International Journal of Pediatric Otorhinolaryngology 78 (2014) 82–87

Contents lists available at ScienceDirect

International Journal of Pediatric Otorhinolaryngology

journal homepage: www.elsevier.com/locate/ijporl

Demonstration of the role of an implantable bioscaffold in airway reconstruction: A pilot study utilizing an animal model

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ARTICLE INFO

Article history:
Received 28 July 2013
Received in revised form 12 October 2013
Accepted 15 October 2013
Available online 14 November 2013

Keywords:
Laryngotracheal reconstruction
Subglottic stenosis
Bioscaffold

ABSTRACT

Introduction: Laryngotracheal reconstruction is a common procedure to repair subglottic stenosis. Despite a success rate upwards of 85%, this procedure has significant morbidity associated with it, specifically with the site of the graft harvest and recurrence of stenosis. We propose that a recently described cellular bioscaffold xenograft may be useful in reducing these complications.
Methods and materials: 10 Sprague Dawley rats were divided into 2 groups of 5. One group underwent incision through the cricoid and the first two tracheal rings followed by primary closure (G1); the second group underwent incision through the cricoid and the first two tracheal rings followed by placement of the xenograft (G2); additionally, a specimen was harvested from an animal which did not undergo any surgical procedure to compare to the two surgical groups. Specimen harvest occurred on post-operative days 1, 7, 14, 21, and 28.
Results: 6 of 10 animals provided usable data. All animals receiving the xenograft survived until the time of specimen harvest. Only 1 animal undergoing primary closure survived beyond post-operative day one. On histology review, the xenograft animals showed a progressive decrease in fibrosis relative to the animals that underwent primary closure. On POD 28, restoration of the respiratory epithelium and intact basement membrane was noted in the xenograft group.
Conclusion: We believe that this pilot study shows the potential of utilizing bio-implantable biomaterials, specifically a cellular bioscaffold which encourages the ingrowth of native tissue instead of fibrosis. Histologic analysis shows that use of the xenograft can initiate the proliferation of native tissues decreasing the amount of fibrosis present post-operatively, although significant further analysis is needed before definitively concluding that this approach is superior to utilization of a graft.
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Introduction

Subglottic stenosis is commonly encountered in term and preterm infants, with an incidence of 0.0–2.0% in live births [1]. Although there are numerous strategies for surgical intervention, augmentation grafts remain the mainstay of treatment. A variety of autologous cartilage grafts have been described in the literature, including: costal, auricular, thyroid alar, and nasal septal cartilage. Contemporary advances in biomaterials have led to multiple investigators utilizing alternative materials in laryngotracheal reconstructions, including bio-absorbable mini-plates and synthetic or irradiated cadaveric cartilage [2,3].Mini-plates composed of poly-L-lactic-acid-polyglycolic-acid were successfully utilized in ten children for laryngotracheal reconstruction [2]. All patients were successfully decannulated, and repeated bronchoscopy revealed well-healed and fully mucosalized anterior tracheal walls. While irradiated cartilage does offer an alternative to autologous cartilage, it may undergo partial resorption making autologous grafts the preferred material for cartilage-based reconstruction [3].

MatriStem (Acell, Columbia, MD) is a stem-cell bioscaffold derived from porcine tissue. When placed onto a wound, it is resorbed and replaced with new native tissue rather than scarring. This technology gained FDA approval for use in human subjects in 2009 for wound care, soft tissue repair, and general surgery.

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http://dx.doi.org/10.1016/j.ijporl.2013.10.059

Our primary objective was to evaluate the potential role of MatriStem as an augmentation graft in laryngotracheal reconstruction utilizing an animal model.

Methods and materials

Prior to initiation of the study, approval was obtained from the Wayne State Institutional Animal Care and Use Committee. All surgical intervention was performed using aseptic techniques in accordance with IACUC standards.

21 Sprague Dawley outbred rats were obtained from a licensed vendor and randomized into two treatment groups and 1 control group (Table 1). The animals were then divided into two groups of five. Animals in group 1 (G1) underwent incision through the cricoid and the first two tracheal rings followed by primary closure while individuals in G2 underwent incision through the cricoid and the first two tracheal rings followed by placement of the MatriStem xenograft (G2). A specimen was harvested from a control animal that had not undergone any surgical intervention. Specimen harvest occurred for all surviving animals on post-operative days 1, 7, 14, 21, and 28

Presurgical preparation of animals

The rats were administered with ketamine and xylazine subcutaneously (66.7 mg/kg and 6/67 mg/kg) immediately prior to the procedure to induce anesthesia and were additionally given 0.01–0.05 mg/kg buprenorphine subcutaneously for analgesia. The neck and thorax were shaved 2–3 cm around the incision.

Surgical procedure

After securing each rat to the operating surface, a midline ventral cervical incision was made. Blunt dissection was used to approach the trachea, which was carefully exposed and dissected so as not to damage adjacent structures. A small Wheatlander self-retractor was then placed to hold incision. The operating microscope was brought into the surgical field and used for the remainder of the case. The first animal to undergo the procedure underwent intubation at this point, using a rat intubation pack from Hallowell. However, the small pipette used for intubation was found to be in the esophagus and the subsequent animals did not undergo intubation. An anterior cricoid split was performed. A ventral incision was made in the cricoid and extended inferiorly to include the first two tracheal rings. At this point, the graft was placed depending on the experimental group. Group 1 underwent primary closure using 6-0 PDS suture. Group 2 underwent placement of the xenograft which was secured in place with 6-0 PDS suture. The cervical fascia was closed using 6-0 vicryl suture. The skin was closed using 6-0 fast sutures.

Post-operative care

The rats were transferred back to their cages and given access to food and 5% dextrose in Lactated Ringer's solution. Animals were monitored at 15–30 min intervals until regaining consciousness as well as the ability to lift their heads. Frequent monitoring continued until the animals could maintain itself in sternal recumbence. Warm Lactated Ringer's solution was administered subcutaneously (4–8 ml per 100 gm body weight per 24 h) immediately post-operatively to prevent dehydration. Analgesics (post-operative buprenorphine at 0.01–0.05 mg/kg) were administered subcutaneously once anesthetic recovery was observed to be progressing normally and repeated every 6–12 h as necessary. Criteria used to determine whether the animal was experiencing discomfort are noted in Table 2. Any rat that displayed uncontrolled post-operative distress (infection, depressed vital functions) was sacrificed by CO_2narcosis until death followed by bilateral pneumothorax.

Specimen collection

Specimen collection of each experimental group occurred on post-operative days 1, 7, 14, 21, and 28. The animals were sacrificed by CO_2 narcosis until death followed by removal of the larynx and trachea. Photographs of the airway were taken prior to removal. The specimens were then turned over to the Wayne State Department of Pathology for histologic processing and evaluation by a board certified pathologist. The tracheal specimens were sectioned, routinely processed for histological examination, and stained with Hematoxylin and Eosin (H&E), Trichrome and Peridoc Acid-Schiff (PAS) stains.

The following parameters were histologically assessed: (1) airway lumen diameter; (2) degree of scar tissue formation; (3) degree of bioscaffold degradation; and (4) extent of fibrous tissue replacement by native tissue.

Results

6 of 10 experimental animals provided usable data. All of the animals receiving the xenograft (G2) survived until specimen collection. Significantly, 4 of the 5 control G1 animals developed respiratory compromise and did not survive for specimen harvest (Table 1). Postmortem examination of these animals demonstrated significant fibrosis in the subglottic region. Only one G1 animal survived beyond post-operative day one after undergoing primary closure of the airway incision.

Intra-operative examination of the xenograft animals revealed progressive resorption of the xenograft and return of intact tracheal airway (Fig. 1). Histologic examination of the xenograft specimens revealed a definite injury to the tissue and violation of the basement membrane (Fig. 2A and B). Collagen deposition appeared to reach its maximum level by post-operative day 14 (Fig. 2C and D) and this was followed by decrease over the next 2 weeks until post-operative day 28 (Fig. 2E and F). The specimen obtained from the animal that survived post-operative day 28 showed an intact intraluminal respiratory epithelium along with an intact basement membrane (Fig. 2E and F, Fig. 3). An animal that did not undergo any reconstruction was used as a normal anatomic comparison to the reconstruction groups, which is shown in Fig. 4A.

Table 1
Characteristics of treatment groups.

Group	Surgical intervention	Number of animals at start of experiment	Number of animals with usable data
1	Primary tracheal closure using 6-0 PDS suture	5	1[a]
2	Xenograft placement secured with 6-0 PDS suture	5	5
Control	None	1	1

[a] Four animals in this group died on post-operative day 1.

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