Chapter 6

A Novel Tracheal Tissue Connector for Fixation of Laryngeal Prostheses

by A.A. Geertsema, H.K. Schutte, G. Rakhorst, M.J.A. van Luyn, H.F. Mahieu, G.J. Verkerke

Abstract

A tissue connector (TC), basically consisting of a ring that will be integrated into the trachea, is under development to study the fixation of laryngeal prostheses.

Two experiments have been performed to test the TC in goats. In experiment 1, a polypropylene mesh was implanted around the trachea. The meshes were explanted after 6 and 12 weeks. In experiment 2, the actual TC consisted of 2 titanium rings (inner ring and outer ring) executed as quarter rings, fixed on each other, and a polypropylene mesh like a sandwich in between. The titanium inner ring was implanted between two tracheal rings thus penetrating the trachea with the mesh around the trachea and the fixed titanium outer ring on the outside of the trachea. The TCs were removed after 12 weeks.

Experiment 1 showed that the mesh was entirely infiltrated by host tissue. Inflammatory cells and high vascularisation were observed in 3 of 4 implants. However, in experiment 2, the mesh was completely incorporated by mature connective tissue without inflammation reaction. At some areas, deposition of cartilage tissue was observed.

In conclusion, the TC was firmly embedded in the trachea thus being appropriate for its intended use.
6.1 Introduction

Alternative fixation methods for laryngeal prostheses like tracheostoma valves (TSVs) and shunt valves are necessary. The most-used method to fix TSVs is gluing the valve on the skin around the tracheostoma. However, this fixation is only suitable for a selected group of patients. For this reason, TSVs are not often applied. Shunt valves are fixed by a form-closed connection; a fistula is created in the tracheoesophageal wall and a shunt valve with a suitable diameter is inserted. Sometimes, the diameter of the fistula increases after insertion, causing air leakage along the shunt valve. Another disadvantage of the form-closed connection is the replacement of the shunt valve, which is difficult to perform and uncomfortable for the patient.

The so-called tissue connector (TC) is an alternative fixation device that was developed to solve these problems. The TC basically consists of a titanium ring penetrating the tracheal epithelium. Thereafter, the valves can be placed in the ring (Fig. 1).

![Figure 1: The implanted tissue connector and its intended use; the fixation of a tracheostoma valve (left) and the fixation of a shunt valve (right).](image-url)
The titanium ring is fixed to a polypropylene (Marlex®) mesh, which is used clinically, for repair of abdominal wall defects\textsuperscript{2,3} for tracheobronchomalacia\textsuperscript{4,5,5} and experimentally for tracheal defects.\textsuperscript{6,7,7} The polypropylene mesh allows the ingrowth of soft tissue, thus anchoring the titanium rings to the trachea. The biocompatibility of the TC has been tested by implanting the TC percutaneously in the backskin of rats and proved to be appropriate for its intended use, as previously reported.\textsuperscript{1}

This paper describes the next two steps in the development of the TC penetrating the trachea:

1) Testing the polypropylene mesh, meant as the anchorage part of the TC, by implanting the polypropylene mesh around the trachea of goats.

2) Testing the TC in situ in the trachea of goats by implanting the polypropylene mesh with fixed titanium rings, that penetrates the tracheal epithelium.

6.2 Materials and Methods

6.2.1 Implants

The TC was tested in two experiments; in each experiment a different implant was used. The implant used in the first experiment was the base of the tissue connector (TC); a polypropylene mesh (Bard® Marlex® mesh, Bard Benelux N.V., Leuven, Belgium) with dimensions 2.5 * 10 cm (Fig. 2).
In the second experiment, the complete TC was implanted (Fig. 3). It consists of two titanium ($\text{TiAl}_6\text{V}_4$) rings, a titanium inner ring and a titanium outer ring both enclosing a polypropylene mesh (Fig. 4). The inner ring penetrates the tracheal epithelium when placed in situ. The titanium rings consisted of only a quarter of the ring to limit complications during the experiments. The titanium rings were connected to each other by means of 3 titanium screws. The polypropylene mesh was sterilised by the manufacturer. The titanium rings of the implants were thoroughly cleaned, followed by autoclave sterilisation.

### 6.2.2 Surgical procedure

The two experiments described in this section were approved by the faculty animal ethical committee.

![Figure 3: Photo of complete Tissue Connector](image)

**Figure 3:** Photo of complete Tissue Connector

![Figure 4: Tissue Connector implanted in the trachea; (a) anterior view, (b) top view and (c) cross-section of the rings of the tissue connector; grade of epithelium downgrowth: from minimal epithelium downgrowth (4) to maximal epithelium downgrowth (0).](image)

**Figure 4:** Tissue Connector implanted in the trachea; (a) anterior view, (b) top view and (c) cross-section of the rings of the tissue connector; grade of epithelium downgrowth: from minimal epithelium downgrowth (4) to maximal epithelium downgrowth (0).
Experiment 1

Four goats were used in this experiment. The ventral side of the neck of the goat was shaved, cleaned, and disinfected. The skin was incised at the midline between mandible and sternum. The pre-tracheal muscles were dissected and the upper tracheal rings of the cervical trachea exposed. The polypropylene mesh was cut thus fitting around 50% to 75% of the circumference of a 2.5-cm segment of the anterior trachea. The polypropylene mesh was bonded to the external surface of the trachea with fibrin glue (Tissucol Kit, Immuno N.V., Brussel). The pre-tracheal muscles and the skin were closed by sutures. All surgeries were done under full sterile conditions.

Experiment 2

In this experiment again, four goats were used undergoing almost the same surgical procedure as in the first experiment. In this experiment, however, the TC was implanted. After cutting to fit the polypropylene mesh like in experiment 1, the titanium outer ring was screwed on the titanium inner ring with the polypropylene mesh in between (Fig. 4).

An incision was made between 2 tracheal cartilage rings in such a way that the titanium inner ring fitted between these 2 tracheal rings. The tissue connector was placed in the trachea. The edges of the polypropylene mesh were sutured to the trachea. A mattress suture over the titanium outer ring was used to close the trachea under mild tension. Finally, the polypropylene mesh and titanium rings were bonded to the trachea with fibrin glue. Tracheoscopic examination was performed periodically by a flexible endoscope (model 7220 Wolf 181155, Richard Wolf GMBH, Knittlingen, Germany) under general anaesthesia.

The characteristics of the implants are listed in Table 1.

<table>
<thead>
<tr>
<th>Implant</th>
<th>Material</th>
<th>Implantation time (weeks)</th>
<th>Experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC 1, TC 3</td>
<td>Polypropylene mesh</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>TC 2, TC 4</td>
<td>Polypropylene mesh</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>TC 5, TC 6, TC 7, TC 8</td>
<td>Polypropylene mesh and titanium rings</td>
<td>12</td>
<td>2</td>
</tr>
</tbody>
</table>
The general health condition of the goats was checked by monitoring their eating pattern and weight control. In experiment 1, two goats were sacrificed six weeks after the operation and the other two goats twelve weeks after the operation. In experiment 2, all the goats were sacrificed after twelve weeks. The goats were sacrificed by injection with Nembutal and the TCs with surrounding tracheal tissue were surgically removed.

6.2.3 Histological procedures

Immediately after explantation, the TCs with their surrounding tissue were fixed in 4% P-formaldehyde buffer (PFA) in 0.1 M phosphate buffer. Successively, the implants were trimmed to remove superfluous tissue followed by ethanol dehydration and embedded in methylmethacrylate. Thin sections (20-30 µm) were cut on a sawing blade machine (Leica 1600, Rijswijk, Holland) in the longitudinal direction for experiment 1, in both longitudinal and transversal direction for experiment 2. Sections were stained with basic fuchsine and toluidine blue for examination by light microscopy.

6.2.4 Histological Assessment

Three evaluation criteria were defined to assess the implants:

1) Quantification of epithelium downgrowth along the titanium inner ring;
2) Morphology of soft tissue around the titanium outer ring;
3) Morphology of soft tissue around the polypropylene fibers.

A histologic grading scale (Table 2) was used to quantify these categories. The downgrowth of epithelium is subdivided in 5 areas (Fig. 4c).

No downgrowth scores 4 points, downgrowth till or further than the edge of the ring scores 0 points. The other categories (2 and 3) consist of two subcategories; occurrence of inflammatory cells (like granulocytes, macrophages, giant cells) and the development of fibrous tissue (stage of fibroblasts). Soft tissue in category 2 and 3 scores 4 points, when connective tissue is mature (2 points) without inflammatory cells (2 points). Zero points means no connective tissue (0 points) and many inflammatory cells (0 points). For obvious reasons, the implants of the first experiment were only quantified for category 3. The implants of the second experiment were quantified for all categories.
Table 2: Histologic Grading Scale

<table>
<thead>
<tr>
<th>Evaluation Criteria</th>
<th>Outcome</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epithelial downgrowth (Fig. 4c)</td>
<td>no downgrowth</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>along 1/3 titanium part</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>along 2/3 titanium part</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>along complete titanium part</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>along polypropylene mesh</td>
<td>0</td>
</tr>
<tr>
<td>Soft tissue at surface outer ring</td>
<td>Mature connective tissue</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Immature connective tissue</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>No connective tissue</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>No inflammatory cells</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Few inflammatory cells</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Many inflammatory cells</td>
<td>0</td>
</tr>
<tr>
<td>Soft tissue around polypropylene fibers</td>
<td>Mature connective tissue</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Immature connective tissue</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>No connective tissue</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>No inflammatory cells</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Few inflammatory cells</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Many inflammatory cells</td>
<td>0</td>
</tr>
</tbody>
</table>

6.3 Results

6.3.1 Macroscopic results

The animals of both experiments remained in good general health during the entire experiment. They took normal amounts of food and did not lose weight. The recovery of the operation sites after the surgical procedure went well. When the implants of experiment 1 and 2 were explanted, the polypropylene mesh was surrounded by a fibrous capsule and was well attached to the trachea. No infection, redness, or swelling of the tracheal tissue was observed macroscopically.

The tracheoscopic examination of experiment 2 showed the development of white-coloured tissue with clinical aspects of granulation tissue of about 3-mm high around the inner ring of TC 5. After one week this tissue had almost disappeared. The titanium inner ring was observed to be in close contact with the tracheal tissue during the rest of the implantation period. After explantation, TC 5 appeared to be in close contact with the mucosal tracheal tissue in the transversal direction, but
was retracted in the longitudinal direction. In contrast to TC 5, the inner ring of TC 6, TC 7, and TC 8 was overgrown with tracheal epithelium in less than three weeks. Tracheoscopic examination of these TCs showed also development of granulation tissue near and over the inner ring, which also disappeared after some weeks.

6.3.2 Microscopic results

The implants were evaluated microscopically by applying the histologic grading scale (Table 1). Histological examination of experiment 1 showed that the meshes were entirely infiltrated by host tissue and completely incorporated by the body. In TC 1, TC 2, and TC 3, inflammation cells like macrophages, giant cells, granulocytes and occasionally plasma cells indicating tissue remodelling were observed in combination with many blood vessels in TC 2 and TC 3 (Fig. 5). In TC 4, regression of blood vessels was observed without inflammatory cells, but in combination with mature connective tissue (Fig. 6). The scores of soft tissue around and in between the polypropylene fibers are shown in Table 3. Expected higher scores for 12 weeks versus 6 weeks does occur for 12-weeks implant TC 4 compared to 6-weeks implants TC 1 and TC 3, but not for 12-weeks implant TC 2 which scores lower than TC 1 and TC 3.

![Figure 5: Longitudinal section of soft tissue with polypropylene fibers (P) of TC 2. A high vascularization (V) in the surrounding tissue (S) is observed. Furthermore, some inflammatory cells (I) and connective tissue (C) surrounded the fibers.](image-url)
A novel tracheal tissue connector for fixation of laryngeal prostheses

Figure 6: Longitudinal section of soft tissue with polypropylene fibers (P) of TC 4. Polypropylene fibers are surrounded by connective tissue (C).

Table 3: Histologic grading of experiment 1.

<table>
<thead>
<tr>
<th>Tissue Connector</th>
<th>TC 1</th>
<th>TC 2</th>
<th>TC 3</th>
<th>TC 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Implantation time</td>
<td>6</td>
<td>12</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>Soft tissue at polypropylene fibres</td>
<td>1.5</td>
<td>0.9</td>
<td>1.0</td>
<td>1.9</td>
</tr>
</tbody>
</table>

Table 4: Histologic grading of experiment 2.

<table>
<thead>
<tr>
<th>Tissue Connector</th>
<th>TC 5</th>
<th>TC 6</th>
<th>TC 7</th>
<th>TC 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epithelial downgrowth</td>
<td>2.4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Soft tissue at titanium outer ring</td>
<td>1.9</td>
<td>2.5</td>
<td>3.1</td>
<td>2.6</td>
</tr>
<tr>
<td>Soft tissue at polypropylene fibres</td>
<td>3.4</td>
<td>3.7</td>
<td>3.6</td>
<td>3.5</td>
</tr>
</tbody>
</table>

Table 4 gives the results of the microscopic assessment of experiment 2. Epithelium downgrowth only occurred in the longitudinal section of TC 5 (Fig. 7). In the transversal section the mucosa is in close contact with titanium (Fig. 8). Epithelium had grown over the titanium inner ring of TC 6, TC 7 and TC 8 (Fig. 9), thus scoring 4 points. In the longitudinal section mucosal tissue is not connected to
the titanium inner ring in contrast to the transversal section, where mucosal tissue is always in close contact to the titanium inner ring.

Figure 7: Picture of the gap between soft tissue and the titanium inner ring (T) of TC 5 in a longitudinal section. Downgrowth of epithelium (E) along the titanium is shown (C = connective tissue).

Figure 8: Soft tissue near the titanium inner ring (T) of TC 5 in a transversal section. Fibrous tissue (F) has formed near the titanium, epithelium (E) shows no tendency to grow down (C = connective tissue; A = artefact).
A novel tracheal tissue connector for fixation of laryngeal prostheses

Figure 9: Soft tissue on top of the titanium inner ring (T) of TC 8 in a transversal section. Epithelium (E) with connective tissue (C) has completely overgrown the titanium inner ring. A fibrous capsule (F) has formed around the titanium.

The results of the soft tissue assessment at the titanium outer ring of experiment 2 are also presented in Table 4. In all TCs a thick fibrous capsule mainly containing fibroblasts and collagen-like structures surrounded the titanium outer ring. Bundles of mature connective tissue were observed parallel to the surface (Fig. 10a). Regularly, a layer of fibrous tissue with cells like fibroblasts and macrophages were detected near the titanium ring (Figure 10b). The layer sometimes also contained a few granulocytes, but never granuloma. The scores of the soft tissue of the TC at the titanium outer ring of experiment 2 vary from 1.9 to 3.1 indicating the differences in maturity and the infiltration of inflammatory cells in the fibrous capsules.

The space between the fibers of the polypropylene meshes of experiment 2 was filled with mature connective tissue (Fig. 11) and deposition of new cartilage tissue was observed as well (Fig 12) in all TCs. Only occasionally, scattered foci of macrophages and granulocytes were detected. The scores of soft tissue formed around the polypropylene fibers were higher compared to the scores of soft tissue around the titanium outer ring, as the connective tissue is mature and nearly free from inflammatory cells.

Between the titanium inner ring and outer ring and on the place where polypropylene is fixed to the titanium, cell debris and some inflammatory cells were observed without vascularisation.
6.4 Discussion

The developed TC was previously implanted in the backskin of rats by a 2-stage surgical procedure: first the subcutaneous implantation of the mesh and 6 weeks later the percutaneous implantation of the titanium stylus. The TC was found to be biocompatible.\textsuperscript{1} In this article, the next step, the function of the TC was tested in the trachea of goats.

One of the most apparent phenomena are the much higher scores of soft tissue at the polypropylene fibers in experiment 2 compared to experiment 1 (Table 3, 4). These scores are also higher compared to the scores of soft tissue at the polypropylene fibers in the backskin of rats, which ranged from 2.4 to 2.8. A possible explanation for these differences is the mechanic forces on the polypropylene mesh caused by the relative movement of the trachea to the surrounding skin. In experiment 1 the polypropylene mesh was only fixed by fibrin glue, whereas in experiment 2 also sutures have been used and the titanium inner ring was fixed between two tracheal rings offering extra anchorage and stabilisation. This leads to a milder inflammatory body response resulting in high scores for the soft tissue reaction; even higher than the scores of soft tissue at polypropylene fibers in the backskin of rats.

The goat was chosen as experimental animal because the anatomy and size of its trachea is comparable to humans. However, one should realize that the goat is a difficult laboratory animal, as the goat is a ruminant. This means increased tracheal
movements compared to humans thus providing a very sensitive model. These movements are probably also a reason for the gap between tissue and titanium inner ring. This gap is only observed in the longitudinal sections (Fig. 7), as these movements mainly causes tension in the longitudinal direction of the trachea. Extra mattress sutures over the titanium outer ring could possibly prevent this gap formation.

One of the major problems in this experiment is the epithelium overgrowth of the TC, which could probably be prevented by redesigning the titanium inner ring. A suggestion for a new design is using appropriate microgrooves \(^8\text{-}^{10}\) to prevent epithelium overgrowth. Another approach of this problem could be the surgical removal of the inner ring covering tissue during implantation.

The most positive outcome of these experiments is the finding of cartilage in the neighbourhood of polypropylene in experiment 2. This cartilage tissue could provide an excellent anchorage of the tissue connector to the trachea. Longer implantation periods should be used to make clear if the deposition of cartilage tissue will continue. This deposition of cartilage tissue was not observed by Okumura et al \(^6\text{-}^{7,11,12}\), who studied trachea reconstruction by a new tracheal prosthesis made from polypropylene mesh and coated with collagen. It was completely incorporated by connective tissue, but deposition of cartilage tissue was not observed.
Between the titanium rings (Fig. 4) and on the place where polypropylene mesh is fixed to the titanium cell debris and some inflammatory cells appeared. This can be explained by the limited space between the titanium rings making vascularisation impossible. Possible solutions are increasing this space or sealing this space.

Figure 12 Cartilage tissue (CT) and connective tissue (C) near polypropylene fibers (P) of TC 5; inlay: detail of cartilage tissue.

The endoscopy was performed with a flexible endoscope, but it was rather difficult to see the tissue near the inner ring of the TC. In the future, endoscopy performed by a 90° endoscope with short-distance focus could make it easier to get more distinct pictures from that area.

In contrast to the implantation of the TC in the backskin of rats, which was performed by a 2-stage surgical procedure\(^1\), experiment 2 is performed by a 1-stage surgical procedure. This experiment proves that a 2-stage operation procedure is not necessary for gaining good histological and functional results.

The intended use of the novel TC is the fixation of laryngeal prostheses like tracheostoma valves and shunt valves to the trachea. These valves are exposed to forces due to the present tracheal pressure during speaking and forces due to opening and closing the tracheostoma valve. This experiment showed that this TC appeared to be firmly embedded in the trachea, which seems to make the TC appropriate for its intended use and a potential improvement of existing fixation methods. So, application of the TC in humans is promising, and the fact that the goat is a very sensitive model contributes to this. In the future, the next step will be
the implantation of a complete ring, as the implantation of a quarter of a ring has been proven successful.

Acknowledgements

We would like to thank Dr. D. Mihaylov for performing the experimental surgery. We would also like to express our gratitude to Ir. A. van der Plaats and to the co-workers of the Central Animal Laboratory for their assistance.

References


