Berlin, Germany) were administered as and when required. The sutures

2.2. Bone-substitute material

Bio-Oss® is a xenogenic bone-substitute consisting of sterilized anorganic bovine bone in the form of granules with a particle size of 0.25–2.0 mm.

2.3. Membranes

2.3.1. Collagen membrane

A conventionally used non-cross-linked collagen membrane, i.e. Bio-Gide® (porcine collagen types I and III), was used for the covering of the buccal window of the maxillary sinuses.

2.3.2. PRF membrane

The PRF membranes were prepared according to the method of Dohan et al. (2006a). Patients donated 40 ml of blood in 10 ml tubes without anticoagulant (Vacuette 455092, Greiner Bio-One GmbH, Frickenhausen, Germany). Blood samples were immediately centrifuged for 12 min at 400×g. After activation of the coagulation cascade by contact of the blood platelets with the tube walls, a fibrin clot was obtained in the middle between the platelet poor plasma at the top and red blood cells at the bottom of the receptacle. The different steps of the PRF membrane preparation are shown in Fig. 1.

2.4. Surgical technique

All operations were performed by one surgeon with long-standing experience of this technique. Patients were treated under local anaesthesia. Sinus floor augmentation was performed by creating a small buccal window in the lateral wall of the maxillary sinus, using a round diamond bur under sterile saline irrigation. The Schneiderian membrane was separated from the inner surface of the maxillary sinus with curets of different shapes until the lateral and inferior sinus walls were completely detached.

A corticocancellous mandibular ramus graft obtained from the linea obliqua was milled (R. Quetin Bone-Mill, H.C. Grosse GmbH & Co. KG, Daldorf, Germany) and mixed with bone-substitute material in a 1:1 ratio according to the method of Clavero and Lundgren, 2003. Then, 2 ml of blood was added and the mixture was carefully placed in the sinus cavity. An absorbable membrane, collagen or PRF, was placed against the packed maxillary sinus windows. The decision as to whether to place PRF on the left and collagen on the right or collagen on the left and PRF on the right was randomized by envelope. After replacing the mucoperiosteal flap, several non-absorbable single button sutures (4/0) were placed. The clinical photographs illustrating the application of collagen and PRF membrane are shown in Figs. 2 and 3.

Fig. 1. Photographs of PRF membrane preparation: (a) fibrin clot in the middle of the tube, between the red blood cells at the bottom and platelet poor plasma at the top; (b) stable fibrin clot adherent to the red blood cells; (c) separation of PRF clot from the red blood cells using a sterile syringe and scissors; (d) resistant autologous PRF membrane after squeezing out serum.
were removed 1 week later. Five months later, threaded titanium implants were inserted into the augmented sinuses. At that moment, a specimen of the graft was harvested from the lateral wall using a 3.5/10 mm diameter trephine (Hager & Meisinger GmbH, Neuss, Germany) and evaluated histologically, observer-blinded. A schematic drawing describing the harvesting of specimens is shown in Fig. 4. A total of 32 titanium implants were inserted, whereby all were placed in free-end situations. The prosthetic restoration took place 5 months after implant placement and thus followed a delayed loading protocol. The prerequisites for this are stable implants and the absence of any peri-implant inflammation. Finally, removable partial dentures were incorporated in all patients. The inserted implants were evaluated 1 year after placement by panoramic X-rays. Bleeding, mucosal recessions, and probing depth were also measured at four positions (mesial, distal, vestibular, and oral). An increased probing depth over 3 mm in connection with bleeding on probing was interpreted as peri-implant tissue inflammation.

2.5. Specimen processing

Specimen processing was carried out as described by Roldan et al. (2004) with minor modifications. Primary specimens were fixed in a 4% buffered formalin–glutaraldehyde solution. The specimens were then prepared for polymerization using automated embedding for 42 h (Pool of Scientific Instruments Ltd.; Type 1.412.00, PSI Grünenthal GmbH & Co. KG, Laudenbach, Germany). The polymerization was performed in embedding glasses at 38 °C in a water bath. After polymerization, tissue blocks were cut into shape with a diamond band-saw (Metabowerke GmbH, Wiesmoor, Germany) and fixed on slides. One approximately 150 μm thick slice of the tissue block was cut out of the middle of the block in longitudinal direction of the trephine bur using a diamond-coated pendulum saw (Exakt GmbH, Norderstedt, Germany). Afterwards, the slice was ground and polished down to a final thickness of 40 μm using a wet grinding device (DP-U4®, Struers GmbH, Erkrath, Germany).

Pre-treatment of the toluidine blue staining was performed by etching with 0.1% formic acid for 4 min, rinsing with 20% methanol for 2 h and staining with 1% toluidine blue (1% toluidine blue and 1% sodium tetraborate in Aqua dest.) for 4 min. The saw-ground preparations were evaluated histomorphometrically after digitalization of the microscopic images. The software used permitted evaluation according to the degree of strength of the staining (Q500MC®, Leach Cambridge Ltd., Cambridge, England), which allowed a distinction between the bone-substitute and the newly mineralized bone. The amount of bone-substitute material and newly mineralized bone are expressed in percent of the total area evaluated. The primary outcome of the present study is the vital bone formation.

2.6. Statistical analysis

The statistical analysis was performed with the statistical programming language R, version 2.13.1. Wilcoxon Signed-Rank test was chosen with respect to the uncertainty of the distribution caused by the small number of values. However, the Wilcoxon Signed-Rank test was not able to prove a significant difference in the amount of vital bone in PRF or the collagen membrane. Afterwards, we conducted a linear regression of the bone ratio in dependency of the patient, the amount of bone-substitute material and the membrane type. Stepwise parameter reduction, performed according to the Akaike information criterion (Akaike, 1972), first removed the factors amount of bone-substitute material and
membrane type. This suggests that differences between the patients contribute to the amount of vital bone to a larger extent than the amount of bone-substitute material and the membrane type.

3. Results

3.1. Wound healing period

The wound had completely healed after 7 days when the sutures were removed. No dehiscences or membrane exposures could be detected at any site in any of the treated patients. Between primary and secondary surgeries no local or systemically adverse events were observed.

3.2. Clinical observations at implant placement

Five months after sinus floor elevation, threaded titanium implants were inserted into the augmented sinuses. During preparation of the implant bed, clinically no differences of bone quality could be observed at both sites. Primary stability was achieved in all implants. At the former lateral osteotomy site no soft tissue ingrowth was seen. The surfaces seem to be homogeneous with the visible bone-substitute material embedded in the newly formed bone at both sites. All graft specimens harvested from the lateral walls showed a dense character.

3.3. Histomorphometry

The graft specimens were evaluated for the percentage of vital bone, and the percentage of residual graft material. The histomorphometric examination of specimens revealed vital bone of a woven type adjacent to the bone-substitute particles in all samples. A representative sample of the collagen site and PRF site is shown in Figs. 5 and 6, respectively. At the site covered with a PRF membrane the average amount of vital bone was 17.0% and of bone-substitute 15.9%, at the collagen site the average amount of vital bone was 17.2% and of bone-substitute 17.3%. The values for vital bone formation and residual bone-substitute are shown in Table 1.

3.4. Time for PRF preparation

The preparation of the PRF membrane only takes approximately 15 min and blood sampling, centrifugation, and PRF membrane preparation take place during the sinus lift preparation carried out by an assistant. Hence, no valuable time is lost. Intraoral application is the same procedure as with a standard collagen membrane (Figs. 2 and 3). The production costs for one PRF membrane is about 5 Euros without the cost of centrifuging.

4. Discussion

This study aimed to evaluate the vital bone formation of composite grafts (autologous bone and bone-substitute) in maxillary sinus floor augmentation after placement of two different absorbable membranes at the site of the lateral sinus wall osteotomy. On the assumption that the bone formation starts from the walls and floor of the maxillary sinus and is completed at the lateral osteotomy site, the hypothesis of the above-mentioned technique is that membrane coverage could enhance the bone quality in this area (Avera et al., 1997; Margolin et al., 1998). Conversely, it can be concluded that the lateral wall is the optimal site for biopsy to evaluate the quality of bone grafts, because it is one of the last areas to mineralize.

This hypothesis could be confirmed and good results have been reported by different research groups when comparing membrane and non-membrane coverage of the lateral sinus wall osteotomy (Froum et al., 1998; Tarnow et al., 2000; Tawil and Mawla, 2001).
Tawil and Mawla (2001) showed that the concomitant use of a collagen membrane as a barrier at the lateral osteotomy site led to better bone graft quality and higher implant survival rates of approximately 93.1% survival in the membrane group and 78.1% in the non-membrane group. A few years ago, a systematic review was performed to determine the efficacy of sinus augmentations on implant survival with a particular view to the applied surgical technique, grafting materials, and implants (Wallace and Froum, 2003). One of the results was that the implant survival rate in grafted sinuses was higher when a membrane was placed over the lateral sinus window. It was shown that implant survival in 5 studies utilizing a membrane over the lateral osteotomy was 93.6% for 919 implants, in comparison to 15 studies without any membrane coverage which revealed a survival rate of 88.7% for 2,436 implants. In general, it has been shown that there are no significant differences between non-absorbable and absorbable membranes regarding vital bone and implant failure (Wallace et al., 2005).

One of the most frequently applied absorbable membrane materials used to close the lateral window of the maxillary sinus is collagen. Marinucci et al. (2001) found that bioabsorbable membranes, particularly collagen and hyaluronic acid, enhance the secretion of type I collagen, TGF-β1 and alkaline phosphatase, and may promote bone regeneration through their activity on osteoblasts more than non-absorbable membranes (e.g. polytetrafluoroethylene). Thus, it could be supposed that the good results mentioned above are not only caused by the mechanical shielding of the lateral sinus window against the ingrowth of soft tissue (McAllister et al., 1998), but are also due to membrane-specific features which support new bone formation.

The rationale for the application of PRF membranes to cover the lateral osteotomy site in sinus floor augmentation procedures is based on the natural involvement of fibrin in wound healing processes. The ability of PRF to polymerize and form three-dimensional supramolecular assemblies with entrapped platelet cytokines (intrinsic cytokines) seems to be a fundamental advantage for the bone graft healing process (Dohan et al., 2006b). It has been shown that these cytokines have mitogenic properties for osteoblastic cells (Slater et al., 1995) and mediate the chemotaxis of undifferentiated multipotent mesenchymal stem cells (MSCs) and as a result lead to a differentiation of cells with regard to the osteoblastic phenotype (Gould et al., 2000). In vitro examinations have also shown that PRF gradually releases autologous growth factors, and expresses strong and durable effects on the proliferation and differentiation of rat osteoblasts (He et al., 2009). A recently published study comparing PRF and conventionally collagen membranes as scaffolds for cell seeding procedures has revealed that PRF seems to be more suitable for in vitro cultivation of periosteal cells and thus may support bone graft healing in vivo (Gassling et al., 2010). Thus, the abundance of cytokines present in PRF seems to function as a drug delivery system which has profound effects on cell development and the composition of the extracellular matrix and thus may support new bone formation in sinus floor augmentation (Dohan Ehrenfest et al., 2009).

Within its limits, the results of this study have shown that the amount of vital bone formation at both sites of grafted sinuses show comparable values. The mean value of vital bone formation on the PRF-covered site was 17.0% and on the collagen site 17.2%, respectively. In attempting to compare the present results with those of other studies, it must be mentioned that sample collection

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Mean (range) of vital bone in %</th>
<th>Mean (range) of residual bone-substitute material in %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen membrane</td>
<td>17.2 (8.5–24.2)</td>
<td>17.3 (0.7–33.5)</td>
</tr>
<tr>
<td>PRF</td>
<td>17.0 (7.8–27.8)</td>
<td>15.9 (0.9–33.4)</td>
</tr>
</tbody>
</table>

Table 1

Values (%) for vital bone formation and residual bone-substitute material. The mean values and the range (minimum and maximum values) are shown.
in the same anatomical position is a particular precondition. In many publications, drilling cores have been harvested at the alveolar crest site, thus leading to a higher degree of vital bone formation caused by faster mineralization at this localization. In addition, it is possible to obtain previously existing crestal bone using this technique. In contrast, taking samples from the cranial aspect of the lateral window, as in the present study (see Fig. 4), leads to graft specimens far from multiple bony walls (e.g. crestal region), and thus present the worst possible scenario for vital bone formation (Froum et al., 1998).

With this in mind, the review of literature revealed similar values for vital bone formation in sinus grafting as the present study has. Wallace et al. (2005) presented results of a histomorphometric study of sinus augmentation utilizing absorbable and non-absorbable membranes in sinus augmentation procedures to cover the lateral sinus window. They showed that mean vital bone formation was 17.6% for collagen, 16.5% for e-PTFE (Gore-Tex®), and 12.1% without any membrane coverage, respectively. Similar results concerning the amount of vital bone formation have been described by other research groups (Froum et al., 1998; Tarnow et al., 2000). The investigation of 12 bilateral sinus floor elevations in the presence or absence of e-PTFE barrier membranes showed higher values of vital bone formation on the site covered with e-PTFE (Tarnow et al., 2000). An evaluation of 113 sinus floor elevations with different grafting materials showed that a lateral barrier membrane leads to a substantial increase in vital bone formation (Froum et al., 1998). In particular, the use of OsteoGraf/N, an anorganic bovine bone matrix material used in combination with autogenous bone, showed a mean vital bone formation of 29.1%. Nevertheless, the comparison of vital bone formation from different studies with different test designs is seemingly difficult. Possible reasons for this are the use of different bone-substitute materials, a larger or smaller sinus graft volume which leads to faster or slower vital bone formation, and different ratios of autogenous bone-to-bone-substitute materials.

Nowadays, the time factor plays an important role in monetary considerations of treatment costs. The PRF membrane seems to have no drawback in this context.

To summarize, our data suggest that sealing the lateral osteotomy site has a positive effect on vital bone formation in sinus floor elevation compared to non-membrane coverage. There are at least three explanations for this. Firstly, the membrane helps to prevent the dislocation of graft material. Secondly, it acts as a shield against soft tissue ingrowth as could be seen particularly when a periosteal releasing incision of the mucoperiosteal flap was performed to achieve wound-closure over the grafted area. Thirdly, membrane-specific features may help to enhance vital bone formation in the grafted area.

Besides the above-mentioned considerations there are monetary aspects which should be discussed. Dental implants are today a commonly accepted method of treatment in partially or completely edentulous patients (Romeo et al., 2004). It is estimated that the number of dental implants carried out in Germany exceeds more than 1,000,000/year. Thus, the consideration of treatment costs is one essential duty of the treating physician, not only for economic reasons, but also to make it possible for more patients with limited financial resources to receive such treatment. In this respect, an economic evaluation of different sinus lift techniques was recently performed by Listl et al. (2010). It was shown that the lateral approach in sinus floor augmentation procedures in general is more expensive than the transalveolar technique. Therefore, costs within this technique should be controlled, particularly in patients with limited monetary resources and in some indications, PRF, an autologous biomaterial, may be a safe and cost-effective alternative to the commonly applied absorbable membrane systems.

5. Conclusions

Within the limits of the present study, coverage of the lateral window with two different absorbable membranes has been shown to result in a similar amount of vital bone formation.

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Conflict of interest

The authors have declared no conflicts of interest.

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