Tracheal cartilage regeneration by slow release of basic fibroblast growth factor from a gelatin sponge

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Objective: We investigated whether implantation of a gelatin sponge, releasing basic fibroblast growth factor slowly (b-FGF) into a tracheal cartilage defect, would induce regeneration of autologous tracheal cartilage.

Methods: We created a 1-cm defect in the midventral portion of each of 10 consecutive cervical tracheal cartilage rings in 12 experimental dogs. In the control group (n = 4), the resulting defects were left untreated. In the gelatin group (n = 4), empty gelatin sponges were implanted in the defects. In the basic fibroblast growth factor group (n = 4), gelatin sponges incorporating 100 μg of b-FGF solution were implanted in the defects. We killed the 4 dogs in each group at 1, 3, 6, and 12 months after implantation, respectively, and examined the implant sites macro- and microscopically.

Results: In the control and gelatin groups, no regenerated cartilage was observed in the tracheal cartilage defects, and the width of the gap between the host cartilage stumps had shrunk. In the b-FGF group, regenerated cartilage was observed in all dogs. The proportion of the defect in the host cartilage occupied by regenerated cartilage was 13%, 84%, 75%, and 69% at 1, 3, 6, and 12 months, respectively. The regenerated cartilage was fibrous cartilage covered with perichondrium, which grew from the host perichondrium and showed continuity with the host cartilage stumps.

Conclusions: Implantation of a gelatin sponge slowly releasing basic fibroblast growth factor induces tracheal cartilage regeneration, which subsequently fills a large proportion of experimentally created tracheal cartilage defects within 12 months after implantation.

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The surgical management of tracheal reconstruction is one of the most difficult problems associated with extensive tracheal resection in patients with malignant or benign disease. Several approaches have been attempted, including tracheal transplantation[1-3] and use of prosthetic materials,[4,5] but success has been limited because of graft ischemia and immune rejection, leading to anastomotic dehiscence and stenosis.

We have been investigating the use of artificial organs made of bioabsorbable materials to induce regeneration of defective or missing host tissues.[6-8] In a previous study, we focused on inducing regeneration of the trachea to produce an artificial prosthesis.[9]

Abbreviations and Acronyms
AB = alcian blue
b-FGF = basic fibroblast growth factor
BMP = bone morphogenetic protein
HE = hematoxylin and eosin

The normal canine trachea, as is the case in humans, is composed of a mucosal layer, a submucosal layer, tracheal glands, a smooth muscle layer, and tracheal cartilage rings. The chief function of tracheal cartilage is to maintain the integrity of the tracheal lumen. Therefore, we have investigated the possibility of tracheal cartilage regeneration using biodegradable materials as a first step in attempting to produce an artificial trachea.

Bone morphogenetic protein (BMP)-2 or basic fibroblast growth factor (b-FGF) promotes the regeneration of chondrocytes in articular cartilage defects in vivo.[10-15] However, to our knowledge, few reports have indicated that these growth factors induce cartilage regeneration in tracheal cartilage defects. We have reported previously that implantation of gelatin sponge releasing BMP-2 or b-FGF slowly induces regeneration of tracheal cartilage.[16-18] However, implantation of a gelatin sponge incorporating BMP-2 did not achieve a sufficient result because regenerated cartilage was observed only at the ends of the host cartilage stumps.[17] On the other hand, implantation of a gelatin sponge incorporating b-FGF resulted in regeneration of cartilage that occupied 84% of cartilage defects created experimentally, although the results were based on short-term observations made over 3 months.[18] In the present study, we investigated the long-term results in dogs receiving b-FGF implantation for repair of tracheal defects and whether the regenerated cartilage would be absorbed as a result of foreign body reaction.

Materials and Methods

Preparation of Gelatin Sponge Incorporating b-FGF

Gelatin as a 5 wt% aqueous solution with an isoelectric point of 5.0 (Nitta Gelatin Co, Osaka, Japan) containing 0.05 wt% glutaraldehyde (Wako Pure Chemical Industries, Osaka, Japan) was cast into a Teflon mold, then stored at 4°C for 12 hours to allow complete chemical cross-linking. The resulting material was immersed in an aqueous solution of glycine (Nakarai Tesque Inc, Kyoto, Japan) at 37°C for 1 hour to block any residual glutaraldehyde, rinsed in distilled water, freeze-dried, and finally sterilized by exposure to ethylene oxide gas. The prepared gelatin sponge was trimmed into pieces measuring approximately $10 \times 50 \times 2$ mm^3 (Figure 1). Just before implantation, 100 μg of aqueous b-FGF solution was dissolved in 0.3 mL of saline solution, and the resulting solution was applied dropwise to the empty gelatin sponge. The sponge was then left to stand for 15 minutes to allow the b-FGF solution to soak in completely. B-FGF ionically immobilized on the gelatin sponge is released slowly for about 2 weeks as it degrades.[19]

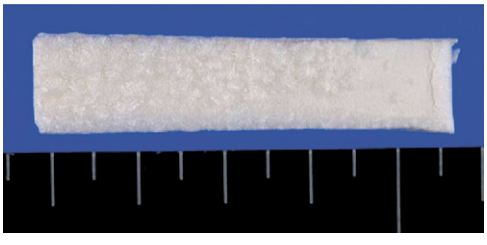

Figure 1. Macroscopic appearance of the gelatin sponge. The material was hard when in a dry state but was converted to a gel form when soaked with b-FGF solution.

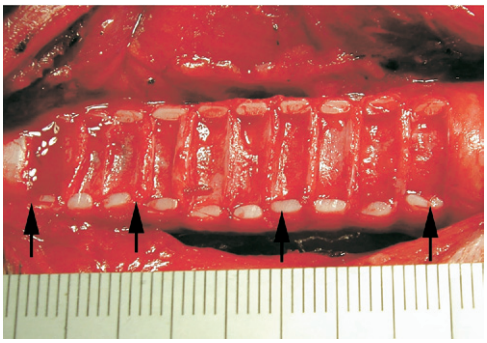

Figure 2. Operative appearance of a 1-cm gap made in the midventral portion of each of 10 consecutive cervical tracheal cartilages from rings 4 to 13, extending for a total length of about 5 cm. The tracheal mucosa was carefully preserved. (*Arrows* show the tracheal cartilage defect.)

Operative Procedures

Twelve hybrid beagle dogs aged 2 to 3 years and weighing between 10 and 13 kg were anesthetized by intramuscular administration of 10 mg/kg ketamine (Sankyo Co, Tokyo, Japan) and 5 mg/kg xylazine (Bayer Japan, Tokyo, Japan), with 2 μg/kg atropine sulfate (Tanabe Seiyaku Co, Osaka, Japan) added to control secretions. All dogs were intubated via the trachea and ventilated

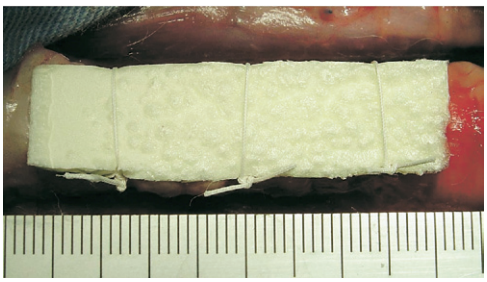

Figure 3. Operative appearance of the gelatin sponge, containing 100 μg of b-FGF solution, implanted in the tracheal cartilage defect. The gelatin sponge was fixed by 1-0 silk sutures to prevent dislodgment.

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