Tissue Engineering for Voice Disorder

JMAJ 54(4): 254–257, 2011

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Abstract

The role of regenerative medicine for voice disorder is reviewed in this paper. Voice disorder is caused by disorders of the vocal fold mucosae, the origin of the voice. The cause varies greatly, but severe voice disorder occurs when the vocal folds, which are an oscillating body, undergo histologic changes disabling the vibration. Examples include vocal fold scarring and atrophy, and there is no effective treatment for these lesions. The concept of regenerative medicine is to promote the revitalization of the tissues and functions by administering cells, scaffolds, and growth factors. Recent studies have revealed that cells such as embryonic stem cells, bone marrow-derived mesenchymal cells, and adipose-derived stem cells, as well as growth factors such as hepatocyte growth factor and basic fibroblast growth factor are promising materials, and some of them are already clinically applied. In addition, *in situ* tissue engineering that aims to regenerate the tissues only by providing appropriate regeneration scaffolds and thereby supplying the necessary cells or growth factors through the surrounding tissues, is also a powerful regeneration approach. The required materials should have high biological affinity, promote the influx of cells, and be highly absorbable, and various biological materials have been examined.

Key words Voice disorder, Regenerative medicine, Tissue engineering

Introduction

The vocal fold mucosae are mucosae that vibrate. They vibrate at 100 to 200 times per second, and in some singers, rapid vibration of almost 1,000 times per second can be achieved. Only the vocal fold mucosae can produce such vibration, and this property is attributed to the unique structure of the vocal folds. Beginning from the surface, vocal fold mucosae consist of the epithelium, the lamina propria, and the vocal muscle. The lamina propria is subdivided into three layers: superficial, intermediate, and deep. Within each layer, extracellular matrices are distributed in an orderly manner. The superficial layer abounds in soft matrices, mainly hyaluronic acid. The intermediate and deep layers are composed of fibrous proteins such as elastin and collagen, forming the vocal ligament. Rapid vibration of the vocal folds is made possible by the vibratory movement of the viscoelastic superficial layer supported by the muscles and the ligaments as scaffolds.

Voice disorder is caused by various diseases of the vocal fold mucosae, and the diseases are broadly divided into organic, functional, and neurogenic disorders. The vocal fold mucosae are altered by these disorders, become harder and less viscoelastic, and lose their ability to vibrate rapidly, resulting in permanent hoarseness. Lesions like vocal fold scarring, atrophy, or sulcus vocalis may be direct causes. Vocal fold scarring occurs after inflammation or injury, and is frequently observed in people with occupations requiring very hard daily use of the vocal folds, such as singers. Vocal fold atrophy often develops with age.

These voice disorders are associated with changes in the tissues of the vocal fold mucosae. Excessive collagen deposition in the lamina propria and a decrease or loss of hyaluronic acid are confirmed with these disorders. Along with these changes, the normal layered structure

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This article is a revised English version of a paper originally published in the Journal of the Japan Medical Association (Vol.139, No.4, 2010, pages 827–830).

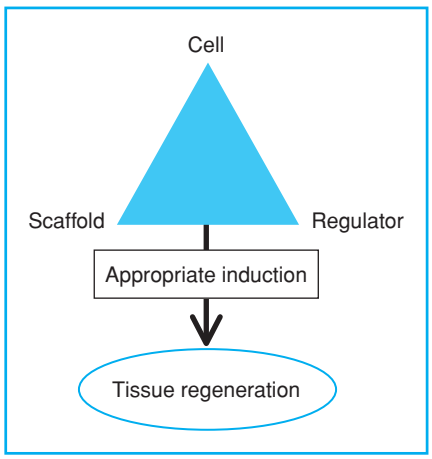

Fig. 1 Basic concept of regenerative medicine

of the vocal fold mucosae disappears, and the mucosae become harder. Once this happens, there is no way at present to restore them to normal, and no effective treatment has been established. Regeneration of the changed tissue would be required to treat this condition.

In this paper, attempts to regenerate the tissues of degenerated vocal folds by means of regenerative medicine, including basic experiments and clinical findings, are reviewed.

Concept of Regenerative Medicine

In recent years, the expectations for regenerative medicine including the development of induced pluripotent stem cells (iPS cells) are increasing. In regenerative medicine, lost tissues and functions are regenerated mainly through cell transplantation, and three important elements are the cells, scaffolds, and regulators (**Fig. 1**). Appropriate induction of these elements promotes regeneration of the tissues. Not all of the three elements are essential; only one of them can be effective for regeneration.

Cell types used for cell transplantation include stem cells, progenitor cells, and mature cells. In terms of regenerative potential, as young cells as possible are preferred. However, because of the carcinogenic risk associated with stem cells, it is difficult to put them into clinical application unless a means to avoid the risk is found. On the other hand, mature cell transplantation is safe, but sufficient regeneration may not be achieved. Selection of the cell type should depend on the requirements for regeneration of each tissue.

Among the stem cells, embryonic stem cells (ES cells), iPS cells, mesenchymal stem cells (MSCs), and adipose-derived stem cells (ASCs) are considered to be promising. In addition to carcinogenicity, there is still a too strong ethical concern over ES cells to use these in the future. Since MSCs and ASCs are autologous, they are good candidates in terms of safety. Although iPS cells have the problem of carcinogenicity, it may be possible to reduce the risk by transplanting them after differentiation and induction.

As regulators of cells, cell growth factors are useful. Cell growth factors play various roles, not only in cell proliferation, but also in cell function. Some treatments known as growth factor treatments, are already covered by insurance. Basic fibroblast growth factors (bFGFs) and insulin-like growth factors (IGF), for example, can be clinically applied in Japan.

There is another concept called *in situ* tissue engineering, in which no such cells or regulators are used, and the transplantation of scaffolds alone induces the necessary cells and regulators from the tissue. The advantage of this approach is that it does not have the problems with cell therapies or growth factor treatments. Since the ideal scaffold for regeneration varies with each organ, the key is the selection and preparation of the scaffold.

Regeneration of Vocal Fold Mucosae

Cell therapy

Cell transplantations have been performed to regenerate tissues for vocal fold scarring and damaged vocal folds at the level of animal experiments. Fibroblasts, MSCs, ASCs, and ES cells have been used.

A research team from UCLA performed an experiment in a canine model to transplant autologous fibroblasts that were cultured and expanded in the laboratory from a buccal mucosal biopsy to the injured vocal folds.[1] As a result, the mucosal waves became normal or almost normal after the cell therapy. The treated vocal folds demonstrated an increased density of fibroblasts and collagen, and a decreased density of elastin, which were not favorable compared with the normal structure of the vocal folds. Similarly, the author transplanted vocal fold fibroblasts to scarred

vocal folds in a canine model. The results showed that the scar worsened after the therapy, which was an adverse effect.[2] Since fibroblasts produce numerous extracellular matrices, it is important to control such production. In mature cell transplantation, it is considered difficult to predict the results of this production.

Stem cells can divide and differentiate into diverse specialized cell types and can self-renew to produce more stem cells. When transplanted, they are expected to differentiate themselves into cells required in the environment and perform positive biological functions. A Swedish research group attempted to regenerate vocal fold mucosae by ES cell transplantation.[3] The researchers transplanted human ES cells to scarred vocal folds of rabbits, and the human cells were found to be engrafted at 1 month later, with improved viscoelasticity of the treated vocal folds.

As mentioned above, although regenerative ability of ES cells has great potential, it is difficult to apply them to clinical practice, because of ethical concerns and carcinogenicity. Thus, an experiment using MSCs, which are autologous, was performed. Our research team transplanted bone marrow-derived MSCs to injured vocal folds in dogs.[4,5] Morphological and histologic evaluations showed improved regeneration of the mucosae in the treatment group compared with the control group without transplantation. Furthermore, the transplanted cells were found to differentiate into epithelial cells, mesenchymal cells, and muscle cells. Since MSCs are autologous and, therefore, have fewer safety problems, they are a promising material for future clinical application.

Growth factor treatment

As mentioned earlier, extracellular matrices of the lamina propria play a key role in the vibration of the vocal fold mucosae. Fibroblasts are important cells that produce these extracellular matrices, and growth factor treatment is a promising treatment to regulate the function of fibroblasts. The treatment is expected to restore the properties of the vocal fold mucosae if there is a measure to promote production of hyaluronic acid required by the vocal fold mucosae and decrease the excessive collagen. We have conducted some experiments with a focus on hepatocyte growth factor (HGF) and bFGF, and applied some of the results to clinical practice.

HGF has strong antifibrotic activity, and was found to promote the production of collagen-degrading enzyme from fibroblasts and hyaluronic acid from vocal fold fibroblasts. We have conducted experiments on the regeneration of scarred vocal folds using HGF. We have developed a canine model of acute and chronic scarred vocal folds, administered HGF aqueous solution or hydrogel-based sustained-release preparation to the scarred vocal folds, and evaluated the regenerative effect. The results showed that significantly better vibrations of mucosae and regeneration of tissues were observed in the HGF-treated group, and HGF's potential to prevent scarring and a therapeutic effect were reported.[2,6,7]

Besides its proliferative action on fibroblasts, bFGF has regulatory action on extracellular matrices. It is already available on the market for clinical use in the treatment of skin ulcers. It has been reported that bFGF promotes wound healing by causing proliferation of fibroblasts, and decreases scarring by regulating collagen distribution. The author and colleagues reported that bFGF was useful to treat vocal fold atrophy associated with aging. We injected bFGF transorally into the lamina propria of aged rats' vocal folds, and found that bFGF significantly increased the hyaluronic acid content in the lamina propria.[8] Based on this result, we conducted a clinical human trial approved by the institutional review board at Kyoto University. The first case has already been reported,[9] showing regeneration of atrophied vocal folds by injecting bFGF with improvements in phonation and vibrations of vocal folds. Regeneration medicine like this may be applied as anti-aging treatment of the voice in the future.

In situ tissue engineering

Some studies to regenerate the larynges by transplanting regeneration scaffolds have been reported. Requirements for ideal regeneration scaffolds are as follows: influx of cells is sufficient, no foreign body reaction is observed, absorption occurs over time, and tissue affinity is high. Development of such materials has been ongoing, and extracellular matrix preparations, hyaluronic acid gel, and collagen sheets have been examined for regeneration of the vocal fold mucosae.

Our research team has been studying the regenerative effects of cross-linked atelocollagen

in vitro and *in vivo*. In the *in vitro* study, bone marrow-derived MSCs were seeded into atelocollagen sheets to evaluate their affinity for the cells. The results showed that MSCs were successfully implanted into the sheets, and functioned well by producing extracellular matrices such as fibronectin.[10] It is considered that implantation of a regeneration scaffold like this into scarred vocal folds leads to mucosa regeneration. A significant advantage of this treatment, unlike cell therapies or growth factor treatments, is that it is easy to apply for clinical use.

Conclusion

Approaches of regenerative medicine to the vocal fold mucosae that have lost their original properties are reviewed. Cell transplantation focusing on stem cells seems to be the most powerful regenerative approach, and bone marrow-derived MSCs and ASCs, which are autologous, are good candidates for future clinical application. Growth factor treatment is also an effective means to promote tissue regeneration, and HGF and bFGF are promising for regeneration of scarred or atrophied vocal folds. Some of these approaches have already been put into clinical application.

On the other hand, *in situ* tissue engineering is an approach that aims to regenerate tissues only by providing an appropriate regeneration scaffold, without using cells or growth factors. The concept of the approach is to implant a regeneration scaffold, and the necessary cells and regulators are to be supplied by the surrounding tissues. The use of extracellular matrix preparations, hyaluronic acid gel, and atelocollagen sheets has been examined for regeneration of the vocal fold mucosae and some animal experiments have reported their efficacy. These approaches are expected to enable the treatment of degenerated vocal fold mucosae, which has been a therapeutic challenge, by regenerating the mucosae.

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