22

Bioengineering the Vocal Fold: A Review of Mesenchymal Stem Cell Applications

Rebecca S. Bartlett, MA and Susan L. Thibeault, Phd
University of Wisconsin-Madison
United States

1. Introduction

The vocal folds are a remarkably unique tissue in the body, in that they can produce vibration at rates between 100-1000 hertz throughout the course of a day (Guimaraes & Abberton, 2005; Hunter et al., 2006). When this robust tissue is disrupted by scarring associated with vocal fold lesion excision, carcinoma, radiation, trauma, or inflammatory disease, the associated degradation in vocal quality can be devastating. More than half of individuals with voice disorders attribute their dysphonia as having a negative impact on their social interactions, career and mental health (Smith et al., 1995). Traditional surgical treatments and voice therapy have been suboptimal for improving the dysphonia and fatigue associated with vocal fold scarring (Hansen & Thibeault, 2006). Over the past decade, a number of promising pre-clinical studies targeted at vocal fold regeneration using mesenchymal stem cells (MSCs) have been completed. As nearly all of the dedicated stem cell investigation has been completed in vitro and in animal models, there exists a great opportunity for innovation and landmark work in the future of this field. Our intention is to present the therapeutic interventions explored in the literature to date for mesenchymal stem cell (MSC) based approaches for vocal fold regeneration, in order to inform optimal approaches of the future.

2. Key aspects of vocal fold function

2.1 Basic cellular constitution of the vocal mucosa

The vocal folds have a defined multilayered structure; whereby the vocal mucosa, made up of the epithelium, basement membrane zone and superficial lamina propria, is anchored to the underlying intermediate and deep layers of the lamina propria and thyroartenoid muscle. The vocal mucosa vibrates during phonation and is the most common site for injury and scarring. As such, the anatomical target of the majority of bioengineered constructs for the larynx is the vocal mucosa. The lamina propria is home to fibroblasts, myofibroblasts and macrophages, and the epithelium contains epithelial cells and some dendritic cells that gain entry via the basement membrane zone (Catten et al., 1998). Due to their abundance

and the utility of their unique functions, the two cell types of primary interest for vocal fold tissue engineers are epithelial cells and vocal fold fibroblasts. The vocal folds are covered by stratified squamous epithelium, which serves as a protective barrier against chemical, pathogenic and mechanical insults (Sivasankar et al., 2010; Mogi et al., 1979; Gray & Titze, 1988; Gipson et al., 1995; Johnston et al., 2003). Laryngeal epithelial cells also help maintain local hydration, which is essential for healthy voice production (Fisher et al., 2001). The most abundant cell type in the lamina propria is the vocal fold fibroblast. These cells are largely evenly spread throughout the depth of the tissue (Catten et al, 1998). During a wound healing response, vocal fold fibroblasts migrate to the site of injury and remodel the tissue by generating extracellular matrix (ECM) (Hirano et al., 1999).

2.2 Basic ECM constitution of the vocal mucosa

The ECM profile determines the biomechanical properties of vocal fold tissue, which, in turn, influences vocal quality (Thibeault et al., 2002). Vocal fold scarring can be identified by the fibrotic conversion of the native ECM (Benninger et al., 1996). While this later phase in injury and repair processes is innate, it can have a deleterious effect on vocal quality by altering the viscoelasticity of the tissue. As such, vocal fold bioengineers must utilize cell sources, scaffolds, and growth factors that will remodel ECM profiles to mimic the viscoelasticity of healthy tissue. Accurate measurement of the elastic properties of vocal fold mucosa is difficult due to its small size and difficult to access location. Investigators have used various models (cadaveric human larynges, *in vivo* and *ex vivo* canine larynx, etc) and techniques (nerve stimulation, parallel plate rheometry, indentation, etc), and have reported a range of shear moduli results (Hirano, 1981; Chhetri et al., 2010).

The layered structure of vocal fold tissue can be defined by its relative constituent makeup; the vocal fold mucosa is primarily composed of ECM, and the underlying thyroartenoid is largely composed of cells (Gray et al., 2000b). The robust ECM of the vocal fold mucosa is made up of fibrous proteins (i.e., collagens, elastins), interstitial proteins (i.e., hyaluronic acid, decorin, fibromodulin, decorin, versican) and other molecules such as lipids and carbohydrates.

The fibrous proteins of the vocal fold ECM have traditionally received a large amount of attention, as they contribute significantly to viscoelasticity. Collagens provide tensile strength to vocal fold tissue. The lamina propria hosts collagen type I, II and III and the basement membrane zone contains type IV and VII (Gray, et al., 1993; Mossallam, et al., 1986; Courey, et al., 1996; Gray, 1991). Elastins afford vocal tissue the ability to be stretched and then return to its original shape. The interstitial proteins are found between the collagens and elastins, and provide a variety of functions to the tissue. The large size, porous quality and high water content of hyaluronic acid (HA) are thought to offset the chronic vibration associated with phonation via shock absorption (Gray et al., 1999). HA also affects the viscosity of tissue, as a higher content corresponds to a higher tissue viscosity. Decorin and fibromodulin also have a role in wound healing, as they are able to bind to collagen and regulate fibril synthesis (Gray, et al., 1999). Versican has an ability to bind water molecules and plays a role as a space-filler in the lamina propria (Gray, 2000b).

ECM remodeling in the vocal folds is a finely coordinated, complex process, which is not yet fully understood. Further investigation into how to modulate this process following injury is warranted.

3. Therapeutic interventions explored to date

Reference	MSC type	Cell Therapy Length	Model	Scaffold	Growth factor	Outcomes
Quinchia-Johnson et al., 2010	BM MSCs (murine)	4 weeks	18 rats	HA hydrogel	-	Improved ECM and TGF-β1 production, and increased hyaluronan metabolism over 3 other treatment combinations
Hertegård et al., 2006a	BM MSCs (human)	4 weeks	10 rabbits	-	-	Improved tissue viscoelasticity and reduced collagen type 1 in treated folds
Kanemaru et al., 2005	BM MSCs (murine)	8 weeks	4 rats	-	-	MSCs differentiated into cells + for epithelial and muscle markers
Kanemaru et al., 2003	BM MSCs (canine)	8 weeks	8 dogs	1% HCl atelo-collagen	-	2/8 dogs had granulation and 2/8 dogs had atrophic changes on untreated, scarred fold, while none of the treated vocal folds showed signs of damage
Svensson et al., 2010	BM MSCs (human)	12 weeks	11 rabbits	-	-	Improved tissue viscoelasticity and reduced collagen type I in treated folds
Lee et al, 2006	ASCs (canine)	24 weeks	10 dogs	atelo-collagen	-	Less granulation tissue and atrophic changes in the treated folds

Table 1. Animal models for vocal fold regeneration using MSCs

3.1 Cells

MSCs have many favorable characteristics, including an ability to migrate to a site of injury and to encourage proliferation and differentiation of progenitor cells. They have also been shown to exert control over the microenvironment via secretion of growth factors and matrix remodeling (Caplan, 2007; Uccelli et al., 2007). There is evidence that the activity of T-lymphocytes, B-lymphocytes, and natural killer cells can be inhibited by MSCs, which suggests their potential allogeneic use without risk of immune response (Le Blanc & Ringden, 2007; Rasmussen et al., 2007). Interestingly, bone marrow derived mesenchymal stem cells (BM MSCs), adipose derived mesenchymal stem cells (ASCs) and human vocal fold fibroblasts found in the lamina propria demonstrate similar immunophenotypic properties, differentiation potential and cell surface markers (Long et al., 2009; Puissant et al., 2005; Hanson et al., 2010). This shared cell surface profile included a positive expression for MSC markers (i.e., CD29, CD44, CD73, CD90 and CD105) and a negative expression for hematopoietic stem cell markers (i.e., CD14, CD31, CD34, CD45). Additionally, BM MSCs and ASCs have been shown to differentiate down epithelial and fibroblast lineages (Kanemaru et al., 2005; Long et al., 2009; Ohno et al., 2009). Collectively, these findings support the use of BM MSCs and ASCs in future vocal fold regeneration interventions. Further inquiry distinguishing the utility of these two cell sources is necessary, as there have been no *in vivo* reports directly comparing them in laryngeal research.

There have been a few reports of injecting MSCs directly into the vocal fold, without a scaffold or soluble factors, in order to encourage regeneration following injury (see Table 1). These *in vivo* animal models have generally used a common investigative paradigm, which involves first creating a lesion unilaterally or bilaterally in the membranous portion of the vocal fold. After a period of days to weeks which allows the mature scar to form, the investigator then injects the cell therapy into the scarred region. Outcomes are determined with morphological assessment to characterize the anatomical structure of the tissue, rheometric analysis to determine the tissue's viscoelasticity, and/or with gene or protein expression levels to analyze extracellular matrix component production.

Measures of tissue biomechanics may be the most meaningful outcome to report in vocal fold literature, as viscoelasticity is known to significantly affect voice quality and patient outcomes. Thus, rheometric assessment is becoming an important functional measure in laryngeal literature. Improved viscoelastic measures have been reported for vocal folds treated with BM MSCs alone in a rabbit model (Hertegård et al., 2006a; Svensson et al., 2010).

Improved tissue morphology (less granulation tissue, less rough vocal fold edge) has been reported in injured vocal folds treated with BM MSCs as compared with controls, over a recovery periods lasting from four and twelve weeks (Hertegård et al., 2006a; Kanemaru et al., 2005, Svensson et al., 2010). Figure 1 demonstrates the resolution of one dog's granuloma and rough vocal fold morphology over a 24 week recovery period.

Protein and gene expression analysis can provide useful information about signaling pathways and ECM production. The literature to date has focused largely on the expression and some quantification of the ECM components known to be involved with wound healing, such as collagen, fibronectin, and hyaluronic acid. A reduction in collagen I content (Hertegård et al., 2006a, Svensson et al., 2010) and an increase in TGF-β1 production (Quinchia-Johnson et al., 2010) have been reported in vocal folds injected with BM MSCs alone versus scarred, untreated controls. Additionally, it has been found *in vitro* that a co-culture of ASCs and scar fibroblasts (SF) had decreased collagen production levels and

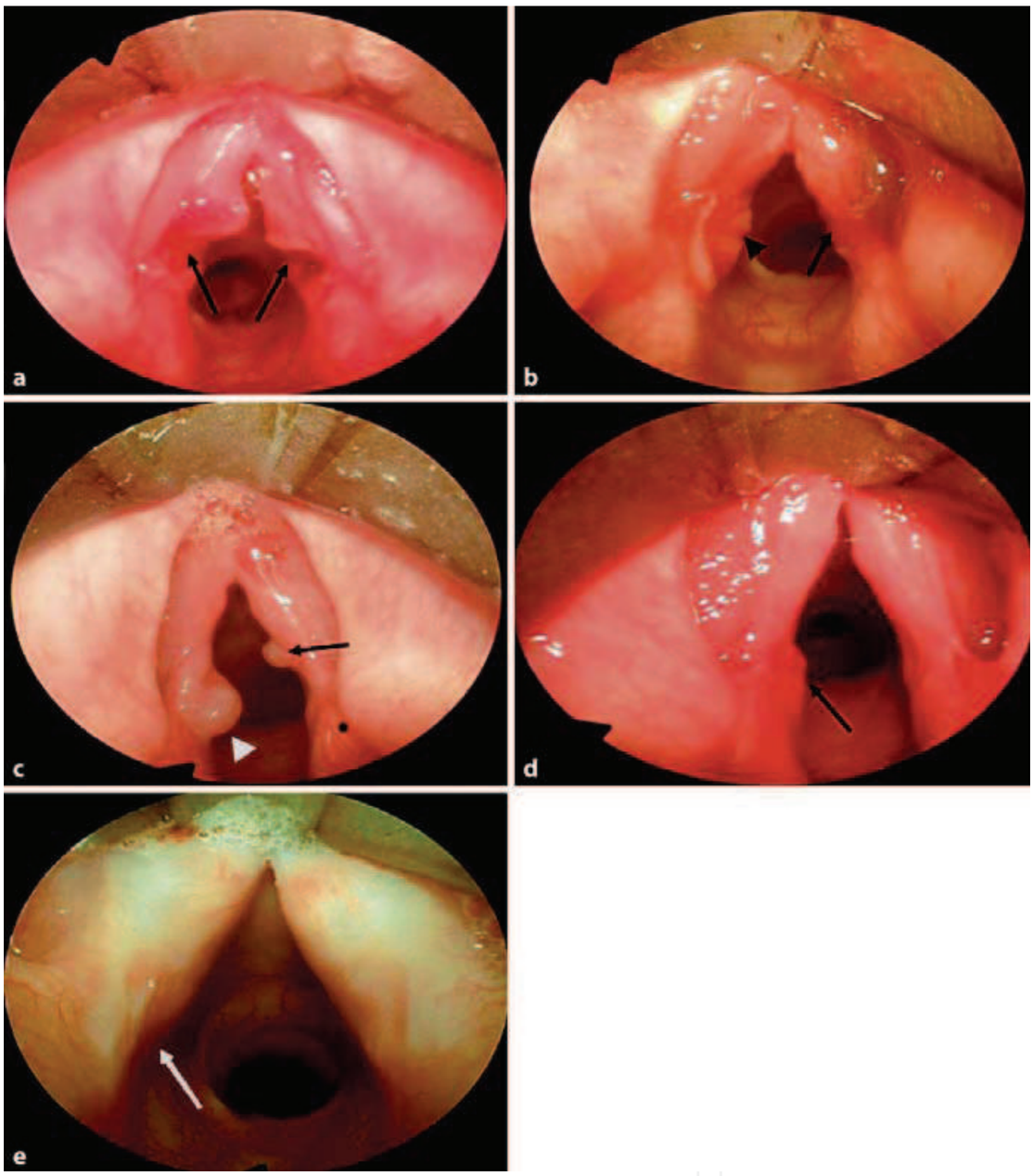


Fig. 1. Reprinted from *Cells Tissues and Organs,* Vol. 184/No. 3-4. Lee., B.Y., Wang, S.G., Lee, J.C., Jung, J.S., Bae, Y.C., Jeong, H.J., Kim, H.W., Lorenz, R.R. The prevention of vocal fold scarring using autologous adipose tissue-derived stromal cells, pp. 198-204, (2006), with permission from S Karger AG, Basel. Improved vocal fold morphology was observed in the scarred, treated right vocal fold, as compared with the scarred, untreated left vocal fold. *a:* On day 4, bilateral vocal fold lesions (see arrows) were created via electrocautery. *b*: On day 14, edema noted in right vocal fold, with irregular vocal mucosa noted in left vocal fold. *c:* On day 28, granuloma was larger on the left than on the right. *d:* On day 57, right-sided granuloma has resolved, but left-sided granuloma remains. *e:* During week 24, the authors noted the appearance of scarring in the left vocal fold, but not in the right vocal fold.

SF proliferation rates when compared with SFs in monoculture (Kumai et al., 2009). These data suggest that BM MSCs and ASCs support an anti-fibrotic profile when exposed to a scar environment. Further protein and gene expression investigation is needed to uncover signaling pathways that may allow future bioengineers to control these processes.

An optimal cell source to remediate vocal fold scarring and promote regeneration would be non-immunogenic, remain viable amidst significant vibration, migrate to the site of injury, encourage remodeling of ECM to reduce the fibrotic scar profile and efficiently differentiate into vocal fold fibroblasts and epithelial cells. To date, a handful of initial studies have indicated the therapeutic potential of injecting BM MSCs or ASCs alone for vocal fold regeneration, but further characterization using the above criteria may prove useful. Investigation using pluripotent cells (embryonic stem cells, induced pluripotent stem cells) and MSCs isolated from other sources (i.e., spleen, thymus, umbilical cord blood, amniotic fluid and dental pulp) may also be considered.

3.2 Scaffolds without cells

Scaffold materials have long been used in injection laryngoplasty procedures by otolaryngologists for the treatment of patients with vocal fold paresis, paralysis or anatomical defects. In this treatment, the biomaterial/scaffold is primarily used as a bulking agent to push the affected vocal fold toward midline and allow for a closer physical approximation during phonation. Synthetic materials (silicone, polytetrafluoroethylene-Teflon, polyhydroxyethylmethacrylate) have largely been eschewed for clinical use because of their propensity for foreign body response, migration of material and non-biodegradability (Ejnell et al., 1984; Nakayama et al., 1993). These materials have been replaced in the clinic by more degradable biomaterials, such as collagen matrix (Kriesel et al., 2002) and fat (McCulloch et al., 2002; Lo Cicero et al., 2008). These materials have viscoelastic properties more similar to vocal mucosa than the previous injectables. Additionally, the site of injection has changed from the thyroartenoid muscle to the lamina propria.

Interestingly, it has recently reported that abdominal adipose tissue harvested for clinical use as a fat injection contains a local population of MSCs. These stem cells have a high proliferative potential and the ability to differentiate down multiple lineages (Lo Cicero et al., 2008). These data suggest that a resident MSC population in vocal fold fat injections may contribute to long-term graft survival, which is suspected to occur in 55-100% of patients (McCulloch et al., 2002; Lo Cicero et al., 2008).

Commercially available composite materials have also been commonly used in clinic, such as Gelfoam paste (Schramm et al., 1978), Cymetra (Milstein et al., 2005), calcium hydroxylapatite (Rosen et al., 2007), Restylane and Hylaform (Hertegard et al., 2006b). While these materials have all been reported to improve vocal function, they are not traditionally thought to grow and remodel the tissue, as many of them biodegrade in the body. Resorption is an advantage for patients with temporary vocal fold pathology and for those interested in a short-term trial, but it is a significant limitation for patients that are interested in long-term treatment, as they can necessitate repeated injections.

A promising line of research is the investigation of gels and hydrogels as vocal fold injectables, as they may offer greater potential for vocal fold regeneration and improved viscoelastic properties over current scaffold materials. To date, hydrogels have primarily been HA based (Duflo et al., 2006a, Duflo et al., 2006b, Jia et al., 2004) and collagen based (Hahn et al., 2006). Figure 2 demonstrates the distinct rheometric measurements obtained from four treatment groups that included a semi-synthetic gel matrix (Thibeault et al., 2009).

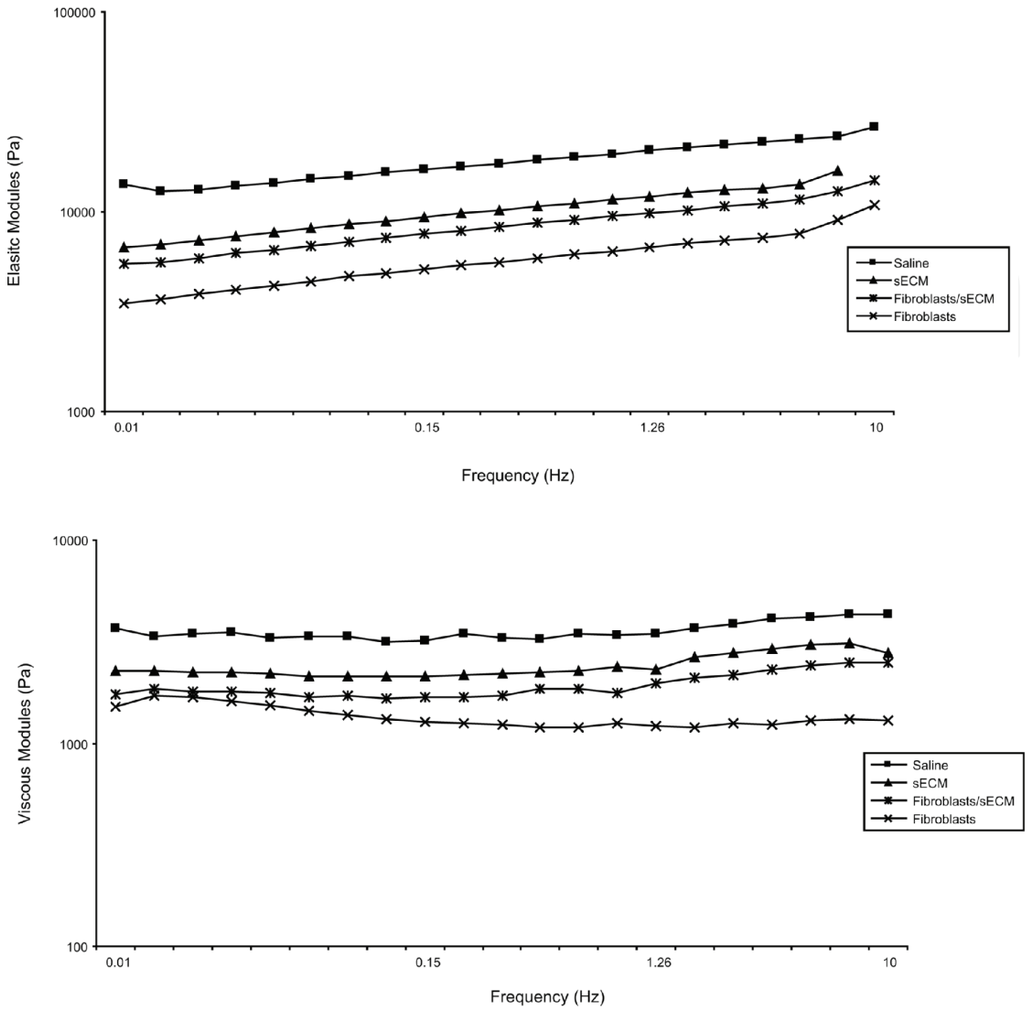


Fig. 2. Reprinted from *Tissue Engineering Part A*, Vol. 15/No. 7, Thibeault, S.L., Klemuk, S.A., Smith, M.E., Leugers, C., Prestwich, G. In vivo comparison of biomimetic approaches for tissue regeneration of the scarred vocal fold, 1481-1487, (2009), with permission from Mary Ann Liebert, Inc. Using a stress-controlled rheometer, the authors were able to demonstrate distinct elastic and viscous moduli associated with the four treatment groups.

Decellularized xenogeneic matrices derived from porcine small intestine, urinary bladder and bovine vocal fold tissue have also been investigated as potential laryngeal scaffolds (Ringel et al., 2006; Xu et al., 2007). In this model, the tissue is decellularized, leaving the potential for ECM-based repair of native via release of endogenous growth factors and activation of local cells.

3.3 Scaffolds with cells

There are many well characterized biomaterials used for injection laryngoplasty, though, only a few substances (i.e., HA based hydrogels, atelocollagen, fibrin) have been investigated for use as scaffolds in vocal fold MSC based therapies (Long et al., 2009; Kanemaru et al., 2003; Lee et al., 2010; Quinchia-Johnson et al., 2010; Ohno et al., 2009).

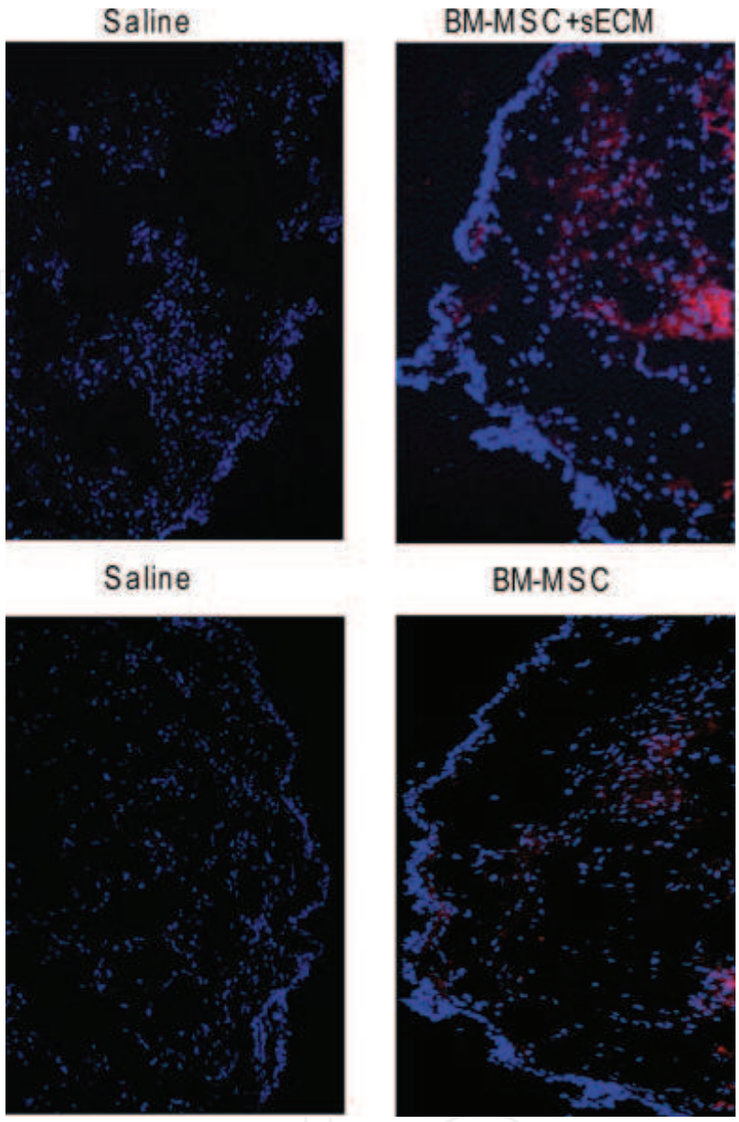


Fig. 3. Reprinted from *The Laryngoscope*, Vol. 120/No. 3, Quinchia-Johnson, B.H., Fox, R., Chen, X., Thibeault, S.L. Tissue regeneration of the vocal fold using bone marrow mesenchymal stem cells and synthetic extracellular matrix injections in rats, pp. 537-545, (2010), with permission from John Wiley and Sons (publisher). More BM MSCs tagged with green fluorescent protein (shown in red) remain in the tissue 30 days postinjection for the combination therapy than the cells alone condition.

HA based hydrogels have been shown to be noninflammatory, nonimmunogenic and biocompatible, as well as have significant shock absorption ability. Carbylan-GSX (a thinly-modified semisynthetic GAG derived of HA mixed with a thiolated gelatin, and cross linked with polyethylene glycol), has viscoelastic properties similar to vocal mucosa when injected *in vivo* (Duflo et al., 2006a). When encapsulated in Extracel, BM MSCs maintained a high viability (96% or better), and were able to sustain their cell growth over a three day period (Duflo et al., 2006b). They remained alive 30 days post injection (See Fig. 3). In addition, this construct injected *in vivo* produced an ECM profile more supportive of wound healing (i.e.,

increased expression of fibronectin and pro-collagen III) than a cells only approach and the control condition (Quinchia-Johnson et al., 2010).

Atelocollagen, a biomaterial found in a few recent reports, is derived from calf dermis and can be degraded *in vivo* by endogenous collagenase at a slower rate than collagen scaffolds. It can be used for tissue engineering purposes in an unmodified form (Lee et al., 2006), made into a sponge (Ohno et al., 2009) or into a gel (Kanemaru et al., 2003). Adherence of cells to the material is an important criterion for potential scaffold biomaterials. Approximately 20% of BM MSCs initially adhered to an atelocollagen sponge *in vitro*, with significant proliferation noted on days 3 and 5 (Ohno et al., 2009). The authors credited the cellular entry to the large pore size. BM MSCs labeled with a fluorochrome and seeded within a 1% hydrochloric acid atelocollagen gel were found to have "good proliferation" *in vitro* (Kanemaru et al., 2003). Similarly, " a large number" of fluorochrome-labeled cells were observed in canine vocal folds eight weeks after having been injected with ASCs within an atelocollagen scaffold (Lee et al., 2006). Despite this, atelocollagen has the distinct disadvantage of being an animal derived substance, which brings with it the threat of immune response activation or transmission of an endogenous retrovirus.

A third reported scaffold biomaterial for vocal fold stem cell applications is fibrin. Long et al. (2009, 2010) was able to demonstrate *in vitro* that a fibrin-based construct seeded with ASCs have good physical integrity and handling characteristics, slow degradation patterns and an ability to support cell viability. Recent findings demonstrate that these constructs have the elastic modulus and collagen orientation similar to native vocal mucosa, and are able to withstand oscillation when sutured onto an excised cadaveric larynx on top of pipe through which air can flow (Long et al., 2010). See Figure 4.

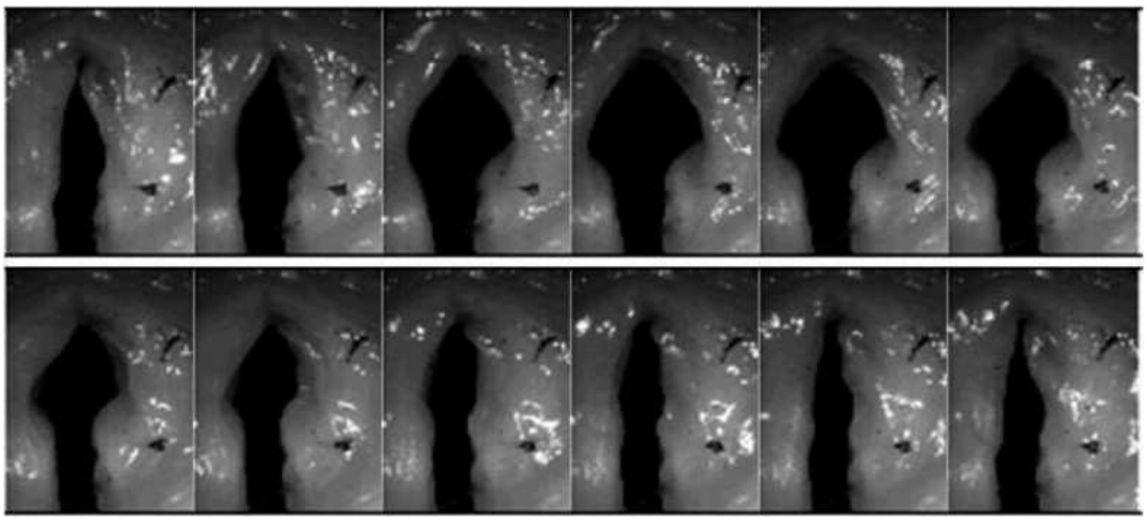


Fig. 4. Reprinted from *Otolaryngology-Head and Neck Surgery*, Vol. 142 /No. 3, Long, J.L., Neubauer, J., Zhang Z., Zuk, P., Berke, G.S., Chhetri, D.K. Functional testing of a tissue-engineered vocal fold cover replacement, 438-440, 2010, with permission from Elsevier. Native vocal fold cover of cadaveric larynx has been replaced with a tissue engineered construct and vibrated with air from an underlying pipe. Arrows mark the edges of the construct. The top of images is the anterior commissure. These images were captured with a high speed camera.

An optimal scaffold for stem cell applications for vocal fold regeneration would be noninflammatory, nonimmunogenic, encourage adherence and viability of resident cells, support appropriate cell-cell signaling, biodegrade at an acceptable rate, remain intact during investigator handling, as well as be able to sustain vocal fold vibration. The scaffold materials listed previously have demonstrated some of these attributes in animal models, but applications in conjunction with stem cell approaches is scant, currently. There exists a great opportunity to advance vocal fold regeneration strategies by finding an optimal scaffold to deliver cells and growth factors.

3.4 Growth factor delivery

To date, the delivery of only a few growth factors, including epidermal growth factor (EGF) fibroblast growth factor (FGF) and hepatocyte growth factor (HGF) have been investigated within MSC-based therapies for vocal fold regeneration. All of this work has been completed *in vitro*.

The effect of soluble signaling has been used to examine the differentiation potential of ASCs. A bilayered, three dimensional construct was created *in vitro* by seeding ASCs within fibrin hydrogels, and once gelation was complete, additional ASCs were added directly on top. When EGF, FGF and retinoic acid were added to the media surrounding these constructs, it was found that EGF encouraged differentiation of ASCs into epithelial cells more efficiently than the other soluble signals (Long et al., 2009). The authors found that the cells on the top, epithelial-like surface stained positive for E-cadherin and cytokeratin 8, epithelial phenotype markers. It was found that these cells differentiated along this lineage only when they had an air interface and exposure to EGF. Interestingly, the authors hypothesized that mechanotransduction may have also played a role in differentiation, as the cells were cultured on a matrix with similar stiffness to the lamina propria. The cells on the inside of the hydrogel stained positive for vimentin, a cytoskeletal protein expressed by cells of mesenchymal origin. It should be noted that during the two week culture period, the epithelial cells did not form a confluent layer, suggestive of reduced efficiency of differentiation and proliferation of epithelial cells.

HGF is known to have strong anti-fibrotic activity, and has been investigated in the voice literature as a stand-alone injection to remediate vocal fold scarring in an animal model (Hirano et al., 2004). In this study, the HGF treated vocal folds had improved rheometric measurements and less collagen deposition than the scarred, untreated vocal folds. In the MSC literature, HGF has been implicated as being secreted by ASCs and encouraging an anti-fibrotic extracellular matrix profile when they are in co-culture with scar fibroblasts (Kumai, 2009). Following vocal fold scarring, ASCs and scar fibroblasts (SF) were isolated from male ferrets, and then co-cultured in a variety of conditions to investigate their relationship with HGF. In order to demonstrate that HGF was one of the growth factors implicated in reducing the production of collagen, a neutralization assay was used. Following four days of co-culture of ASCs and SFs with an anti HGF antibody in the medium, the SFs had significantly higher amounts of collagen secretion than in the control condition. This condition did not affect HA secretion, and thus it was concluded that the HGF secreted by ASCs encourages the anti-fibrotic profile of SFs by downregulating collagen production, but not by upregulating HA production. Additionally, the authors suggested that a tissue engineering construct delivering HGF through ASCs to the vocal fold microenvironment rather than through an exogenous agent is preferable because of the

slow release associated with having residency in the tissue and the potential activation of concurrent endogenous facilitatory factors. So, while there have been few studies of introducing growth factors exogenously to tissue engineering for vocal fold regeneration, endogenous growth factors are often thought to be present.

4. Future directions

4.1 Bioreactors

Bioreactors provide *ex vivo* mechanical stimulation that mimics a specific tissue's microenvironment for cells in media. With regard to laryngeal research, bioreactors can provide a unique model for studying the effects of vibration (similar to phonation) on cells in a controlled environment. For the custom designed bioreactors currently used in this line of research, frequency, amplitude and duration of vibration and tension of the substrate which cells are adherent to can often be programmed according to the experimental question of interest. There are many potential applications of this technology, including examination of the effects of dosage of vibration on cells of various laryngeal diseases, investigation of scar fibroblast activity at varying time intervals post laryngeal surgery (to inform recommendations about when to resume voicing post-operatively) and to compare the effects different laryngeal configurations during phonation on healing (to mimic different voice therapies at the cellular level), etc.

While there have been several reports of the effects of stem cell therapies on ECM production, few studies have investigated the mechanisms for encouraging specific vocal fold ECM profiles. Bioreactors may provide a mode of inquiry toward these ends. Interestingly, recent literature suggests that fibroblasts are able to convert mechanical stimuli into ECM modifications, and thereby induce tissue remodeling via mechanotransduction (Ingber, 2006). Recent voice research using bioreactors have found significant vibration induced changes in the ECM profile. For example, human dermal fibroblasts vibrated in hydrogels for periods of five and ten days demonstrated increased expression of HA synthase 2, decorin and fibromodulin (Kutty & Webb, 2010). Human laryngeal fibroblasts vibrated for periods between 1-21 days showed an increased production of fibronectin and collagen type I (Wolchok et al., 2009). Finally, human vocal fold fibroblasts vibrated for 6 hours showed an upregulation of fibronectin and HA-associated genes (Titze et al., 2004). Comparison of the ECM produced by multiple cell types exposed to vibration that mimics phonation may help scientists determine an optimal cell source for vocal fold bioengineering.

Currently bioreactors provide a research model, but in the future they may be utilized in therapeutic inventions. It may be found that cells can be primed in a bioreactor to create an optimal ECM profile before they are implanted into an organism with scarring or other vocal pathology. The use of bioreactors is a promising line of research that could shape future tissue regeneration approaches.

5. Conclusion

The regenerative potential of vocal fold tissue is a topic that is currently being investigated by an increasing number of teams internationally. While the literature to date has merely scratched the surface of the basic parameters involved in laryngeal tissue engineering, there is great opportunity for advancement of the knowledge base with the advent of high

throughput experimental techniques, systems biology approaches and their associated statistical analysis. These developments allow for more efficient and comprehensive assessments of cell/scaffold interactions and ECM production profiles. Current themes in the literature include morphological and rheological outcomes of cell based therapies and how to use scaffolds and bioreactors to encourage optimal ECM regeneration. Future topics may include how to encourage efficient differentiation into epithelial cells via signaling mechanisms, how to engineer confluent and distinct layers that mimic normal vocal fold anatomy, how to induce angiogenesis that will be able to withstand vibration without hemorrhage and how to innervate the tissue.

6. Acknowledgements

The authors would like to acknowledge the National Institute of Deafness and Other Communication Disorders-R01 DC4336 for supporting this work.

7. References

Benninger, M.S., Alessi, D., Archer, S, Bastian, R., Ford, C., Koufman, J. (1996). Vocal fold scarring: current concepts and management. *Otolaryngology- Head Neck Surgery,* Vol.115, No. 5, (Nov 1996) 474-482, 0194-5998

Caplan, A. (2007). Adult mesenchymal stem cells for tissue engineering versus regenerative medicine. *Journal of Cellular Physiology,*Vol. 213, No. 2, (Nov 2007) 341-7, 0021-9541

Catten, M., Gray, S.D., Hammond, T.H., Zhou, R., Hammond, E. (1998). Analysis of cellular location and concentrations in vocal fold lamina propria. *Otolaryngology- Head Neck Surgery,* Vol., 118, No.5, (May 1998) 663-667, 0194-5998

Chhetri, D.K., Zhang, Z., Neubauer, J. (2010). Measurement of young's modulus of vocal folds by indentation. *Journal of Voice,* article in press

Courey, M., Shohet, J., Scott, M., Ossoff, R. (1996). Immunohistochemical characterization of benign laryngeal lesions. *Annals of Otology, Rhinology & Laryngology,* Vol. 105, No.7, (July 1996) 525-531, 0003-4894

Duflo, S., Thibeault, S.L., Li, W., Shu, X.Z., Prestwich, G.D. (2006a). Vocal fold tissue repair *in vivo* using a synthetic extracellular matrix. *Tissue Engineering,* Vol. 12, No.8, (Aug 1996) 2171-2179, 2152-4955

Duflo, S., Thibeault, S.L., Li, W., Shu, X.Z., Prestwich, G.D. (2006b). Effect of a synthetic extracellular matrix on vocal fold lamina propria gene expression in early wound healing. *Tissue Engineering,* Vol. 12, No. 11, (Nov 2006) 3201-3207, 2152-4955

Ejnell, H., Mansson, I., Blake, B., Stenborg, R. (1984). Laryngeal obstruction after Teflon injection. *Acta Laryngologica,* Vol. 98, No. 3-4, (Sept-Oct 1984) 374-379, 1758-5368.

Fisher, K.V., Telser, A., Phillips, J.E., Yeates, D.B. (2001). Regulation of vocal fold transepithelial water fluxes. *Journal of Applied Physiology,* Vol. 91, No. 3, (Sept 2001) 1401-1411, 8750-7587

Gipson, I.K., Spurr-Michaud, S.J., Tisdale, A.S., Kublin, C., Cintron, C., Keutmann, H. (1995). Stratified squamous epithlelial produce mucin-like glycoproteins. *Tissue and Cell,* Vol. 27, No. 4, (Aug 1995) 397-404, 0040-8166

Gray, S.D., Titze, I. (1988). Histologic investigation of hyperphonated canine vocal cords. *The Annals of Otology, Rhinology and Laryngology,* Vol. 97, No. 4, (July-Aug 1988) 381-8, 0003-4894

Gray, S. D. (1991) Basement membrane zone injury in vocal nodules. *Vocal Fold Physiology Conference,* pp. 21-28, Singular Publishing Group, San Diego

Gray, S.D., Hirano, M., Sato, K. (1993). Molecular and cellular structure of vocal fold tissue, In: *Vocal fold physiology: frontiers of basic science, 1st ed.,* Titze, I. (Ed.), 1-34., Singular Publishing, 1870332997, San Diego

Gray, S.D., Titze, I., Chan, R., Hammond, T.H. (1999). Vocal fold proteoglycans and their influence on biomechanics. *The Laryngoscope,* Vol. 109, No. 6, (June 1999) 845-854, 1531-4995

Gray, S.D. (2000a). Cellular physiology of the vocal folds. *Otolaryngologic Clinics of North America,* Vol. 33, No. 4 (Aug 2000), 679-697, 0030-6665

Gray, S.D., Titze, I., Alipour, F., Hammond, T.H. (2000b). Biomechanical and histologic observations of vocal fold fibrous proteins. *Annals of Otology, Rhinology & Laryngology,* Vol. 109, No. 1, (Jan 2000) 77-84, 0003-4894

Guimaraes, I., Abberton, E. (2005). Fundamental frequency in speakers of Portuguese for different voice samples. *Journal of Voice,* Vol. 19, No. 4, (Dec 2005) 592-606, 0892-1997

Hahn, M.S., Teply, B.A., Stevens, M.M., Zeitels, S.M., Langer, R. (2006). Collagen composite hydrogels for vocal fold lamina propria restoration. *Biomaterials,* Vol. 27, No. 7, (Mar 2006), 1104-1109, 0142-9612

Hansen, J.K., Thibeault, S.L. (2006). Current understanding and review of the literature: vocal fold scarring. *Journal of Voice*, Vol. 20, No. 1, (March 2006) 110-120, 0892-1997

Hanson, S., Kim., J., Quinchia-Johnson, B., Bradley, B., Breunig, M., Hematti, P., Thibeault, S. (2010). Characterization of mesenchymal stem cells from human vocal fold fibroblasts. *The Laryngoscope,* Vol. 120, No. 3, (March 2010) 546-551, 1531-4995

Hertegård, S., Cedervall, J., Svensson, B., Forsberg, K., Maurer, F.H.J., Vidovska, D., Olivius, P., Ahrlund-Richter, L., Le Blanc, K. (2006a). Viscoelastic and histologic properties in scarred rabbit vocal folds after mesenchymal stem cell injection. *The Laryngoscope,* Vol. 116, No. 7, (July 2006) 1248-1254, 1531-4995

Hertegård, S., Dahlqvist, A., Goodyer, E. (2006b). Viscoelastic measurements after vocal fold scarring in rabbits—short-term results after hyaluronan injection. *Acta Otolaryngologica,* Vol. 126, No. 7, (July 2006b) 758-763, 0365-5237

Hirano, M. (1981). Structure of the vocal fold in normal and disease states. Anatomical and physical study. *ASHA Rep*; 11:11-30, 1981

Hirano, M., Sato, K., Nakashima, T. (1999). Fibroblasts in human vocal fold mucosa. *Acta Oto-Laryngologica*, Vol. 119, No. 2, (Mar 1999) 271-276, 1758-5368

Hirano, S., Bless, D.M., Rousseau, B., Welham, N., Montequin, D., Chan, R.W., Ford, C.N. (2004). Prevention of vocal fold scarring by topical injection of hepatocyte growth factor in a rabbit model. *The Laryngoscope*, Vol. 114, No. 3, (Mar 2004) 548-556, 1531-4995

Hunter, E.J., Svec, J.G., Titze, I.R. (2006). Comparison of the produced and perceived voice range profiles in untrained and trained classical singers. *Journal of Voice*, Vol. 20, No. 4, (Dec 2006) 513-526, 0892-1997

Ingber, DE. (2006). Cellular mechanotransduction: putting all the pieces together again. *The FASEB Journal,* Vol. 20, No. 7, (May 2006) 811-827, 0892-6638

Jia, X., Burdick, J.A., Kobler, J., Clifton, R.J., Rosowski, J.J., Zeitels, S.M., Langer, R. (2004). Synthesis and characterization of in situ cross-linkable hyaluronic acid-based

hydrogels with potential application for vocal fold regeneration. *Macromolecules,* Vol. 37, No. 9, (Apr 2004), 3239-3248, 1874-3439

Johnston, N., Bulmer, D., Ross, P.E., Axford, S.E., Pearson, J.P., Dietmar, P.W., Panetti, M., Pignatelli, M, Koufman, J.A. (2003). Cell biology of laryngeal epithelial defenses in health and disease: further studies. *Annals of Otology, Rhinology Laryngology,* Vol. 112, No. 6, (Jun 2003) 481-491, 0003-4894

Kanemaru, S., Nakamura, T., Omori, K., Kojima, H., Magrufov, A., Yiratsuka, Y., Hirano, S., Ito, J., Shimizu, Y. (2003). Regeneration of the vocal fold using autologous mesenchymal stem cells. *Annals of Otology, Rhinology & Laryngology,* Vol. 112, No. 11, (Nov 2003) 915-920, 0003-4894

Kanemaru, S., Nakamura, T., Yamashita, M., Magrufov, A., Kita, T., Tamaki, H., Tamura, Y., Iguchi, F., Kim, T.S., Kishimoto, M., Omori, K., Ito, J. (2005). Destiny of autologous bone marrow-derived stromal cells implanted in the vocal fold. *Annals of Otology, Rhinology & Laryngology,* Vol. 114, No. 12, (Dec 2005) 907-912, 0003-4894

Kriesel, K., Thibeault, S.L., Chan, R.W., Suzuki, T., Van Groll, P.J., Bless, D.M., Ford, C.N. (2002). Treatment of vocal fold scarring: rheological and histological measures of homologous collagen matrix. *Annals of Otology, Rhinology and Laryngology,* Vol. 111, No. 10, (Oct 2002) 884-889, 0003-4894

Kumai, Y., Kobler, J.B., Park, H., Lopez-Guerra, G., Karajanagi, S., Herrera, V., Zeitels, S. (2009). Crosstalk between adipose-derived stem/stromal cells and vocal fold fibroblasts in vitro. *The Laryngoscope,* Vol. 119, No. 4, (Mar 2009), 799-805, 1531-4995

Kutty, J.K. & Webb, K. (2010). Vibration stimulates vocal mucosa-like matrix expression by hydrogel-encapsulated fibroblasts. *Journal of Tissue Engineering and Regenerative Medicine,* Vol. 4, No. 1, (Jan 2010) 62-72, 1932-6254

Le Blanc, K., Ringden, O. (2007). Immunomodulation by mesenchymal stem cells and clinical experience. *Journal of Internal Medicine,* Vol. 262, No. 5, (Nov 2007) 509-525, 1539-3704

Lee., B.Y., Wang, S.G., Lee, J.C., Jung, J.S., Bae, Y.C., Jeong, H.J., Kim, H.W., Lorenz, R.R. (2006). The prevention of vocal fold scarring using autologous adipose tissue-derived stromal cells. *Cells, Tissues and Organs,* Vol. 184, No. 3-4, (2006) 198-204, 1422-6405

Lo Cicero, V.L., Montelatici, E., Cantarella, G., Mazzola, R., Sambataro, G., Rebulla, P., Lazzari, L. (2008). Do mesenchymal stem cells play a role in vocal fold fat graft survival? *Cell Proliferation,* Vol. 41, No. 3, (Jun 2008), 460-473, 0960-7722

Long, J.L., Zuk, P., Berke, G.S., Chhetri, D.K. (2009). Epithelial differentiation of adipose-derived stem cells for laryngeal tissue engineering. *The Laryngoscope,* Vol. 120, No. 1, (Jan 2010), 125-131, 1531-4995

Long, J.L., Neubauer, J., Zhang, Z., Zuk, P., Berke, G.S., Chhetri, D.K. (2010). Functional testing of a tissue-engineered vocal fold cover replacement. *Otolaryngology-Head and Neck Surgery,* Vol. 142, No. 3, (Mar 2010) 438-440, 0194-5998

McCulloch, T.M., Andrews, B.T., Hoffman, H.T., Graham, S.M., Karnell, M.P., Minnick, C. (2002). Long-term follow-up of fat injection laryngoplasty for unilateral vocal cord paralysis. *The Laryngoscope,* Vol. 112, No. 7, (Jul 2002) 1235-1238, 1531-4995

Milstein, C.F., Akst, L.M., Hicks, M.D., Abelson, T.I., Strome, M. (2005). Long-term effects of micro-ionized alloderm injection for unilateral vocal fold paralysis. *The Larygoscope,* Vol.115, No. 9, (Sept 2005) 1691-1696, 1531-4995

Mogi, G., Watanabe, N., Maeda, S. Umehara, T. (1979). Laryngeal secretions: an immunochemical and immunohistological study. *Acta Oto-laryngologica,* Vol. 87, No. (1-2), (Jan-Feb 1979) 129-141, 0365-5237

Mossallam, I., Kotby, M., Ghaly, A., Nassar, A., Barakah, M. (1986). Histopathological aspects of benign vocal fold lesions associated with dysphonia. In: *Vocal fold histopathological: a symposium, 1st ed.,* Kirchner J., (Ed.), pp. 65-80, College Hill Press, San Diego

Nakayama, M., Ford, C.N., Bless, D.M. (1993). Teflon vocal fold augmentation: failures and management in 28 cases. *Otolaryngology-Head and Neck Surgery,* Vol. 109, No. 3 Pt 1, (Sept 1993), 493-498, 0194-5998

Ohno, S., Hirano, S., Tateya, I., Kanemaru, S., Umeda, H., Suehiro, A., Kitani, Y., Kishimoto, Y., Kojima, T., Nakamura, T., Ito, J. (2009). Atelocollagen sponge as a stem cell implantation scaffold for the treatment of scarred vocal folds. *Annals of Otology, Rhinology & Laryngology,* Vol. 118, No. 11, (Nov 2009) 805-810, 0003-4894

Puissant, B., Barreau, C., Bourin, P., Clavel, C., Corre, J., Bousquet, C., Taureau, C., Cousin, B., Abbal, M., Laharrague, P., Penicaud, L, Casteilla, L., Blancher, A. (2005). Immunomodulatory effect of human adipose tissue-derived adult stem cells: comparison with bone marrow mesenchymal stem cells. *British Journal of Haematology,* Vol. 129, No. 1, (Apr 2005) 118-129, 0007-1048

Quinchia-Johnson, B.H., Fox, R., Chen, X., Thibeault, S.L. (2010). Tissue regeneration of the vocal fold using bone marrow mesenchymal stem cells and synthetic extracellular matrix injections in rats. *The Laryngoscope,* Vol. 120, No. 3, (Mar 2010) 537-545, 1531-4995

Rasmussen, I., Uhlin, M., Le Blanc, K., Levitsky, V. (2007). Mesenchymal stem cells fail to trigger effector functions of cytotoxic T lymphocytes. *Journal of Leukocyte Biology*, Vol. 82, No. 4, (Oct 2007) 887-893, 0741-5400

Ringel, R.L., Kahane, J.C., Hillsamer, P.J., Lee, A.S., Badylak, S.F. (2006) The application of tissue engineering procedures to repair the larynx. *Journal of Speech, Language and Hearing Research,* Vol. 49, No. 1, (Feb 2006), 194-208, 1558-9102

Rosen, C.A., Gartner-Schmidt, J., Casiano, R., Anderson, T.D., Johnson, F., Reussner, L., Remacle, M., Sataloff, R.T., Abitbol, J., Shaw, G., Archer, S., McWhorter, A. (2007). Vocal fold augmentation with calcium hydroxyapatite. *Otolaryngology-Head and Neck Surgery,* Vol. 136, No. 2, (Feb 2007) 198.e1-198e.12, 0194-5998

Schramm, V.L., May, M., Lavorato, A.S. (1978). Gelfoam paste injection for vocal cord paralysis: temporary rehabilitation of glottic incompetence. *The Laryngoscope,* Vol. 88, No. 8, (Aug 1978) 1268-1273, 1531-4995

Sivasankar, M., Erickson, E., Rosenblatt, M., Branski, R. (2010) Hypertonic challenge to porcine vocal folds: effects on epithelial barrier function. *Otolaryngology-Head and Neck Surgery,* Vol. 142, No. 1, (Jan 2010) 79-84, 0194-5998

Smith, E., Gray, S., Verdolini, K, Lemke, J. (1995). Effects of voice disorders on quality of life. *Otolaryngology-Head and Neck Surgery*, Vol. 113, No. 2, (Aug 1995) 121, 0194-5998

Svensson, B., Nagubothu, R.S., Cedervall, J., Le Blanc, K.L., Ahrlund-Richter, L., Tolf, A., Hertegård, S. (2010). Injection of human mesenchymal stem cells improves healing of scarred vocal folds: analysis using a xenograft model. *The Laryngoscope*, Vol. 120, No. 7, (Jul 2010) 1370-1375, 1531-4995

Thibeault, S.L., Gray, S.D., Bless, D.M., Chan, R.W., Ford, C. (2002). Histologic and rheologic characterization of vocal fold scarring. *Journal of Voice,* Vol. 16, No. 1, (Mar 2002) 96-104, 0892-1997

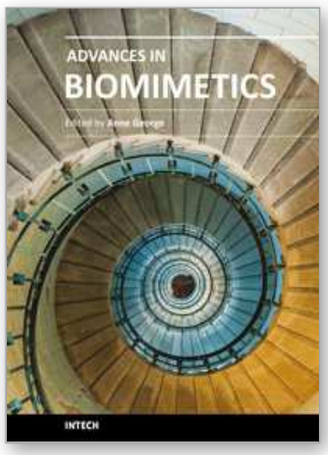
Thibeault, S.L., Klemuk, S.A., Smith, M.E., Leugers, C., Prestwich, G. (2009). In vivo comparison of biomimetic approaches for tissue regeneration of the scarred vocal fold. *Tissue Engineering Part A,* Vol. 15, No. 7, (Jul 2009), 1481-1487, 2152-4947

Titze, I.R., Hitchcock, R.W., Broadhead, K., Webb, K., Li, W., Gray, S.D., Tresco, P.A. (2004). Design and validation of a bioreactor for engineering vocal fold tissues under combined tensile and vibrational stresses. *Journal of Biomechanics,* Vol. 37, No. 10, (Oct 2004) 1521-1529, 0021-9290

Uccelli, A., Pistoia, V., Moretta, L. (2007). Mesenchymal stem cells: a new strategy for immunosuppression? (2010). *Trends in Immunology,* Vol. 28, No. 5, (May 2010) 219-226, 1471-4906

Wolchok, J.C., Brokopp, C., Underwood, C.J., Tresco, P.A. (2009). The effect of bioreactor induced vibrational stimulation on extracellular matrix production from human derived fibroblasts. *Biomaterials,* Vol. 30, No. 3, (Jan 2009) 327-335, 0142-9612

Xu, C.C., Chan, R.W., Tirunagari, N. (2007). A biodegradable, acellular xenogeneic scaffold for regeneration of the vocal fold lamina propria. *Tissue Engineering,* Vol. 13, No. 3, (Mar 2007), 551-566, 1937-3368



Advances in Biomimetics

Edited by Prof. Marko Cavrak

ISBN 978-953-307-191-6
Hard cover, 522 pages
Publisher InTech
Published online 26, April, 2011
Published in print edition April, 2011

The interaction between cells, tissues and biomaterial surfaces are the highlights of the book "Advances in Biomimetics". In this regard the effect of nanostructures and nanotopographies and their effect on the development of a new generation of biomaterials including advanced multifunctional scaffolds for tissue engineering are discussed. The 2 volumes contain articles that cover a wide spectrum of subject matter such as different aspects of the development of scaffolds and coatings with enhanced performance and bioactivity, including investigations of material surface-cell interactions.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Rebecca S. Bartlett and Susan L. Thibeault (2011). Bioengineering the Vocal Fold: A Review of Mesenchymal Stem Cell Applications, Advances in Biomimetics, Prof. Marko Cavrak (Ed.), ISBN: 978-953-307-191-6, InTech, Available from: http://www.intechopen.com/books/advances-in-biomimetics/bioengineering-the-vocal-fold-a-review-of-mesenchymal-stem-cell-applications

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen