Chapter 4b

Biodegradable polyurethane foam for closure of oroantral communications in rabbits: a 4 year light- and electronmicroscopic study.

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Submitted
Abstract

Oroantral communications (OACs) are connections between the oral cavity and maxillary sinus and usually caused by extraction of posterior teeth. OACs require surgical treatment in order to minimize the risk of fistula formation and chronic sinus disease.

A new non-surgical method for closure of OACs using degradable polyurethane (PU) foam was studied. OACs were created in the edentulous part of the maxilla in 19 New Zealand White rabbits and subsequently closed with PU foam. This PU foam is composed of hard urethane segments synthesized with 1,4 butanediisocyanate and butanediol, and soft segments made of D/L (50:50) lactide, ε-caprolactone, and 5 % polyethylene glycol.

Time intervals up to 4 years were included to analyze the long term degradation process. The OACs recovered in all rabbits, with both soft tissue and bony regeneration. The degradation process of the PU was not fully completed after 4 years but a decreasing number of macrophages with internalized PU and the aspect of the internalized PU suggest further degradation in time to an ultimate end stage. Human studies will follow for further development and evaluation of this nonsurgical closure of OACs.

Introduction

Oroantral communications (OACs) can be defined as open connections between the oral cavity and maxillary sinus. Usually, OACs are caused by extraction of (pre)molars in the maxilla due to the close relationship of the apices and the sinus floor (1). It is important to close an OAC as soon as possible to minimize the risk of the development of a maxillary sinusitis and epithelisation of the connection, resulting in an oroantral fistula (2). Closure of OACs is in general performed surgically.

Ideally, an alternative strategy for surgical closure of OACs gives predictable results, is easy and quick to perform, and gives rise to little postoperative complaints. Also, it would be interesting for both the patient and the dentist if the latter would be able to treat OACs instead of a maxillofacial or oral surgeon.

Biodegradable polyurethane (PU) foam offers the right characteristics for an alternative treatment strategy for OACs. A long term subcutaneous implantation study of this PU foam in rats and rabbits showed that after 3 years only an occasional macrophage containing PU could be observed; the samples had thus resorbed almost completely (3). The fully synthetic PU foam can be placed in the OAC and loosely secured on the oral side with a suture. The highly porous foam fills with blood upon placement thus forming a solid barrier. It retains its mechanical properties for 2 weeks, enabling mucosal overgrowth of the perforation, after which disintegration sets in. In a subsequent in vivo study, the degradation of the PU foam for this specific application has already been studied in New Zealand White (NZW) rabbits for time intervals up to 1 year, and showed promising results (4). However, the end stage of degradation had not been reached in that study. Therefore, it was concluded that longer time intervals were needed.

In the present in vivo study OACs were created in NZW rabbits in analogue to the preceding study (4), and closed with the same PU foam consisting of hard urethane segments synthesized with 1,4 butanediisocyanate (BDI) and butanediol (BDO) and soft segments consisting of D/L lactide, ε-caprolactone and polyethylene glycol (PEG). Also, part of the created OACs were closed surgically with a buccal flap to facilitate comparison between the healing process after both the surgical treatment and this experimental nonsurgical treatment. In theory, PU based on 1,4 butanediisocyanate will degrade into substances that already occur in the body (5). This characteristic of the PU foam implies the capacity to degrade completely and become excreted by the body through natural pathways (6). In order to allow documentation of the end stage of degradation, time intervals up to 4 years after implantation were included. The samples were evaluated with both light microscopy (LM) and electron microscopy (EM).
Materials and methods

Polyurethane foams
The PU foam used in this study consisted of repeating units of hard urethane segments which give the foam its strength, and soft segments of (50/50) D/L lactide, ε-caprolactone and (6.5 Wt %) PEG (Mn = 1000 g/ mol). The PEG was added to make the PU more hydrophilic. The urethane segments had a uniform length of 5 urethane moieties (BDI-BDO-BDI-BDO-BDI) and an overall PEG content of 5.0 Wt %.

The PU was dissolved in 1.4 dioxane, till a concentration of 4 Wt % PU. After addition of water (7.5 Wt %) the solution was poured in a mould and cooled down to -18 °C. Next, the solution was freeze dried (3 mbar) to remove water and dioxane crystals. The end product is cylindrically shaped PU foam with pore sizes of 100-300 μm, interconnected pores of 10-30 μm and a porosity of 97 %. The foams were synthesized by Polyganics BV, Groningen, the Netherlands and sterilized with ethylene oxide prior to the study.

Methods
Approval for the animal study was acquired by the involved committee for animal experiments (University of Groningen, the Netherlands). Nineteen female NZW rabbits were obtained from Harlan BV, the Netherlands, which included extra rabbits to compensate for potential early loss due to dying of old age. The rabbits were housed in accordance with Dutch legislation for animal welfare.

All procedures (pre-, postoperative) and the surgical technique, were performed in accordance with the preceding study (4). In short, on all rabbits 5 mm perforations were created into the maxillary sinus with a drill, directly mesial of the left and right first premolars in the edentulous part of the maxilla (Figure 1). Next, all perforations were closed, either with the polyurethane foam, or conventionally with a buccal mucoperiosteal flap (Table 1). The latter being a common surgical treatment strategy for closure of OACs in clinical practice.

Figure 1 Lateral view of the rabbit’s skull after surgery, the arrow indicates the site of the bilaterally created 5 mm perforations in the edentulous part of the maxilla directly mesial of the premolars.

Table 1 Overview of the healing periods and the type of treatment applied in the rabbits, abbreviations: PU, polyurethane, L, left, R right, EM, electron microscope

<table>
<thead>
<tr>
<th>Time intervals</th>
<th>Rabbit #</th>
<th>Type of treatment</th>
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<tbody>
<tr>
<td>Group 1: 2 years</td>
<td>1-3</td>
<td>L &amp; R: PU foam</td>
</tr>
<tr>
<td>Group 2: 3 years</td>
<td>4-6</td>
<td>L &amp; R: PU foam</td>
</tr>
<tr>
<td></td>
<td>7 (EM)</td>
<td>L: PU foam</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: surgical closure</td>
</tr>
<tr>
<td>Group 3: 3 ½ years</td>
<td>8-10</td>
<td>L &amp; R: PU foam</td>
</tr>
<tr>
<td></td>
<td>11-13 (9 EM)</td>
<td>L: PU foam</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: surgical closure</td>
</tr>
<tr>
<td>Group 4: 4 years</td>
<td>14-16 (16 EM)</td>
<td>L &amp; R: PU foam</td>
</tr>
<tr>
<td></td>
<td>17-19</td>
<td>L: PU foam</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: surgical closure</td>
</tr>
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</table>

After sacrificing the animals at 4 different time intervals the surgical sites and sinuses were dissected and macroscopically inspected for abnormalities. Initially 3 time intervals were planned but because little loss of the animals occurred, an extra interval (3½ yrs) was included.

Specimen preparation and analysis
Samples were obtained from both the left and right maxilla and fixed in either 4 % paraformaldehyde in 0.1 M phosphate buffer for LM evaluation, or fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer for EM evaluation. All samples were decalcified in RDO varying from 80-120 hours.

The samples for LM were embedded in GMA. 2 μm sections were cut and stained with Toluidin Blue or Toluidin Blue/ Basic Fuchsin. The samples for EM were treated with 1 % osmium tetroxide, dehydrated in ethanol and embedded in Epox. Subsequently 2 μm samples were cut to determine the area with possible PU remnants. Selected areas were further cut into 60-90 nm ultrathin sections. These sections were placed on copper grids whereafter contrasting for 20 minutes with a 5 % solution of uranyl acetate in aqua bidest took place. Next, the samples were contrasted with a lead citrate according to Reynolds. Finally, inspection in a Philips (EM-CM 100) microscope at an accelerating voltage of 80 kv was carried out. Both the aspect and phase of degradation of any present PU foam were evaluated.
Results

Clinical observations
The creation of the OACs and subsequent treatment with either PU foam or a buccal flap procedure was carried out without complications in all rabbits. The animals were active post-operatively, gained weight in the course of the study, and showed no signs of wound infection, nasal discharge or maxillary sinusitis. Two rabbits however, died unexpectedly after respectively 3 and 3½ years. Autopsy showed in both cases that the cause of death was in all probability not related to the experimental treatment, as no abnormalities were observed in the region of the maxilla and maxillary sinuses.

Macroscopic observations
Upon dissection of the maxillary sinuses, a white mucus-like clot was seen inside the sinus of 5 rabbits at time intervals up to 4 years (#2-right, #9, #10, #11, and #20), appearing to be macroscopic remains of the PU foam (Figure 2). Besides this, no abnormalities were discovered macroscopically; all defects were closed on the oral side with normal palatal mucosa and the epithelium of the maxillary sinuses appeared healthy.

Light microscopic observations
Two years after surgery the left and right maxilla of rabbit #1 and the left maxilla of rabbit #2 showed that the antral mucosal lining had fully regenerated into ciliated epithelium with normal appearing submucous glands, supported by a connective tissue layer. No PU was found inside the maxillary sinus. The bony defect was fully bridged with newly formed bone. Very small PU particles, no longer with the typical porous structure, were identified in the fatty tissue located directly below the lower border of the maxillary sinus. Macrophages were found in their vicinity. The number and size of the PU particles seemed reduced compared to the PU fragments seen within the 1 year group of the preceding study.

In rabbit #2 a different view was seen in the right maxilla; the PU foam had most likely initially been located mainly inside the maxillary sinus, or had dislocated into the sinus shortly after surgery. A rather large part of the PU foam was still present in the sinus (Figure 3) and little PU fragments were found in the tissue below sinus level. Besides this, the perforation had fully closed with newly formed bone and the sinus epithelium was restored. The sinus epithelium showed signs of irritation (thickening and increase in number and size of goblet cells).

In rabbit #3 PU foam had probably also been placed partly inside both the left and right maxillary sinus, but only small PU particles could still be found. Also, macrophages containing PU were identified.

At 3 years, in all rabbits (#4-7) very small PU fragments were located mainly in the fatty tissue below the lower border of the maxillary sinus (Figure 4). Marked formation of new blood vessels was seen in the implantation area. Macrophages with PU particles were predominantly seen in the vicinity of small blood vessels. In all rabbits within this healing period the maxillary sinuses were free from PU foam.

Three and a half years after surgery varying observations were made. In most samples the degradation process had further proceeded. Single macrophages were iden-
Identified near blood vessels containing PU remains. Also, PU particles were seen in the wall of small blood vessels. Obviously, there was far less PU foam present at this stage compared to the samples obtained 2 years after surgery.

In a few cases however, the PU foam had most likely been pushed completely into the maxillary sinus like in rabbit #2 and its greater part was still present inside the sinus at this stage (rabbit #20 left maxilla, rabbit #10).

At 4 years, macrophages containing PU could still be identified, mostly in the fatty tissue. In none of the rabbits in this group PU fragments could be found inside the maxillary sinus.

The surgically closed defects in all 4 time intervals (Table 1) showed normal healing, e.g. no OACs reoccurred, bony closure was accomplished, and the architecture of the sinus was restored with the formation of ciliated epithelium supported by a connective tissue layer. No histological differences were observed between the healing of the surgically closed OACs and the OACs treated with PU foam.

Electron microscopic observations

Initially electron microscopy was scheduled to facilitate identification of PU fragments which would be too small to identify with the light microscope alone. However, within the samples of the 4 years group, structures that were highly suspected of PU fragments could still be identified with the light microscope. The electron micrographs confirmed that these structures were indeed PU fragments. Also, the micrographs of this study and the preceding study (3) were compared to optimize the identification procedure and showed that the PU fragments were indeed similar in size and distribution in macrophages.

Electron micrographs of the left maxilla of rabbit #7 (Figure 5) show intracellular PU remnants in different stages of degradation. This rabbit was sacrificed 3 years after surgery. Figure 6a+b show electron micrographs of the left maxilla of rabbit #7; an occasional macrophage with PU remnants enclosed is centrally located. Figure 7a+b show PU containing profiles of a macrophage 4 years after surgery (rabbit #16).

Discussion

In the preceding in vivo study (4) it was concluded that application of PU foam with 5% PEG for closure of OACs in NZW rabbits led to an uncomplicated recovery. In our opinion the PU foam proved in that study to be a suitable strategy for closure of OACs, although the presence of the PU foam did seem to prolong the process of the bony regeneration across the defect. In the same study, PU foam could still be identified 1 year after treatment. However, in earlier subcutaneous implantation study it was demonstrated that the degradation proceeds further in time and seems to be almost complete after 3 years (4). Therefore, in order to accomplish documentation of the end stage of degradation in this application, time intervals up to 4 years were included in the present study. Unexpectedly however, PU fragments could be found even 4 years after degradation. Although the very slow progress of degradation did not give rise to complications, the ultimate goal of total resorption has not been reached.

Based on the results of the present study 3 different scenario’s concerning the healing process of the OACs which were closed with PU can be pointed out. Firstly,
in rabbits in which the PU foam was fully incorporated in the tissue below the level of the maxillary sinus, PU particles were found mostly in the vicinity of blood vessels and enclosed in macrophages after 3 and 4 years. Although taking longer than expected, this represents the most ideal and normal process of PU degradation and removal.

Secondly, in other rabbits, the PU was displaced into the sinus probably in a very early stage due to mastication, resulting in a complete different course of degradation and restoration. As seen in the preceding study, when the PU foam bulged into the sinus, the regeneration of the sinus epithelium took longer to complete because it had to grow along the outline of the PU foam. Although this situation requires more time, no adverse events occur and healing is completed successfully in the end.

Lastly, when the foam is dislocated completely into the sinus, as occurred in rabbit #2 on the right side, the least favourable situation exists. The role of macrophages and certain enzymes in the degradation process of polyurethanes has been studied thoroughly in the past (7;8). Also, a humid environment is needed for the PU foam in order to fragment and degrade. Apparently these requirements for degradation are not met in the maxillary sinus; the entrapped free PU foam remains present, even 3 years after treatment. Although this situation did not lead to obvious clinical problems, the sinus epithelium showed signs of irritation because of the entrapped foreign body. Initially, it was expected that the ciliairy epithelium would be able to transport PU out of the maxillary sinus. However, this was not possible in these rabbits because the PU foam apparently did not disintegrate into transportable fragments.
In humans, such a situation is far less likely to occur as the human sinus floor is much thicker, which enables better fixation of the foam and thus diminishing the risk of dislocation. Also, patients can be instructed to minimize strain on the operated site. Furthermore, the relative amount of PU used in patients is very small compared to the rabbits.

The amount of remaining PU foam at the different time intervals was not measured quantitatively in this study, simply because the amount of PU may vary in each individual sample. However, in a large number of obtained samples it became clear that, although more slowly than expected, the number and size of the PU remnants as well as the number of macrophages containing PU diminished in time. Besides, a statement of total resorption of a biomaterial always seems risky because intracellular remnants of a biomaterial might be found long after expiration of the claimed resorption period, as demonstrated in earlier studies into a comparable PU foam (3).

The surgically closed defects were of value in 2 different ways. It facilitated a comparison between this commonly performed treatment and the PU treatment in terms of duration of the healing process and the quality of the regenerated tissues. No clear differences were observed between the 2 treatment strategies although the bony regeneration took longer in some rabbits because the bone grew around the PU foam instead of through it. Furthermore, the surgically treated defects helped in identifying the very small PU fragments by comparison of the samples with and without PU foam.

Based on the results of this study it can be concluded that the PU foam is a tissue friendly material and does not give rise to adverse effects. Although the end stage of degradation had still not been reached after 4 years, the gradual pattern of resorption and the tissue response to the PU foam are reassuring. The decreasing number of macrophages with internalised PU foam and the intracellular aspect of the foam suggest further degradation in time to an ultimate disappearance of the implanted material.

Human application of this alternative treatment strategy for OACs seems appealing because of its quickness and easiness and because it is less expensive than the common surgical treatment. However, care should be taken upon fitting of the PU foam to prevent it from displacement into the maxillary sinus with the risk of chronic maxillary sinusitis and subsequent problems. Clinical studies will therefore be implemented for further development and evaluation.

Reference List

(5) C. J. Spaans. Biomedical polyurethanes based on 1,4-butanediisocyanate: an exploratory study State University of Groningen, The Netherlands; 2000.