Biodegradable polyurethane for closure of oroantral communications
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Chapter 4a

Closure of oroantral communications using biodegradable polyurethane foam: a long term study in rabbits.

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Abstract
An oroantral communication (OAC) is an open connection between the oral cavity and maxillary sinus. Closure of oroantral communications is commonly performed with a surgical procedure using a mucoperiosteal flap. An alternative technique using synthetic biodegradable polyurethane (PU) foam for closure of OACs is presented.

This PU foam is composed of hard urethane segments, and soft segments made of D/L lactide (50/50), ε-caprolactone and 5% poly ethyleneglycol (PEG). To evaluate the use of PU foam for this application, OACs were created in the edentulous part of the maxilla in 21 rabbits, after which PU foams were fitted in the defects.

Results showed complete healing of the oral mucosa after 4-10 weeks, healing of the antral mucosal lining after 6 months and complete bony regeneration after 1 year. No reopening of the defects occurred and no maxillary sinusitis was observed. Degradation of the PU foam had not yet reached completion 1 year after implantation.

In conclusion; PU foam with 5% PEG provides adequate closure of an OAC in the rabbit model to support healing of the oral and maxillary sinus mucosa. Longer time intervals are needed to assess the complete degradation of the PU foam.

Introduction
An oroantral communication (OAC) is an open connection between the oral cavity and maxillary sinus. Oroantral communications are most often encountered by extraction of maxillary (pre)molars with roots extending into the maxillary sinus (1). OACs may close spontaneously, particularly when the defect is smaller than 5 mm (2). However, it is difficult to clinically determine the size of the OAC, making it complicated to predict whether an OAC will heal without (surgical) intervention. Consequently, it is generally accepted that all OACs should be surgically closed within 24 to 48 hours to prevent chronic sinusitis and the development of fistulas (3).

Surgical closure of OACs is usually accomplished with a mucoperiosteal flap. Various forms of buccal flaps, palatal flaps and tongue flaps have been described in literature for this purpose (4-8). In general, these surgical flap procedures have high success rates in terms of permanent closure of the OACs (6;9;10). On the other hand, surgical procedures have several disadvantages including postoperative pain, swelling and donor site morbidity. An additional disadvantage of the commonly used buccal flap procedure is the risk of permanently decreasing the buccal sulcus depth (11).

Several alternative treatment strategies for closure of OACs have been described throughout the years. These strategies include the use of gold (12;13), tantalum (14), aluminium (15) polymethylmethacrylate (16), hydroxylapatite (17) and tissucol (18). To our knowledge, none of these proposed strategies is currently routinely applied in a clinical setting, seemingly because either they are too expensive, or no simplification has been accomplished compared to the standard treatment.

Polyurethane (PU) foam is a fully synthetic biodegradable product that might offer a useful alternative for closure of OACs. This highly porous PU foam has excellent elastic and mechanical properties, allowing adaptation to the extraction socket. PUs are polymers of which the polymer chains are built up of two different segments; a hard urethane segment and the more flexible soft segments. The urethane segments of the PU in this study are synthesized with 1,4-butanediisocyanate (BDI) and butane-diol (BDO). When the urethane segments are hydrolyzed, the 1,4 butanediamine is formed. Butanediamine is normally present in mammalian cells and its catabolites are excreted in urine (19).

In a previous in vivo study (20), OACs in New Zealand White (NZW) rabbits were closed with 2 types of PU foam. These 2 foams had identical urethane segments, and were also built up of BDO and BDI, but their soft segments differed. In the first PU foam the soft segments were made of only (50/50) DL-lactide and ε-caprolactone. In the other PU foam, 23 w/w % polyethyleneglycol (PEG) was added to a (50/50) glycolide/ε-caprolactone soft segment to make the PU more hydrophilic and degrade more rapidly. The porosity of these foams was 94 % with pore sizes of 100-300 μm, and interconnected pores of 10-30 μm. Based on the in vivo study (20), it was
acknowledged that the rabbit represents a suitable model for OAC surgery and that closure of OACs in rabbits with PU foam is feasible. Furthermore, it was concluded that both types of PU foams were not capable of guiding the oral as well as the antral mucosal lining (20). Namely, the first PU foam did not result in closure of the oral mucosa in 4 weeks. The second PU foam showed earlier fragmentation of the foam than expected, which hindered the healing of the maxillary sinus mucosa.

Therefore, in vitro studies have been performed to optimize the kinetics of the PU foam for closure of OACs, resulting in a PU foam with an intermediate composition of 5 % PEG. This foam retains its mechanical properties for a period of 2 weeks, which should enable regeneration of the mucosa on the oral side as well as on the antral side. After 2 weeks, disintegration of the PU foam starts.

The purpose of the present study was to evaluate the application of polyurethane foam with 5% PEG for closure of OACs in rabbits. The closure of the mucosa was studied, as well as the degradation of the foam and the regeneration of the maxillary bone. For this reason, both short and long healing intervals were included.

Materials and methods

Polyurethane foams
The polyurethane foams were synthesized by Polyganics BV, Groningen, The Netherlands. The polyester soft segments were synthesized which consisted of (50/50) D/L lactide/ε-caprolactone and (6.5 w/ w %) PEG (Mn = 1000 g/ mol). The soft segments were end-capped with BDI. Chain extension was performed using a BDO-BDI-BDO urethane block as a chain extender. This resulted in polyurethane segments with a uniform length of 5 urethane moieties (BDI-BDO-BDI-BDO-BDI) with an overall PEG content of 5.0 w/ w %.

To produce the foams, the PU was dissolved in 1,4-dioxane, till a concentration of 4 w/ w % polyurethane. After addition of water (7.5 w/ w %) the solution was then poured in a mould and cooled down to -18 °C. The solution was freeze dried (3 mbar) to remove water and dioxane crystals, resulting in a cylindrically shaped PU foam with pore sizes of 100-300 μm, interconnected pores of 10-30 μm and a porosity of 93%. Prior to the in vivo studies the foams were sterilized with ethylene oxide.

Methods

The animal study was approved by the Committee for Animal Experiments (University of Groningen, the Netherlands). Twenty one rabbits in total were obtained from Harlan bv, the Netherlands. All animals were housed according to Dutch national guide- lines for animal welfare. The rabbits were accustomed to softened food during 1 week prior to surgery and the soft food was maintained up to 2 weeks after surgery.

Prior to surgery, the rabbits were weighed and anesthetized intramuscularly with 15 mg/kg ketamine (Ketalin®, Ceva Sante Animale, Libourne, France) and 0.5 mg/kg medetomidine (Domitor®, Pfizer, Exton, United States). Local anesthesia and vasoconstriction was induced by administration of 0.5 – 1.0 ml articain with epinephrine (Ultracain® D-S forte, Aventis Pharma, Hoevelaken, the Netherlands). Subsequently, the gingiva was incised and reflected. A 5 mm drill was used to create a perforation in the edentulous part of the maxilla, directly mesial of the first premolar, into the maxillary sinus (Figure 1). On all animals, OACs were created on both sides of the maxilla. The perforation of the maxillary sinus was checked by visual inspection and probing. In an earlier study in New Zealand White Rabbits, OACs were created and left untreated to evaluate the natural course of healing. These defects showed complicated healing (20). In the present study no defects were left untreated because comparison between untreated and treated defects will also, for ethical reasons, not be made in a human situation.

The majority of the OACs were closed with PU foam (Table 1). The PU foams were cylindrically shaped and resized if necessary to fit the OACs with slight resistance. To prevent early loss of the foams, one loosely tied suture was applied across the defect without approximating the borders of the incised gingiva.

In 4 rabbits OACs were closed with a surgical procedure using a buccal mucoperiosteal flap, a treatment comparable to the current surgical closure in the human situation (Table 1). The surgically closed OACs enabled a comparison to be made between the initial healing of an OAC closed with PU, and healing secondary to surgical closure.

Figure 1 View of a left oroantral communication (OAC). The rabbit is in supine position. The first left upper premolar (PM) is just visible. The hard palate (Pal) and the inferior incisors (i) are indicated.
Results

Clinical observations
The perforations of the maxillary sinus, surgical closures and placement of the PU foams all went without complications except for 1 rabbit. In this rabbit (rabbit #10), the left OAC had been drilled at a more palatal location than intended. This caused massive bleeding and peroperative death of the animal. The remaining rabbits had no postoperative complications, no signs of nasal discharge or sinusitis, and were all active during the postoperative days. The animals started eating immediately after the surgical procedure and lost no weight.

Macroscopical observations
After 1 week, the oral side of the foam treated defects were contaminated with food remnants (Figure 2a). No wound dehiscence was observed of the surgically closed defect. No inflammation of the oral mucosa was observed neither in both the defects treated with PU foam nor in the surgically treated defect. During preparation of the specimens the maxillary sinuses appeared clear.

At the 2 weeks time interval, the defects on the oral side were still visible, but evidently decreasing in size. Healing appeared uneventful at this stage in both the PU treated defects and the surgically closed defect.

At 4 weeks, all defects had closed macroscopically on the oral side (Figure 2b). No difference could be observed between the healed mucosa of the surgically closed defects, and the defects treated with PU foam. In some rabbits, the foams occupied a relatively large part of the maxillary sinus cavity. However, the remaining space of the maxillary sinus showed no abnormalities.

At 10 weeks after closure, the foams had a more pulpy aspect compared to the previous points of evaluation.

Both at 6 months and 1 year after closure, no abnormalities were observed concerning healing. At a time interval of 1 year, in one of the three rabbits PU foam could still macroscopically be identified in the maxillary sinus.

Microscopical observations
The maxillary sinus mucosa of the surgically closed defect had regenerated into a thin continuous layer after 1 week. At 2 weeks, the first signs of regeneration of the maxillary bone were observed. At 4 weeks after surgical closure, the mucosa covering the defect had regained its normal thickness and the defect was almost totally bridged by newly formed bone. At 10 weeks, the closure of the defect by bone was complete in the surgically treated defect.

Histology of the foam treated defects showed that the PU foams were fully saturated with blood. After 1 week, all foams still were rightly positioned with one side partially introduced into the maxillary sinus. The tissue response to the PU foam and the healing of the oral and antral mucosal lining, as well as the regeneration of the maxillary bone at the site of the defect were evaluated.

Table 1 Overview of the healing periods and the type of treatment applied to the different animals

<table>
<thead>
<tr>
<th>Healing periods</th>
<th>Rabbit #</th>
<th>Type of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 : 1 week</td>
<td>1</td>
<td>L: PU foam R: Surgical closure</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>L: PU foam R: PU foam</td>
</tr>
<tr>
<td>Group 2 : 2 weeks</td>
<td>3</td>
<td>L: PU foam R: Surgical closure</td>
</tr>
<tr>
<td></td>
<td>4-7</td>
<td>L: PU foam R: PU foam</td>
</tr>
<tr>
<td>Group 3 : 4 weeks</td>
<td>8</td>
<td>L: PU foam R: Surgical closure</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>L: PU foam R: Surgical closure</td>
</tr>
<tr>
<td>Group 4 : 10 weeks</td>
<td>10-12</td>
<td>L: Surgical closure R: PU foam</td>
</tr>
<tr>
<td></td>
<td>13-15</td>
<td>L: PU foam R: PU foam</td>
</tr>
<tr>
<td>Group 5 : 6 months</td>
<td>16-18</td>
<td>L: PU foam R: PU foam</td>
</tr>
<tr>
<td>Group 6 : 1 year</td>
<td>19-21</td>
<td>L: PU foam R: PU foam</td>
</tr>
</tbody>
</table>

In the last two time intervals, both OACs in each rabbit were closed with PU foam to examine the PU degradation process in the longer term.

After surgery, the animals were given 0.15 mg buprenorphine (Temgesic®, Schering-Plough, Utrecht, the Netherlands) i.m. as an analgesic. During the first postoperative weeks, the animals were weighed and observed daily to monitor their appearance, any nasal discharge, behaviour and eating habits.

The animals were sacrificed by intravenous administration of 2.0 ml embutramide-mebenzoniumjodide-tetracaine HCL solution (T61®, Intervet, Mechelen, Belgium) and weighed preceding dissection of the surgical sites and sinuses. All areas of interest were inspected for abnormalities.

Specimen preparation and analysis
Samples for histological evaluation were taken from both sides of the maxilla. The samples were fixed in 4% phosphate buffered formalin, and decalcified in RDO for 15-20 hours. After embedding in GMA, 2 µm sections were cut and stained with Toluidin Blue or Toluidin Blue/ Basic Fuchsin. The tissue response to the PU foam and the healing of the oral and antral mucosal lining, as well as the regeneration of the maxillary bone at the site of the defect were evaluated.
started to grow across the foam. At this stage, the oral defects had not yet been closed and were contaminated with food remnants.

At 2 weeks, the maxillary sinus mucosa had grown completely across the foam in seven of nine cases. Under the new antral mucosa, connective tissue had grown into the foam. This tissue ingrowth was accompanied by vascular ingrowth. The first signs of degradation of the foam were observed under the surface of this newly formed tissue. The defect on the oral side had grown smaller in eight of nine cases, while one defect had almost kept its original size.

At 4 weeks, the oral mucosa was restored into a continuous lining in two of the three cases, while a minor gap was still observed in one case (Figure 3). It is also at this stage that the first signs of regeneration of the maxillary bone were observed. The bone grew around the foams, which were situated further into the maxillary sinus compared to the position of the foams at 1 week and 2 weeks after implantation. Loss of foam structure was observed in the centre of the foams.

At 10 weeks the oral mucosa had totally closed in all 5 rabbits. The larger part of the foams was inside the maxillary sinus in some animals (Figure 4a). The remaining space of the maxillary sinus was clear in all rabbits. Ciliated epithelium supported by a connective tissue layer was visible on the PU foam (Figure 4b). The epithelial ongrowth had not yet been completed in some cases where the PU foam was located mainly in the maxillary sinus.

The regeneration of the maxillary bone at the original side of the defect had progressed further in comparison to the implantation period of 4 weeks, but was not yet complete. No bone formation was visible within the foams (Figure 4a and c). In one rabbit the foam had totally lost its architecture, and the whole maxillary sinus was occupied by the remnants.

In the 6 months group it was observed that both the oral and antral mucosa had closed completely in all rabbits. In one rabbit, the remains of the PU foam were found enclosed inside the maxillary sinus.

Bony closure of the defect was totally continuous in all animals except in one case. In this rabbit, the foam in the left maxilla was situated more on the oral side. This seemed to have resulted in delayed bony regeneration, because the bone grew around the PU foam and no bone formation occurred into the foam.

At 1 year, the newly formed antral mucosal lining had further matured (Figure 5b) as compared to the implantation period of 6 months. In one of three rabbits the remaining PU foam was found in both the left and right maxillary sinus. The remaining PU foams showed degradation round the edges and in the centre. In the other 2 rabbits, the PU foam itself was no longer present either inside or below the level of the maxillary sinus (Figure 5a). Only smaller PU fragments were found in the tissue below the level of the sinus with newly formed bone visible between the PU fragments.
The epithelial lining regenerated within a period of 1 week. Bony restoration of the surgically treated defect was established after 10 weeks.

The results of the closure with PU foam are encouraging, as the application of PU with 5% PEG leads to an uncomplicated recovery of the oral mucosa. The PU foam reinforces the coagulum and protects it from displacement. The foam retains its mechanical properties for a period of 2 weeks, after which disintegration of the foam sets in. This period seems long enough to stabilize the blood clot and facilitate mucosal overgrowth, which is in contrast to the PU foam with 23% PEG used in the preceding experiment (20). Namely, the 23% PEG foam already disintegrated after 24 hours. For a good reinforcement, the foam had to be saturated with blood. Histology of the foams in this study showed that this is the case. It is likely that the hydrophilic properties of the foam in combination with the interconnective pores contribute to this fine uptake of blood.

The observations of the antral mucosa bring the differences between rabbits and humans into discussion. Although rabbits have a relatively well developed maxillary sinus, the absolute volume of the sinus is small compared to humans.

The suitability of other animal models for this study has also been evaluated; Göt-
tigen mini pigs were found unsuitable because of the position of the infraorbital nerve (21) and extraction of teeth to create an OAC in sheep proved to be too complicated. In the end the rabbit model was chosen, based on its anatomical similarities to the human; e.g. a well-developed maxillary sinus, and its proven suitability for closure of OACs in earlier experiments.

The applied orooral communications measured 5 mm. This is a relatively large defect size for the rabbit when compared to the size of its maxillary sinus. However, a previous study showed that a 5 mm defect is applicable in a rabbit model (20). This previous study also showed delayed and complicated healing when such a 5 mm defect was left untreated (20).

The PU foam that was needed for closure of the defect may fill a large part of the relatively small rabbit sinus. This is caused by the fact that the foam easily bulges into the sinus, because the maxillary sinus floor in rabbits is very thin. On the other hand, it is essential to push the foam partly through the perforation to ensure adequate fixation.

Once the foam is partly placed in the maxillary sinus, it can still easily be pushed further into it. A displacement of a foam may also be the result of the forces during mastication. Furthermore, the rabbits in this study had defects on both sides of the maxilla which made it difficult for the animals to avoid pressure on the OACs.

The largest displacement of the foams probably already occurred during, or shortly after surgery. In one of the rabbits in the 1 year group the remaining foam was located entirely inside the maxillary sinus. However, on account of the rapid degradation onset of the foam, it was not expected that free foam would be found in the maxillary sinus after 6-12 months. The fact that free foam appeared present in this rabbit 1 year after implantation may be explained by a combination of an early dislocation of the foam into the sinus shortly after implantation, and a slower degradation inside the sinus compared to degradation of an incorporated situation. Within the other rabbits of the 1 year time interval, foam remnants were detectable only in the tissue below the level of the maxillary sinus.

Dislocation of the foams and the occupation of the sinus will probably occur less frequently in the human situation. Firstly, many extraction sockets in humans will not allow dislocation of the PU foam into the sinus, because of their conical shape. Secondly, dislocation can be diminished by instructing the patients to avoid forces on the treated area.

After closure of the OACs with PU foam, the maxillary sinus was in no way affected by the foam bulging into it. The ciliated epithelial cells of the antral mucosa regenerated along the surface of the foam, leaving a smaller cavity of the maxillary sinus, which was totally covered with ciliated epithelium. After 4 weeks the first signs of bone regeneration were observed and the oral mucosa was almost healed. After 10 weeks, the oral mucosa was fully restored in all animals. Furthermore, bone regeneration at this stage was still in progress.

Once the oral mucosa had closed, the maxillary bone started to regenerate. No bony ingrowth into the foam was observed, which might elongate the bony bridging time across the defect, when compared to the bone regeneration after surgical closure. This was seen in cases where the PU foam was situated mainly outside the maxillary sinus, resulting in longer regeneration periods of the maxillary bone, contrary to cases where the PU foam was located largely into the sinus. Namely, when the PU foam was placed largely into the sinus, the bony regeneration showed more progress but consequently the healing of the antral epithelial lining took longer to complete.

However, the extended regeneration of the bone probably does not provide discomfort for the patient, and the non-surgical closure of the OAC is likely to evoke less discomfort than the surgical closure. Besides, to the best of our knowledge no studies have been performed concerning how often surgical closure in humans leads to actual regeneration of the maxillary bone at the site of the defect.

After 1 year, histological sections still showed small fragments of PU foam in 2 rabbits. These PU particles were either located inside the maxillary sinus, or in the tissue between the newly formed bony bridge and the regenerated lining of the maxillary sinus. It is anticipated that the degradation of these PU fragments will continue further in time.

Both inside and outside the maxillary sinus, no inflammatory or other negative effects of the PU remnants were observed. Furthermore, the function of the ciliated epithelium will most probably transport the PU particles out of the maxillary space.

In conclusion, PU foam with 5% PEG provides adequate closure of an OAC in the rabbit model to support and guide healing of both the oral and antral mucosa. Regeneration of the maxillary bone is delayed when compared to the surgical closure. The clinical consequences of this observation seem limited to patients in which dental implants are planned. Longer time intervals are necessary to document the end stage of degradation. In addition, human studies will follow for the further development and evaluation of this new and straightforward method for the treatment of OACs. Lastly, the cost of the foam for clinical appliance is likely to be between 40 and 50 euro, which makes closure of OACs with certainty less expensive than surgical closure. This should make the PU foam also interesting from an socio-economical point of view.

Acknowledgements

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