Tenting effect of the elevated sinus membrane over an implant with adjunctive use of a hydroxyapatite-powdered collagen membrane in rabbits

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Abstract

Purpose: The aim of this study was to determine the de novo bone formation beneath the Schneiderian membrane supported by an implant and with the adjunctive use of a hydroxyapatite-powdered collagen (HAC) membrane without bone grafting in rabbit sinuses.

Material and methods: After sinus-floor elevation procedure, an experimentally devised mini-implant (4 mm in length and 3 mm in diameter) was placed in eight rabbits. For the experimental group, an HAC membrane was placed onto the elevated sinus mucosa prior to implant placement. The animals were allowed a healing period of either 4 or 8 weeks. Microcomputed tomography and histologic analyses were performed.

Results: All implants placed at the thin lateral bony wall were histologically osseointegrated. The topography of the newly formed bone appeared to slope gently from the medial side down to the lateral side. The volume of new bone was significantly greater in the experimental group than in the control group at 4 weeks (P < 0.05), but not at 8 weeks. However, none of the samples in the two groups was entirely covered to the apex by bone tissue.

Conclusions: It can be concluded that using the HAC membrane in combination with placement of an implant resulted in substantial bone formation around the implant, which might have been influenced by the proximity of the axial bony wall.

Vertical deficiencies in the posterior maxilla are currently resolved by sinus-floor elevation combined with various bone grafting for placement of appropriate-length implants (Pjetursson et al. 2008, Tan et al. 2008, Jung et al. 2010). Such an advanced procedure requires a high level of surgical skill, and extensive flap reflection might lead to postoperative complications including pain, swelling, and persistent bleeding.

Many clinical studies have also demonstrated that sinus-floor elevation alone without grafting materials using either the lateral or crestal approach could result in substantial bone regeneration around the implants, as observed by radiography [Lundgren et al. 2004, 2008; Schliephake et al. 2004, Leblebicioglu et al. 2005]. Radiopacity that appears around implants after a certain period of healing might be considered to be newly formed mineralized tissue, due to no radiopaque material being added. From these observations, it can be conjectured that space created between the elevated Schneiderian membrane (SM) and the protruding implants, or “tenting,” would secure the blood coagulum and might act as a natural scaffold for bone regeneration. Natural bone formation from the blood coagulum without osteoconductive bone substitutes in the sinus-floor elevation procedure might be attractive for both clinicians and their patients, because it could simplify a highly complex procedure. Skipping the bone-grafting procedure would also reduce the surgical time and cost [Lundgren et al. 2008; Schliephake et al. 2004; Leblebicioglu et al. 2005]. Furthermore, the risk of SM perforation during insertion of the bone graft into the sinus could be avoided, resulting in reduced postoperative complications associated with foreign-body reactions.

However, opponents to such approaches have advocated that sinus-floor elevation alone is not sufficient to augment bone over the apex of implants in the sinus, which is subjected to continuous negative air pressure,
leading to pneumatization (Asai et al. 2002; Xu et al. 2004, 2005; Sul et al. 2008; Kim et al. 2010). Protrusion of the implant by more than 5 mm in primates was found to result in only partial bone formation at the early healing stage after simultaneous implant placement combined with sinus-floor elevation without bone grafting (Boyne 1993; Scala et al. 2010, 2012). Moreover, during a 1-month healing period, the augmented volume gradually shrank as a result of repneumatization. Limited new bone remained around the implants, and some implants penetrated the SM.

A healthy SM is very thin and fragile, with a wide variation in thickness and mechanical strength (van den Bergh et al. 2000). In vitro mechanical tests of the SM obtained from fresh cadavers revealed widely varying elastic properties that depend on the subjects and their pathophysiologic conditions (Pommer et al. 2009). Therefore, a supporting material to help maintain the elevated position of the SM is required to avoid exposure of the implant apex into the sinus cavity.

Collagen membranes have been used extensively for bone regeneration. In spite of its good biocompatibility and cell occlusiveness, it is difficult to maintain space due to softening of the collagen membrane after hydration. In the present study, small hydroxyapatite (HA) particles were incorporated into collagen membranes to promote the formation of new bone during resorption of the collagen portion. It was expected that this HA-powdered collagen (HAC) membrane would reinforce the elevated SM so as to maintain the volume augmented by blood coagulum.

The aim of this study was to determine the de novo bone formation beneath the SM supported by an implant and with the adjunctive use of an HAC membrane without bone grafting in rabbit sinuses.

Material and methods

Animals

Eight male New Zealand white rabbits weighing 2.5–3.0 kg were selected for the experimental model. The animals were allocated to the following two groups according to the duration of the healing time: 4 and 8 weeks. They were maintained in separate cages under standard laboratory conditions, with ad libitum access to water and a standard laboratory pellet diet. Animal selection and care, the surgical protocol, and the preparation procedures were certified by the Institutional Animal Care and Use Committee, Yonsei Medical Center, Seoul, Korea (approval number: 2012-0265).

Experimental materials

Experimental mini-implants

The custom-made mini-implants that were used in this study (Dentium, Seoul, Korea) were cylindrical, with dimensions of 4 mm × 3 mm (length × diameter), and had a moderately roughened surface that had been sandblasted and acid-etched (Fig. 1a).

The HAC membrane

A resorbable collagen membrane containing small, submicron-scale HA particles was prepared (Genoss Institute, Suwon, Korea). Type I collagen fibers derived from bovine tendon were blended with ~50% (wt) HA particles (Fig. 1b). The thickness of the membrane was around 0.3 mm. Tensile strength in the dry state is 2.6 Mpa and is reduced by 0.7 Mpa when hydrated.

Experimental design

In the control condition, the maxillary sinus window on one side was filled only with a blood clot, while for the experimental condition, the SM of the maxillary sinus window on the other side was covered with an HAC membrane prior to being filled with a blood clot. The experimental and control sides were allocated randomly. Two mini-implants were placed at the left and right lateral walls, 3 mm anterior to the windows.

Surgical procedure

All surgeries were performed under general anesthesia, with additional infiltration anesthesia induced at the midline of the nasal dorsum. The window preparation and elevation of the SM followed protocols that have been described previously (Choi et al. 2012).
Briefly, after shaving the dorsum of each rabbit’s cranium, the surgical field was disinfected with a solution of iodine. A midline incision and reflection of the skin and periosseum were made to expose the dorsal surface of the nasal bone. Two identical circular windows that were 5.5 mm in diameter were prepared bilaterally on the nasal bone using a trephine bur (C-reamer; Neobiotech, Seoul, Korea) under copious irrigation. The thickness of the lateral bony wall, measured by a caliper, was around 0.6 ± 0.1 mm (mean ± SD). Care was taken during this procedure to avoid damaging the sinus mucosa. The SM was carefully elevated up to approximately 10 mm anterior to the window. The implant sites were prepared 3 mm in front of the windows using a pilot and final drill (2.7 mm in diameter) while protecting the SM using a surgical curette. Before placement of the mini-implants, an HAC membrane (10 × 15 mm) was laid onto the SM of the experimental side. Implants were placed by manual force at each site until their shoulder was seated securely into the bone. Once placed, there was no rotational movement of the mini-implants by manual digital force. Protrusion of the implant apex into the sinus cavity by approximately 3 mm was observed in all cases (Fig. 2a). The collected peripheral venous blood (approximately 0.2 ml) sampled from the brachial vein of each rabbit’s ear was used to fill the new compartment of the maxillary sinus after implantation (Fig. 2b). The windows were covered only with periosseum.

The periosteum and skin were sutured layer by layer with 4-0 Monosyn (glyconate absorbable monofilament; B-Braun, Aesculap, PA, USA). The animals were sacrificed at either 4 or 8 weeks postoperatively by anesthesia overdose and specimens including the implant and the surrounding tissues were harvested.

**Microcomputed tomography analysis**

All harvested specimens were fixed in 10% formalin for 10 days and scanned with a high-resolution microcomputed tomography (μCT) system (SkyScan 1173, SkyScan, Aartselaar, Belgium) at a resolution of 35 μm (achieved using 100 kV and 100 μA). Measurements were made by one experienced researcher who was blinded as to the group (i.e., control vs. experimental, and 4- vs. 8-week healing period). The scanned data set was reconstructed, and the three-dimensional volume of newly formed bone (NBV; mm³) was calculated from a surface generated by a triangle meshing technique based on the marching-cubes method (Bouxsein et al. 2010).

Linear measurements were also made on the coronal and sagittal cross-sectional views (Fig. 3). The total length and the protruding height (PH) into the sinus cavity of the implant, the thickness of the basal cortical bone (CBT), the height of the exposed implant surface, and the height of the newly formed bone (NBH) were measured on both the medial and lateral sides of the implant. The horizontal distance between the apical end point of the implant’s microthread and the axial bony wall was also measured on a coronal cross-sectional view.

**Histologic analysis**

After μCT scanning, the fixed specimens were dehydrated in ethanol, embedded in methacrylate, and sectioned in the sagittal plane at the center of the augmented sinus using a diamond saw (Exakt; Apparatebau, Norderstedt, Germany). From each implant site, the central section was reduced to a final thickness of about 20 μm. The sections were stained with hematoxylin–eosin and analyzed histologically with the aid of a light microscope (DM-LB; Leica, Wetzlar, Germany). The histologic slides were observed and captured digitally with a camera attached to a light microscope (BX50, Olympus, Tokyo, Japan). The NBH of the bone that formed along both the anterior and posterior surfaces of the implants was measured for histometric analysis. Descriptive statistical
analyses were performed due to the smallness of the sample.

Results

Clinical observations
Minor perforations of the SM with dimensions of <3 mm occurred at two experimental sites and three control sites during removal of the bony windows. As these minor perforations were distant from the implant sites, the presence of an intact SM over the implants was confirmed visually. No special treatment was performed for covering the perforations. Wound healing was generally uneventful in all animals. There was neither wound dehiscence nor exposure of the implants during the entire healing periods.

Radiographic analysis: μCT
The 3D-reconstructed view revealed that cone-shaped new bone was formed around the implants that were protruding into the sinus cavity in both the experimental and control groups (Fig. 4). The topography of this new bone appeared to slope gently away from the high medial side, down to the low lateral side. In two samples of the experimental group, new bone growth over the apex of the implant was observed at 4 weeks.

The NBV was greater in the experimental group [21.89 ± 6.67 mm³] than in the control group [10.33 ± 5.62 mm³] at 4 weeks of healing. The difference in NBV between the two groups diminished at 8 weeks: 9.78 ± 1.48 mm³ in the experimental group and 6.36 ± 1.21 mm³ in the control group.

In the cross-sectional view, radiopaque new bone grew along the implant surface from the basal bone to form a triangular shape (Fig. 5). The average distance from the center of the implant to the medial wall of the cavity was 1.1–1.4 mm, and 2.6–2.8 mm to the lateral wall [Table 1]. The initial CBT ranged from 0.5 to 0.9 mm, and the mean PH of implants was 3.3 mm. The mean NBH was greater on the medial side than the lateral side at both 4 and 8 weeks, and on the medial side, the NBH was higher in the experimental group than in the control group [Table 2]. However, NBH on the lateral side was greater in the control group than in the experimental group.

Histologic findings and histomorphometric analysis
No signs of inflammation were observed histologically at the surgical sites. The preexisting CBT was 0.6 ± 0.1 mm. All of the placed implants were directly in contact with the bone tissue, resulting in osseointegration. The protruding implants and new bone were covered by intact SM, exhibiting normal serous glands and a pseudoluminal ciliary epithelial lining. However, discontinuity of the SM lining at the apex of the implants was observed in five of 16 samples [2 and 3 in the experimental and control groups, respectively]. Exposure of the implant apex without an SM lining was more frequent at 8 weeks (two in each of the experimental and control groups) than at 4 weeks (one in the control group). In such cases, the samples exhibited reduced bone regeneration, even when no inflammation was found [Fig. 6].

Results at 4 weeks of healing
In the experimental group, triangular-shaped trabecular bone had formed along the protruding surface of the implant above the original cortical bone and over the HAC membrane at 4 weeks [Fig. 7a]. The coronal microthreads were engaged with the cortical bone, where the newly formed trabecular bone projected into the space beneath the elevated SM [Fig. 7b]. The dilated HAC membrane was in direct contact with the apex of the implant and the elevated SM, and the projection of new bone penetrated the HAC membrane [Fig. 7c]. The control group exhibited a similar healing pattern, with the exception of the HAC membrane [Fig. 8]. The new bone that had formed at the anterior surface was continuous with the anterior axial bony wall and gradually flattened away from the implant posteriorly.

Results at 8 weeks of healing
Remnants of the HAC membrane were still observed occupying a substantial space at 8 weeks [Fig. 9a]; a layer of new bone had formed over those remnants. A dense connective tissue layer resembling the periosteum was observed beneath the HAC membrane. The new bone had reduced with maturation compared with that observed at 4 weeks. In the control group, the new bone had also undergone maturation and contained lamellar bone and fatty marrow [Fig. 9b].

Histometrically, the NBH varied widely (0.4–5.0 mm), with no difference between either the implant sides (anterior and posterior) or the healing periods [4 and 8 weeks; Table 3].

Fig. 5. Cross-sectional views in the mediolateral plane. Experimental [a] and control [b] groups after 4 weeks of healing, experimental [c] and control [d] groups after 8 weeks of healing. Some radiopaque mineralized tissue was observed over the apex of the protruding implant [arrowheads]. M, medial; L, lateral.
maxillary sinuses and extraction sockets. Bone regeneration, acting as space fillers in these two species. However, in a monkey model (Scala et al. 2012), similar results to those in these two species. However, unstable space control due to the fragility of the SM might lead to limited new bone formation and exposure of the implant apex into the sinus cavity. To obtain sufficient bone growth, the position of the elevated SM should be maintained until the mechanical strength of the newly formed tissue is sufficient for resisting repneumatization. The HAC membrane was used to secure the sinus compartment filled with blood coagulum in the present study. However, the volume augmented at 4 weeks failed to be maintained up to 8 weeks. The NBVs of both groups markedly decreased with healing time. On average, 3.3 mm of the apical portion of the implant protruded into the sinus, and the mean NBH was approximately 1.4 mm, regardless of the use of the barrier membrane. In other words, about 40% of the PH was covered by new bone over the 8-week healing period. Conversely, more than half of the protruding implant surface was either exposed or covered only by a thin SM. Adjunctive use of an HAC membrane to support the SM failed to protect against repneumatization and gain complete coverage of the new bone up to the apex of the implants, although the frequency of SM exposure was lower in the experimental group.

As the rabbit maxillary sinuses are directly connected to the nasal cavity and have well-defined ostium openings, the air pressure exerted on the elevated SM might be higher and more frequent than in humans. When the ostium of the sinus is occluded, there appears to be less shrinkage of the augmented tissue than when the ostium is left open (Asai et al. 2002). The anatomical and physiological differences between rabbits and humans could influence the healing patterns in these two species. However, in a monkey model (Scala et al. 2012), similar results to those seen in humans could be expected.

Therefore, in terms of mimicking natural bone healing, blood coagulum would presumably be a better scaffold than any other bone substitute, provided that the space can be maintained. The development of surgical protocols and advancements in implant surface modifications could contribute to reducing the treatment time for rehabilitation of the edentulous posterior maxilla.

Table 1. The horizontal dimension on the coronal cross-sectional views of microcomputed tomography (mean ± SD; n = 4)

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<td>2.6 ± 0.2</td>
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<td>Experimental</td>
<td>1.4 ± 0.5</td>
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<td>2.6 ± 0.5</td>
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<td>1.1 ± 0.6</td>
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Table 2. The linear measurements on the coronal views of microcomputed tomography (mean ± SD; n = 4)

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<td>New bone height</td>
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<td>M</td>
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Fig. 6. Perforation of the Schneiderian membrane at the implant apex. (a) control group at 4 weeks. (b) experimental group at 8 weeks. Arrowhead, end of the Schneiderian membrane.

Discussion

Osteoconductive bone substitutes can maintain the augmented volume with substantial bone regeneration, acting as space fillers in maxillary sinuses and extraction sockets (Cha et al. 2011; Hong et al. 2014). However, in the early stage of healing, such space fillers might retard angiogenesis and the formation of a provisional matrix, resulting in delayed bone formation (Froum et al. 1998; Hallman et al. 2002; Schliephake et al. 2004). Therefore, in terms of mimicking natural bone healing, blood coagulum would presumably be a better scaffold than any other bone substitute, provided that the space can be maintained. The development of surgical protocols and advancements in implant surface modifications could contribute to reducing the treatment time for rehabilitation of the edentulous posterior maxilla.
those of the present study were observed, in which about 35% of the PH was covered by new bone after 1 month of healing. Whether the window was open or closed might be critical to maintaining the stability of the blood coagulum. It has been demonstrated that it would be beneficial to reposition the bony window following sinus-floor elevation with simultaneous placement of the implant (Cho et al. 2012). Maintaining the continuity of the SM and closure of the window could prevent loss of the initially formed blood coagulum as a spacer in the sinus and exclude the ingrowth of soft tissue. Furthermore, sealing the window with a nonresorbable membrane may be as effective as using an autogenous bony window (Sohn et al. 2008). In the present study, the circular window remained open and was covered only by peristomeum. Flowable blood filling the sinus compartment might be discharged through an open window in the early healing stage, which could decrease the potential amount of new bone.

Previous studies have applied various shapes of resorbable polylactide devices to maintain the elevated SM in monkeys (Cricchio et al. 2009, 2011). A box-shaped device yielded poor results with regard to bone formation, possibly due to the lack of stabilization and the porosity of the device (Cricchio et al. 2009). To overcome these problems, star- and H-shaped devices were used in the latter study (Cricchio et al. 2011). While displacement of the devices was still observed, bone formation occurred in most cases. The modified devices remained in the sinus cavity without any resorption after 6 months of healing, and a large window needs to be created to allow the bulky device to be inserted into the sinus. The flexible HAC membrane used in the present study could be easily adapted and stabilized in itself to the SM without any fixation after hydration and was still lying in its original position at sacrifice. The HAC membrane allows vascular penetration between the SM and the inner compartment. In the experimental group, growth of new bone over and beneath the HAC membrane was found at 4 weeks, and after 8 weeks of healing, there was extensive resorption of the HAC membrane accompanying the reduced new bone formation.

Schweikert et al. (2012) introduced an angled plate titanium device as a space holder in monkeys that can be stabilized on the lateral wall. After 3 and 6 months of healing, most of the devices were exposed into the sinus, perforating the SM, and extensive shrinkage of the newly formed bone was observed. The poor results might be due to the mechanical properties of the device. The titanium device was structurally rigid, but the edges of the plate could not compensate for the shrinkage of the initially formed tissues, which might increase the likelihood of

Fig. 7. Histologic views of the experimental group at 4 weeks [hematoxylin–eosin (H & E) staining]. (a) overall view, (b) higher magnification view of the cortical bone area, (c) higher magnification view of the implant apex. NB, new bone; CB, cortical bone; HAC, hydroxyapatite-powdered collagen membrane.

Fig. 8. Histologic views of the control group at 4 weeks (H & E staining). (a) overall view, (b) higher magnification view of the cortical bone area, (c) higher magnification view of the newly formed bone around the implant. SM, Schneiderian membrane; SG, serous gland.

Fig. 9. Histologic views at 8 weeks (H & E staining). (a) experimental group, (b) control group.
perforation. In the present study, there was no exposure of the HAC materials out of the SM.

New bone formation occurred from the basal/axial bone of the sinus cavity floor from the point of contact with the implant, which bone healing pattern was concurred with a previous study in a circumferential gap defect (Botticelli et al. 2003). The extent of new bone varied according to the direction. The medial aspect exhibited the highest metric differences in the amount of new bone varied according to the direction. It has been acknowledged that the number and proximity of axial walls surrounding the intrabony defect is critical for periodontal regeneration (C. S. Kim et al. 2004). This is because a contained defect type has a structural advantage in containing the fibrin clot which is necessary for connective tissue attachment. Similarly, it can be assumed that a smaller separation between the medial axial wall (1.1–1.4 mm) of the sinus and the implant surface could better contain the blood clot necessary for the formation of the provisional matrix required for bone formation.

Histologic sectioning was performed in the sagittal plane to avoid the influence of the axial bony wall and to evaluate the genuine tenting effect of the SM. Some authors advocate that the SM itself has innate osteogenic potential (Gruber et al. 2004; Srouji et al. 2009, 2010). Palma et al. (2006) also supported this assumption, they found no histometric differences in the amount of new bone in sinus augmentations performed with or without autogenous bone grafts in nonhuman primates. However, the roughness of the implant surface was referred as a critical factor. Roughened surfaces appear to be superior to turned surfaces in terms of bone formation and implant survival. In the present study, the surface of the experimental mini-implants was modified by sandblasting and acid etching. Primary stability was achieved from approximately 0.6 mm of thin cortical bone. In spite of the minimal availability of preexisting bone, all specimens in both groups exhibited osseointegration after 4 weeks of healing. The survival of all of the implants might be attributable to the excellent osteoconductivity of the implant surface.

Within the limitations of this study, it can be concluded that the use of an HAC membrane during implant installation resulted in substantial bone formation around the implant via the tenting effect and that the amount of new bone formed may be influenced by the proximity of the axial bony wall to the implant.

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Conflict of interests

The authors declare no conflict of interest.

References


malignant disease 21: 211–217.


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### Table 3. The histometric linear measurements on the sagittal views (mean ± SD; n = 4)

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Jung et al. Tenting effect of the sinus membrane over an implant


