Autologous Tissue Engineered Trachea with Epithelial Cell Sheets in Ovine Model

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Introduction

A variety of inert materials have been used as tracheal prostheses either alone or in combination with autologous tissue. However, difficulties arise with the use of prosthetic materials because of their propensity for infection and extrusion. Moreover, autologous tissues are often times limited by poor structural integrity and their use often involves high technical complexity (1). Our specific goals are to focus on the creation of an autologous tissue engineered cartilage construct shaped as a helix to form the structural component of a functional tracheal replacement, with tracheal epithelial cell sheets. The study focused on generating this construct utilizing techniques and materials already within FDA regulation to be able to attain a clinically useful therapy within a reasonable timeframe.

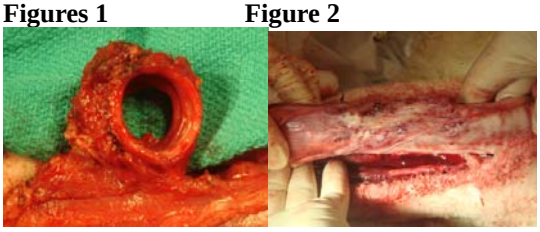
Materials and Methods

5 x 5mm samples of sheep nasal septal were obtained from 2 month-old sheep. Chondrocytes were isolated by digestion of cartilage in collagenase, and epithelial cells were also obtained from the mucosal lining of the same sample. After 2 weeks of culture, epithelial cells were stored and chondrocyte suspensions were placed on 100 mm x 10 mm x 2 mm polyglycolic acid mesh fibers. This chondrocyte-seeded mesh was placed in the grooves of a 20mm diameter x 50mm long helical silicon template and implanted under the sternocleidomastoid (SCM) muscle of the corresponding sheep. 8 weeks post-implantation, the silicon template was removed from the autologous Tissue Engineered Trachea (TET) while keeping the vascularized TET connected to the SCM muscle pedicle (Fig.1). Then, a 7 cm circumferential cervical trachea segment was excised, and the autologous TET was transplanted to the site by an end-to-end anastomosis. The engineered epithelial cell sheet (2 weeks prior to transplantation, epithelial cells were cultured on temperature-responsive culture inserts) was wrapped around the external surface of the TET, including the site of anastomosis. A silicone stent (10 cm) was inserted before completing the distal anastomosis. Internal coverage of the entire length of the TET by the stent was confirmed by bronchoscopy, and the stent was secured in place to prevent migration with two sutures placed at its oral end. Prior to closing the surgical site, the entire vascularized construct was covered with the SCM muscle pedicle. Sheep was euthanized at 4 weeks and the TET was evaluated.

Results

The sheep tolerated the surgical procedure well with no perioperative complications. The proximal and distal anastomosis sites of the TET transplant were clearly visible through the transparent stent, and exhibited no problems at 1 week by bronchoscppy. The gross morphology of the TET was a white, shinny, hard tissue with confirmed epithelization along its entire length, including both anastomosis sites (Fig.2). However, cartilage content of the tissue was less than 10 % of the TET.

Figures 1 **Figure 2**



Discussion and Conclusions

We have demonstrated that a long, circumferential tracheal defect can be successfully transplanted with a TET covered with an epithelial sheet and supported by an inert stent. The external wrapping of the epithelial cell sheet is a key point in our study (2). Our studies in autologous models have shown that viable TETs can be generated with almost any cell source, such as primary chondrocytes or mesenchymal stem cells, in combination with various materials. However, in order to be able to transfer our tissue engineering techniques into the clinic for the benefit of patients, it is also very important to develop appropriate surgical procedures, and ideal cell culture conditions and preparation.

References

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Disclosures

Include disclosure information here, or a statement that authors have nothing to disclose.