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Drug delivery system of basic fibroblast growth factor using gelatin hydrogel for restoration of acute vocal fold scar

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ABSTRACT

Objective: There continue to be therapeutic challenges in the management of vocal fold scarring. We previously showed that basic fibroblast growth factor (bFGF) injection has therapeutic potential for vocal fold scarring. However, the working time of bFGF is relatively short, and multiple injections were required in many cases to obtain the regenerative effect. An efficacious delivery system for bFGF has yet to be established. We designed a method of sustained drug delivery system (DDS) of bFGF by using a gelatin hydrogel. Hydrogel has been developed for targeted delivery and controlled release of bFGF. Hydrogel of the particle type is also injectable and commercially available. The current study aims to investigate the effects of a single injection of bFGF-DDS on acute vocal fold scarring using a canine model.

Methods: Vocal folds from eight beagles were unilaterally scarred by stripping the lamina propria. One month later, hydrogels (0.5 ml) containing 10 μg of bFGF were injected into the scarred vocal folds of four beagles (FGF-hydrogel group). Saline (0.5 ml) was injected into the other four beagles (sham group). Vibratory and histological examination of excised larynges was performed 5 months after treatment. Comparative analysis between the current data and our previous data with repeated injection of bFGF solution was also completed.

Results: Vibratory examination demonstrated significantly improved vibration in the bFGF hydrogel-treated group. Histological examination of the bFGF hydrogel group showed restoration of hyaluronic acid in the lamina propria as compared to sham. Comparison between the DDS system and our previous bFGF solution injection indicated better effects of the DDS system on vibratory amplitude.

Conclusion: A single injection of bFGF hydrogel has regenerative effects on acute vocal fold scarring, which is at least similar to repeated injection of bFGF solution.

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1. Introduction

Vocal fold scarring may occur following injury, inflammation, or phonosurgery. Scarring disrupts the layered structure of the lamina propria leading to changes in the viscoelasticity of the vocal fold mucosa, often resulting in severe and intractable dysphonia [1].

Previous histological studies [2–5] on vocal fold scarring have revealed changes in the organization and distribution of extracellular matrix components (ECM), such as dense and/or disorganized type I collagen deposition, decreased elastin, and decorin, increased fibronectin, and occasional decreases in hyaluronic acid (HA).

Medialization thyroplasty, fat/collagen injection, and scar dissection have been tried in an attempt to restore normal properties to scarred vocal folds [6]. However, these approaches do not restore normal ECM distribution. Due to the difficulty of replacing scar tissue with innate ECM, there is a lack of reliable therapeutic strategies for vocal fold scarring.

Growth factors are a strong tool for inducing tissue regeneration by stimulating and controlling cell behavior. We have previously reported on the therapeutic potential of basic fibroblast growth factor (bFGF) [7–9] and hepatocyte growth factor (HGF) [10,11] for vocal fold scarring.

It has been reported that bFGF, a stimulant for the growth of fibroblasts, decreases the deposition of collagen and increases the production of hyaluronic acid [12,13]. A subsequent in vivo study using canines showed the recovery of vibratory properties of scarred vocal folds after local injection of bFGF [9].

However, the working time of bFGF is relatively short, and in many cases, multiple injections are required to obtain the regenerative effects. Thus it is important to investigate alternative ways of administering growth factors that may enable these effects to be strengthened, such as through the exploration of various drug delivery systems.

A biodegradable hydrogel has been developed to enhance the in vivo regenerative effects of growth factors, such as HGF, bFGF, platelet-derived growth factor, and epidermal growth factor. This hydrogel has proven successful in the controlled release of biologically active growth factors in other parts of the body [14–20]. Moreover, gelatin hydrogel is commercially available (Medgel; Wako Pure Chemical Industries, Osaka, Japan), and this particular type is easily injectable.

The current study aims to investigate the effects of a single injection of bFGF hydrogel on acute vocal fold scarring using a canine model. Furthermore, comparative analyses were performed between the effects of b-FGF hydrogel obtained from the current study and injection of a bFGF solution obtained from our previous data [9].

2. Materials and methods

2.1. Animals

Eight beagle dogs, weighing 8–10 kg, were used in this study. All experimental protocols were approved by the Animal Research Committee of the Graduate School of Medicine, Kyoto University. Animal care was provided under the supervision of the Institute of Laboratory Animals of the Graduate School of Medicine, Kyoto University.

2.2. Preparation of bFGF hydrogel

A commercially available form of human recombinant bFGF (Fiblast; Kaken Co., Tokyo, Japan) and gelatin hydrogel of particle type (Medgel P15; Wako Pure Chemical Industries, Osaka, Japan) was prepared. The hydrogel is constituted by chemically cross-linking acidic gelatin with glutaraldehyde, developed by the Department of Biomaterials, Field of Tissue Engineering, Institute for Frontier Medical Sciences, Kyoto University [14,15]. 1 mg of gelatin was mixed with 10 μl of medicinal liquid following the instruction. To formulate the bFGF hydrogel, 10 μg (100 μl) of bFGF was added to 10 mg of gelatin hydrogel, and the compound was then incubated for 1 h at 37 °C to create the final product. It was dissolved in 0.5 ml of saline just before injection.

2.3. Procedure for vocal fold injury

Animals were sedated under general anesthesia with intramuscular injections of ketamine hydrochloride (6 mg/kg; Sankyo Co., Tokyo, Japan) and xylazine hydrochloride (15 mg/kg; Bayer, Tokyo, Japan). Under direct laryngoscopy, right vocal folds were injured by removal of the entire layer of the lamina propria using microscissors and microforceps under a microscope. The contralateral vocal folds were left intact as normal controls. The vocal fold scars were allowed to mature for one month and endoscopic examination under anesthesia was performed to observe wound healing every two weeks.

One month after the initial surgery, bFGF hydrogel was injected into the injured vocal fold of four beagles (bFGF hydrogel group) under direct laryngoscopy using a transoral intracordal injector. The remaining four beagles received injection of saline (0.5 ml) into the injured vocal fold (sham group).

After injection, endoscopic examination under anesthesia was performed every four weeks to observe wound healing. All animals were euthanized 6 months after initial scarring by intracardiac injection of pentobarbital sodium (25 mg/kg; Dainippon Pharmaceutical Co. Ltd, Osaka, Japan). Larynges were immediately harvested and used for vibratory examination and subsequently subjected to histological examination.

2.4. Set-up for vibratory examination of excised larynges

Vocal fold vibration was examined using an excised larynx set-up developed in previous studies [11]. For better visualization of the vocal fold, supraglottic structures, including the epiglottis, false vocal folds, and aryepiglottic folds, were removed after the resection of the superior portion of the thyroid cartilage. The arytenoid cartilages were sutured together, and an arytenoid adduction procedure was performed bilaterally using a 3-0 Prolene suture to close the glottis. The larynx was mounted on a table, and an intubation tube was inserted into the trachea and clamped tightly. Air was pumped through the tube to generate vocal fold vibrations. During the vibratory examination, saline was dripped onto the vocal folds

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