Clinical and experimental aspects of tracheal stenosis
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Total Tracheal Replacement in Rabbits with a New Composite Silicone-Metallic Prosthesis and Biocompatible Inner Lining

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Abstract

A new composite silicone-metallic prosthesis was tested, studying the potential for respiratory epithelial covering over the biocompatible inner lining, in a rabbit survival model. Seven New Zealand White rabbits underwent near-total excision of their trachea and implantation of a sterile prosthesis. After 2 months, they were sacrificed and the prostheses were retrieved. Specimens were fixed and histologically examined for tissue reaction around the prosthesis, at the anastomotic lines, and particularly for the presence or absence of epithelialization of the inner lumen over the biocompatible surface. All rabbits survived the operation. At 2 months, the outer layer of the prostheses was consistently covered with fibrosis and neutrophils. The inner layer showed necrotic cells and scant reepithelialization over the biocompatible lining, up to 5mm beyond the anastomosis, with no evidence of organized respiratory epithelium in the middle sections. The new prosthesis is a viable temporary solution for airway replacement in rabbits. Granulation tissue was not observed at the anastomosis, reepithelialization did occur, but failed to achieve full-length luminal covering. The potential for granulation tissue does not yet make this an ideal long-term solution. Improvements in prosthesis design or biocompatibility are required, and need to be reevaluated before applicability for chronic use.
Introduction

Reconstruction of the major airway has long been a particular challenge, and is preferably performed by resection and direct anastomosis, by repair, or by replacement with autologous material. Be it for congenital long-segment tracheal stenosis, post-intubation or other airway trauma, burns, chemical strictures, or neoplastic lesions of the trachea and bronchi, the extent of diseased airway does not always allow for primary reconstruction, and plasty or replacement with allografts or foreign material becomes inevitable. Beyond the scope of biological materials that are well documented with their respective advantages and disadvantages, their rarity and potential for complications make a commercially available prosthesis an attractive alternative.

Fully synthetic prostheses have been developed and tried in animal studies and human trials for decades, most often with disappointing results. We report our results from a new composite tracheal prosthesis with a biocompatible inner lining in a rabbit survival model, and discuss the advantages, limitations, and possible improvements to be made before its potential clinical use.

Methods

Seven New Zealand White adult-size rabbits underwent surgery according to an established protocol, described elsewhere in detail. The study protocol was reviewed and accepted by the Hospital Research Ethics Committee, and animals received humane care in compliance with the “Guide for Care and Use of Laboratory Animals” published by the Institute of Laboratory Animal Resources, National Research Council, and published by the National Academy Press, revised 1996.

Briefly, using sterile surgical technique and one prophylactic subcutaneous dose of enrofloxacin (5 mg/kg), a collar incision is made, and the trachea is transected in 2 places, leaving a gap of 1.5-2 cm to be bridged by the prosthesis. Using an uncuffed sterile endotracheal tube, the rabbit is intubated retrogradely through the vocal cords and connected to the ventilator. The endotracheal tube is slid through the sterili prosthesis, and its tip is therefore beyond the transected portion into the distal airway, for maintenance of anesthesia and ventilation/oxygenation of the animal. The prosthesis is telescoped into both ends of the transected trachea. End-to-end anastomoses are performed at both ends with interrupted 6-0 polydioxanone (PDS) sutures (Figure 1). The repair is tested for air leaks and appropriately covered with muscle and fascia using interrupted 3-0 Vicryl as necessary, followed by skin closure.
Figure 1. Perioperative aspect of an implanted prosthesis in a rabbit.

Figure 2. Photograph of the tracheal prosthesis with a silicone outer cover, and inner coiled metallic wire with biocompatible inner lining, in comparison to a number 15 surgical blade.

Figure 2 shows a photograph of the prosthesis used in the experiment, consisting of two parts: an inner spiral of metallic wire coated with a biocompatible polymer, and an outer cover tube of medical-grade silicone rubber (Figure 2). The spiral fits into the lumen of the silicone tube, and protrudes by 4 mm from each side. The spiral is made from coiled stainless steel wire (diameter = 432 micrometers) that bears a thin adherent coating of a hydrophilic polymeric biomaterial on its surface. The spiral has several functions: (a), it provides adequate stiffness, thus preventing narrowing of the prosthesis by compression or kinking; (b), it renders the prosthesis visible to x-ray fluoroscopy; (c), the polymer biomaterial coating provides a biocompatible surface which may promote the adherence and growth of an epithelial cell lining in the lumen of the prosthesis. The hydrophilic polymer is SlipSkin biomaterial (MCTec BV, Venlo, The Netherlands), a copolymer of N-vinyl-pyrrolidinone and n-butylmethacrylate in the molar ratio 9:1. The coating was applied to the metallic wire in a continuous extrusion-like procedure, as described previously; the thickness of the coating was in the range 4-6 micrometers. The silicone tube is rubbery, allowing better handling and suture characteristics for fitting the prosthesis into the trachea, and provides a stable and bio-inert outer surface to the prosthesis.
The animals were sacrificed at 8 weeks (ketamine (35-50 mg/kg im) and xylazine (5-10 mg/kg im), followed by intravenous pentobarbital (100 mg/kg)) and their tracheas were harvested in full length. The prostheses were harvested along with the rabbit trachea in a fashion so as not to disturb the 2 anastomotic lines.
Specimens of trachea were fixed in 10% buffered formalin. Microscopic analysis of the tracheal wall assessed the inner lining of the prosthesis with emphasis on reepithelialization, and the presence or absence of granulation tissue at the anastomotic lines. Sections divided the prosthesis in 4 parts, from the proximal end near the vocal cords to the distal end near the carina, each measuring 5mm in length. Staining was performed with haematoxylin-eosine.

**Results**

All rabbits survived the operation. Three rabbits presented with increasing respiratory difficulty and stridor at 3 weeks, requiring early sacrifice. Intraluminal Candida infection was present in 2 of these rabbits.

Histological analysis of the outer silicone covering of the prosthesis consistently revealed diffuse fibrosis in all 7 rabbits.

The inner surface of the biocompatible polymer surface showed infiltration of neutrophils, thrombosis of vessels, local tissue necrosis, with degrees varying from mild to moderate according to the position within the prosthesis. Consistently, the middle halves, in other words beyond 5 mm from the proximal and distal edges of the prostheses, were devoid of any sign of reepithelialization, and showed areas of thrombosis, fibrosis, and necrosis in the lumen. In the other rabbit specimens, scant reepithelialization of the inner coating was apparent at both ends of the prostheses, but in a disorganized fashion, and only up to 5mm.

**Discussion**

The ideal tracheal substitute should be readily available in all sizes, be biocompatible with minimal or absent rejection as a foreign body, be non-carcinogenic, allow for respiratory epithelial overgrowth and patient somatic growth, resist infection, be flexible to avoid erosion into adjacent structures, yet sturdy enough to be noncollapsible, be air and waterproof, and stand the test of time.Ş.
Chapter 7

Covering of the anastomotic line by specialized respiratory epithelium, derived from overgrowth of adjacent normal cells, with a resultant functional intraluminal lining represents the optimal outcome after any surgical procedure on the airway, be it with incorporation of autologous tissue, with allograft material, or with synthetic prostheses. Clinically, this may lead to quicker extubation or decannulation, and shorter hospitalization time. Complete regeneration of the epithelium restores clearing of secretions, making the cough mechanism efficient, with maintenance of a wide-open airway. Also, granulation tissue, the true Achilles' heel of airway surgery, does not appear in the presence of an intact epithelium, but rather results when breaches in the epithelium are present. It seems intuitive that a surgical anastomosis between two epithelialized surfaces, i.e., autologous trachea-to-trachea, would heal the easiest and quickest, yet granulation tissue formation does occur in this setting, by mechanisms as yet not fully understood.

Efforts towards promoting a normally functioning airway have been directed at enhancing tracheal anastomotic healing by various mechanisms, the most obvious of which are meticulous surgical technique, and optimal vascularization. This has been successful either by preservation of the existing tracheal blood supply, manoeuvres of omental wrapping as an additional blood source, or promotion of local angiogenesis by topical vascular growth factors, to name a few. Others have achieved in vitro or in vivo growth of human respiratory epithelium on inert foreign bodies prior to their reimplantation, and propose this as a potential reproducible tracheal substitute, with improved postoperative healing. In the clinical setting, however, this is cumbersome and time consuming, and not always applicable, specifically in an emergency setting.

Various fully synthetic materials have been used in animal models with varying results. Most reports focus their results on the presence or absence of respiratory epithelial overgrowth, which is used as a measuring stick of biocompatibility, and hence success. Without being exhaustive, a few illustrative studies are summarized. In a canine model (n=9), Cull et al. tested polytetrafluoroethylene (PTFE) grafts as tracheal substitutes. After resecting 5 cm of trachea (7-8 tracheal rings), the grafts were implanted and the dogs were followed bronchoscopically with concomitant endoscopic photography. All developed granulation tissue resulting in airway obstruction after 3 to 8 weeks postoperatively. Upon histological examination, no epithelial growth occurred over the graft, making the authors conclude that PTFE is unsuitable for long-segment tracheal reconstruction. This lack of epithelialization over PTFE grafts correlates with the results of Shaha and associates, who were nonetheless satisfied with the mid-term (3-4 months) clinical tolerance of the grafts in dogs. They raised the concern over the intense
inflammatory reaction and resultant fibrosis created at the outer surface of the grafts. To the contrary, other reports using PTFE both in rabbits and in dogs demonstrated epithelialization of the lumen after 2-4 weeks, with emphasis on an anastomotic technique invaginating the trachea within the graft \(^{17,18}\).

Mendak et al. tested a more sophisticated titanium fiber metal prosthesis with a bioabsorbable inner lining in dogs \(^{19}\). Tissue ingrowth was demonstrated, and the inner coatings of polyglactin-910 and copolymer 75% poly-L-lactic acid/ 25% polyglycolic acid achieved the best results. At 2-4 months postoperatively, they found minimal reepithelialization at the anastomosis, but argued that even in the absence of ciliated epithelium, the ability to clear secretions is not hindered, and satisfactory clinical results are achieved \(^{16}\).

The largest clinical experience with a fully prosthetic tracheal substitute has been that with the Neville silicone prosthesis \(^7\). As these were most often used in the setting of tracheo-pulmonary malignancy, patient survival was determined and probably shortened by the underlying disease. However, the authors found the performance of the prostheses in survivors encouraging, with minimal graft-related morbidity. This included suture line granuloma and hemorrhage from erosion of the innominate artery. Despite an absence of epithelial covering on the silicone prosthesis, they concluded that this may not be an essential factor, as the patients demonstrated little clinical discomfort, and were able to clear secretions adequately \(^7\).

Future prospects may involve the abandoning altogether of prosthetic materials, and focusing not only on biomaterials, but bioactive ones \(^{20}\). These materials interact with surrounding tissues according to timed physico-chemical interactions, either by incorporation of adhesion factors, growth factors, chemically based biorecognition, enzymatic recognition and transformation, to name a few \(^{20}\). If some sort of prosthetic support is to be used, at least the extremity or surface to be in contact with the host’s tissues could be coated with bioactive biomaterials along these same principles.

In conclusion, the current prosthesis was easily implantable, had good handling characteristics, and may be commercially produced in all sizes. It proved air and watertight, and was rigid. However, multiple drawbacks still exist. First of all, it doesn’t grow, and carries the same risk of infection common to all foreign bodies. Secondly, the time interval of the study did not allow assessment of rejection phenomenon or carcinogenicity, although this remains speculative, and would not have been expected. More importantly, the primary question of the study as to the true biocompatibility of the inner lining, namely the capacity to allow for epithelial overgrowth, was answered partially in a satisfactory but not optimal manner. The absence of granulation tissue across the anastomotic line was encouraging, and with a longer sacrifice
time, perhaps full reepithelialization would have been achieved. Although others have stated that reepithelialization inside a prosthesis is not required to achieve good clinical results, most argue that it is a sine qua non condition for optimal healing and long-term success. We are therefore cautious as to the future applicability of our prosthesis in its present design.

Two orders of improvement can be brought about in order to enhance the clinical results: the first is of design, and the second involves the biocompatible lining. The telescoping of the prosthesis into the native tracheal lumen is potentially traumatic to the respiratory epithelium, as proposed by others. Inverting the prosthesis design so as have the inner rigid wire layer apposed to the epithelium may enhance healing and reepithelialization. Secondly, the biocompatible lining can be made bioactive, by incorporating growth factors that could stimulate the proliferation of epithelial cells. The development of tissue engineering concepts in parallel to mechanical improvements require further in vivo and in vitro investigation, which are ongoing in our laboratories, and are hoped to improve clinical results in the future.

References


