Tissue-Engineered Cartilage as a Graft Source for Laryngotracheal Reconstruction

A Pig Model

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Objective: To evaluate the feasibility of using tissue-engineered cartilage for laryngotracheal reconstruction in the pig model.

Design: Auricular cartilage was harvested from 3 young swine. The cartilage was digested, processed, and suspended and a cell culture was obtained. The cells were then suspended in 3 mL of a 30% solution of a biodegradable polymer (Pluronic F-127) (polyethylene oxide/polypropylene oxide copolymer) at a cellular concentration of 50 x 10^6 cells/mL. This suspension was then implanted subcutaneously into each pig's dorsum. Eight weeks after implantation, the cartilage was harvested with the surrounding perichondrial capsule. An anterior cartilage graft laryngotracheal reconstruction was performed. Bronchoscopy was performed at 3 postoperative weeks to demonstrate airway patency. The animals were killed at 3 months, and specimens were obtained for histological analysis.

Setting: An animal research facility.

Subjects: Three young Yorkshire swine.

Results: All 3 pigs survived to the 3-month postoperative interval with no evidence of stridor or airway distress. Interval bronchoscopy revealed a normal patent airway with a mucosalized graft. Histopathologic analysis revealed incorporation of the tissue-engineered cartilage graft in the cricoid area, which correlated with results of bronchoscopic evaluation.

Conclusion: Tissue-engineered auricular cartilage served as a viable graft in the pig model and might be an alternative cartilage source for laryngotracheal reconstruction.

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The surgical treatment of subglottic stenosis has evolved during the past century to encompass procedures designed to enlarge the airway diameter by (1) allowing the stenotic segment to remain in situ while augmenting the subglottic region or (2) resecting the subglottic scar with anastomosis of the patent airway segments. The most popular current approach for the laryngotracheal augmentation is the use of autologous cartilage, although local tissue such as hyoid bone has been used successfully. Cartilage has been harvested from the auricle, the thyroid ala, or the costal area. Rib cartilage grafting has become the standard procedure for pediatric laryngotracheal reconstruction.

The disadvantages of the rib graft donor source include donor site pain and scarring, limited tissue availability, and the potential complication of pneumothorax during the harvest of rib cartilage. In an effort to diminish these disadvantages, irradiated costal cartilage and alcohol-stored auricular cartilage have been used in animal models. Both demonstrate significant resorption. Autologous auricular and thyroid ala cartilage grafts have been used with some success, but remain useful primarily for anterior grafting where wide cricoid distraction is not needed. For more complex repairs, autologous rib cartilage remains the standard source of graft material for the cricoid implant. The sculpturing of a graft to fit within the airway lumen and to afford wide distraction requires a larger cartilage tissue block. The theoretical potential to generate a sizable block of rigid cartilage with perichondrium that can be sculpted to provide distraction was the motivation for the present experiment.

Tissue engineering combines living cells with biocompatible and biodegradable polymers to produce new tissue. Cartilage can be generated successfully in vitro and in vivo by using animal and human chondrocytes. In this study, cartilage was generated as a firm mass in the subcutaneous tissue by injection of a biode-
gradable polymer (Pluronic F-127; BASF, Mount Olive, NJ) seeded with autologous auricular chondrocytes. Pluronic F-127 is a thermosensitive polymer liquid at 4°C that polymerizes to a thick gel at body temperature and degrades as the cartilage is generated in the subcutaneous tissues.13,14

METHODS

Autologous auricular cartilage was harvested from 3 pigs under general anesthesia. Perichondrium was removed under sterile conditions, and the cartilage was fragmented into small pieces; washed in phosphate-buffered saline solution containing 100 U/L of penicillin, 100 mg/L of streptomycin, and 0.25 mg/L of amphotericin B (GIBCO, Grand Island, NY); and digested with 0.3% collagenase II (Worthington Biochemical Corp, Lakewood, NJ) for 8 to 12 hours. The resulting cell suspension was passed through a sterile 250-µm mesh filter (Spectra/Mesh 146-426; Spectrum Medical Industries Inc, Rancho Dominguez, Calif). The filtrate was centrifuged, and the resulting cell pellet was washed twice with copious amounts of Dulbecco phosphate-buffered saline solution. Cell number and viability were determined by means of cell count using a hemocytometer and trypan blue dye. These chondrocytes were suspended in Ham F12 culture medium (Invitrogen, Carlsbad, Calif) with L-glutamine, 50-mg/L L-ascorbic acid, 100-U/L penicillin, 100-mg/L streptomycin, and 0.25-mg/L amphotericin B and supplemented with 10% fetal bovine serum (Sigma-Aldrich Corp, St Louis, Mo). The chondrocyte suspensions demonstrated cell viability in excess of 85% and were suspended in 3 mL of polymer (Pluronic F-127) at a concentration of 50 × 10^6 cells/mL.

PLURONIC F-127 AND CHONDROCYTES

Pluronic F-127 consists by weight of approximately 70% ethylene oxide and 30% propylene oxide. This material is soluble in water and becomes a hydrogel at room temperature. An aliquot of chondrocyte suspension was mixed at 4°C with a 30% solution of Pluronic F-127 at a cellular concentration of 50 × 10^6 cells/mL. A total of 3 mL of the polymer was used. At room temperature, this mixture of chondrocytes and Pluronic F-127 became gellike in consistency.

IN VIVO IMPLANTATIONS

Under general anesthesia, the dorsal surfaces of the pigs were cleaned and draped. A mixture of chondrocytes and polymer (Pluronic F-127) was injected into the dorsal subdermal space by means of a 5-mL syringe.

HARVESTING OF CARTILAGE AND CRICOID IMPLANTATION

Implants were removed after 8 to 12 weeks, and the specimens harvested were examined for the formation of cartilage. No signs of inflammatory reaction around the injected sites were observed. The cartilage was harvested as a block with its surrounding perichondrial capsule and was sculpted as illustrated (Figure 1) before its implantation in the cricoid area. Specimens were also sent for histological examination. An anterior cartilage-graft laryngotracheal reconstruction was performed. No prior effort had been made to create subglottic stenosis. A horizontal incision was made in the midline cervical neck region, and the cervical musculature was lateralized to expose the laryngotracheal skeleton. An incision was made in the anterior cricoid plate and the cricoid was slit open in the middle. The tissue-engineered cartilage graft was then sutured between the distracted cricoid plates (Figure 2 and Figure 3). The incision was closed in layers. The animals were extubated at the end of the procedure and were observed for signs of respiratory distress. All the animals were monitored closely for the first 12 hours.

BRONCHOSCOPY

Bronchoscopy was performed on each animal at the 3-week postoperative interval to evaluate the laryngeal mucosa after implantation of tissue-engineered cricoid and to demonstrate graft viability or the presence of any adverse effects such as inflammatory reaction or granulation tissue formation.

SPECIMEN ANALYSIS

All animals were killed 8 to 12 weeks after implantation, and each larynx was removed and analyzed grossly for the pres-
ence of tissue-engineered cartilage and its integration with the normal cricoid cartilage. Samples were obtained for histological analysis and were fixed in 10% phosphate-buffered formalin (Fisher Chemicals, Fairlawn, NJ). Once fixed for at least 24 hours, specimens were embedded in paraffin and sectioned using standard histochemical techniques. Slide sections were stained with hematoxylin-eosin, safranin O, Verhoeff, and trichrome.

RESULTS

All 3 pigs survived to the 3-month postoperative mark with no evidence of stridor or airway distress. Interval bronchoscopy revealed a normal patent airway with a mucosalized graft in all animals. There was no evidence of any adverse effects such as inflammatory reaction or granulation tissue formation.

Results of the histological examination (Figure 4) of the tissue-engineered cartilage graft grown as part of the graft in subcutaneous tissue demonstrated lobular cartilage. Hematoxylin-eosin staining demonstrated the cartilage to be highly cellular with round-to-oval lacunae containing binucleate and single forms. The cytoplasm was abundant and contained condensed linear eosinophilic fragments of materials suggestive of elastin. Areas bordering the lobular cartilage demonstrated flattened collagenous tissue suggestive of perichondrium. Inflammation and foreign-body reaction were not noted. Safranin O staining of the same specimen showed strong, even positivity throughout, suggesting proteoglycan production. The fibrous perichondrium tissue was highlighted by the absence of safranin O staining.

Gross examination by means of palpation of the laryngeal cartilaginous skeleton revealed no defects in the cricoid area, and the grafts appeared well incorporated with the surrounding native cartilage.

Histological examination of the graft and the laryngeal complex demonstrated incorporation of tissue-engineered cartilage with the native cartilage. There was no evidence of inflammatory reaction or necrosis of the graft. The blending of elastic tissue-engineered cartilage with the native hyaline cartilage was evident by the histological findings. Safranin O staining of elastic cartilage demonstrated even positivity for proteoglycan presence in the extracellular matrix. Binucleate forms of chondrocytes were present. The native cartilage was less cellular with single-lacuna nuclei. Proteoglycan content appeared to be similar in both types of cartilage. These differences between native (hyaline) and implanted (elastic) cartilage were noted after staining the specimens with trichome and Verhoeff stains (Figure 5).

COMMENT

The purpose of this novel technique was to evaluate the potential to generate tissue-engineered cartilage in sufficient thickness and size for use in reconstructive surgery. It is hoped that one day a small biopsy specimen of auricular cartilage from a patient might be able to generate enough cartilage for implantation into the larynx to enlarge the airway. Human cartilage has properties exploitable for tissue engineering.14
The use of growth factors, multiples passages, and recycling of the culture media has generated the best histological quality of tissue-engineered cartilage.\textsuperscript{15-18} By using the mixture of Pluronic F-127 and chondrocytes in this study, we were able to generate cartilage in the required rigidity and volume. The tissue-engineered cartilage was easily sculptured for use in the anterior cartilage graft laryngotracheal reconstruction procedure. The cartilage generated was viable and structurally stable.

The preliminary demonstration that the cartilage graft that had been grown from elastic ear cartilage blended seamlessly with the native hyaline cartilage was surprising and encouraging. No evidence existed of a cleft or a boundary zone between the graft and the native tissue. The junction of the 2 types of cartilage was demonstrated only by the elastin stain, which highlighted the auricular donor source (Figure 3). The chief histological difference between the hyaline (cricoid) and ear (elastic) cartilages is the presence or absence of the protein elastin. Verhoeff elastic stain highlights auricular cartilage, the original source of the tissue-engineered cartilage, with the brown matrix stain. The abutting cartilage with pink matrix demonstrates the hyaline (cricoid) cartilage without any elastin stain.

The use of tissue-engineered cartilage as a substitute for costal cartilage would not be an initial graft choice for every patient undergoing airway reconstruction. Tissue-engineered cartilage would seem to have an application when multiple grafts are needed or when previous costal grafts have been harvested. A major advantage of tissue-engineered cartilage would be reduced donor-site morbidity. Disadvantages of tissue-engineered cartilage graft include cost and the time interval to allow for the growth of an adequate piece of cartilage. An additional surgical procedure would be required. However, the ability to generate the cartilage in a desired thickness and size can be regarded as an important alternative step on the road to the successful surgical treatment of subglottic stenosis.

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