Vocal Fold Bioengineering

One of the most remarkable mechanical devices that nature has engineered consists of small folds of very soft tissue that wrap around the two vocal cords. In humans, these vocal folds are approximately 1 cm long and 1 mm thick. The great variety of sounds that can be articulated by humans are produced when the vocal folds move together and apart in a wavelike motion at frequencies of 75 to 1000 Hz.[1]

One of the keys to this great versatility lies in the unique structure of the vocal folds. Each vocal fold is a laminated structure consisting of a pliable vibratory layer of connective tissue, known as the lamina propria (LP), sandwiched between a membrane (epithelium) and a muscle (vocalis muscle or vocal cord) [Fig. 1].

Human LP is further subdivided into superficial (SLP), intermediate (ILP), and deep (DLP) layers; the composite is a functionally graded material, varying from a highly pliable SLP to a relatively stiff DLP. The SLP consists chiefly of an amorphous substance, whereas the intermediate layer and deep layers of the LP (also known as the vocal ligament) are made of fibrous proteins.[2]

Under normal conditions, the vocal folds can sustain up to 60% strain. However, too much strain, due to voice overuse or abuse, can damage this delicate system. The presence of scar tissue disrupts the natural pliability of the lamina propria and causes hoarseness and other symptoms of vocal dysfunction. In severe cases, patients may lose their voice.

An estimated 3 to 9% of the population is affected by some degree of vocal fold scarring.[3] Patients with vocal fold disorders often suffer psychological impairment due to social isolation.

In June 2002, a new research initiative on vocal fold restoration was announced. Julie Andrews, the actress and singer who suffered the loss of her singing voice following vocal cord surgery in 1997, presided over the press conference. This collaborative effort was begun by physicians and researchers from Massachusetts General Hospital, led by Dr. Steven Zeitels, and engineers and scientists from Massachusetts Institute of Technology (MIT), led by Dr. Robert Langer. In Fall 2003, they were joined by applied mechanics contributors at Brown University, led by Dr. Rodney Clifton.

The ultimate goal of this project is to reestablish normal vibratory characteristics to scarred vocal folds to help patients regain their normal voice. Since the announcement of the research initiative, innovative surgical techniques including laser-based precision technology and surgical microinstrumentation have been actively pursued at Massachusetts General Hospital. Meanwhile, considerable research has been directed toward design, synthesis, and characterization of biocompatible SLP substitutes at MIT and Brown University.

Our strategies for voice restoration include three parallel approaches:

- The first involves the creation of injectable materials to improve pliability of damaged vocal folds.
- The second is to synthesize a "designer material" to replace portions of the vocal folds.
- A third uses tissue engineering methods to "grow" new vocal fold tissue.

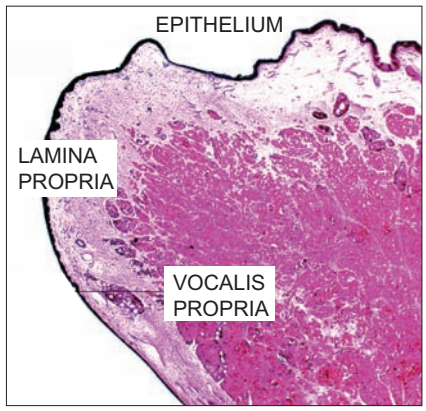

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Fig. 1. Coronal section through normal vocal cord tissue shows the epithelium, lamina propria, and vocalis muscle.

Injectable materials are attractive candidates for vocal fold restoration, because they involve a minimally invasive surgical procedure and can be used to augment the amount of functional tissue. We have identified the SLP as the key target for restoration due to its critical role in vocal fold vibration and the fact that most vocal fold disorders occur in the SLP.

We are exploring the potential of repairing damaged vocal folds with injectable hydrogels derived from a naturally occurring polysaccharide, hyaluronic acid (HA). HA is composed of β-1,4-linked D-glucuronic acid and β-1,3 N-acetyl-D-glucosamine disaccharide units. It is the only nonsulfated glycosaminoglycan in the extracellular matrix of all higher animals and is inherently biocompatible.

In addition to maintaining the hydration state for vocal fold tissue, HA plays a pivotal role in vocal

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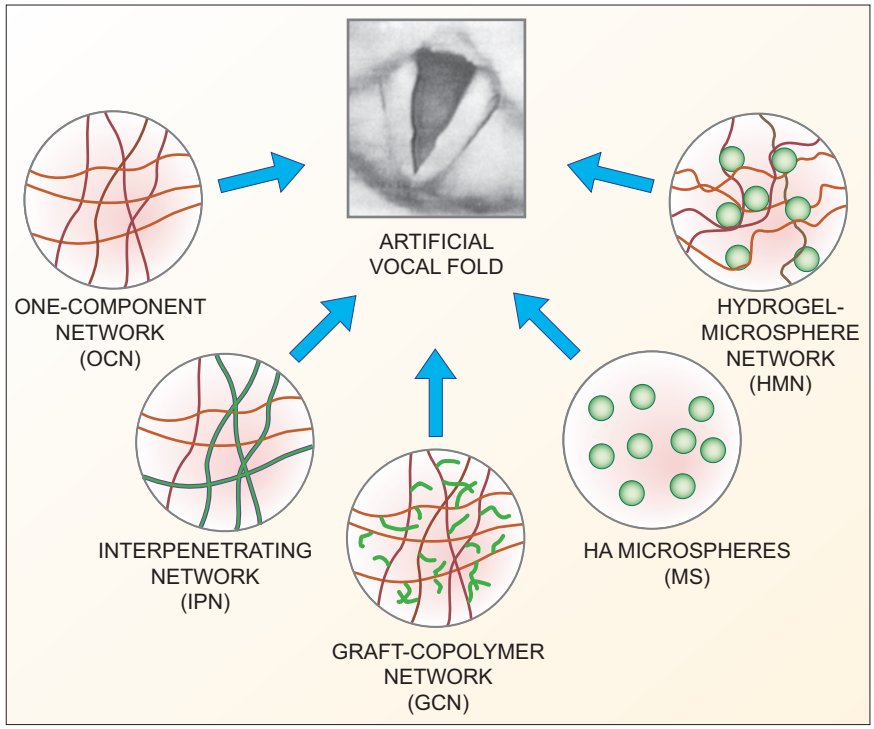

Fig. 2. Hyaluronic acid (HA)-based injectable hydrogels can be synthesized in a variety of forms that exhibit different molecular structures and mechanical properties.

fold biomechanics. Reduced HA synthesis has been associated with vocal fold scarring. However, natural HA lacks mechanical integrity and has a very short in vivo lifetime. To improve HA's applicability for vocal fold regeneration, we have synthesized a series of novel hydrogel networks that exhibit different molecular architectures and tunable mechanical properties [Fig. 2].

The first hydrogel is a one-component hydrogel network (OCN) and consists of HA modified with a photo-crosslinkable group. Hydrogels thus formed are referred to as OCN-UV. Alternatively, one-component hydrogels may be formed by simple mixing of HAs that have been previously modified with mutually reactive groups. The resulting hydrogels are referred to as OCN-Mix.

A second hydrogel consists of an interpenetrating network (IPN) of HA and poly(ethylene glycol). In a third hydrogel, a synthetic polymer is covalently conjugated to an HA backbone to form graft-copolymer networks (GCN), which are subsequently crosslinked on exposure to UV irradiation.

HA microspheres [Fig. 3] are prepared by an inverse emulsion technique, and the resulting hydrogel particles also can be used to prepare a hydrogel microsphere network (HMN).[4]

The versatility of HA chemistry offers opportunities to fine-tune the material's properties. The soft and transparent hydrogels can be easily manipulated and precisely injected into the SLP by a fine needle. In vitro studies indicate that these hydrogels are not only biocompatible but also resistant to enzymatic degradation. In vivo evaluation of functional recovery of damaged vocal folds repaired with these hydrogels will be conducted in the near future.

As a first step toward developing suitable replacement materials, it is important to understand the mechanical response of the natural tissue that the hydrogels will replace, as well as the replacement's own mechanical response at frequencies of human phonation.

The need to measure mechanical responses at phonation frequencies arises because both the natural tissue and the candidate replacement materials are viscoelastic or frequency dependent. Rheometers commonly used for measuring the response of viscoelastic materials are limited to frequencies of approximately 10 to 15 Hz when applied to materials as soft as lamina propria.[5,6] Furthermore, this limitation is not overcome by redesigning a rheometer for higher frequency, because these materials are so soft and consequently have such low wave speeds that the state of stress and deformation is not uniform through the thickness of the sample at the frequencies of interest.[7]

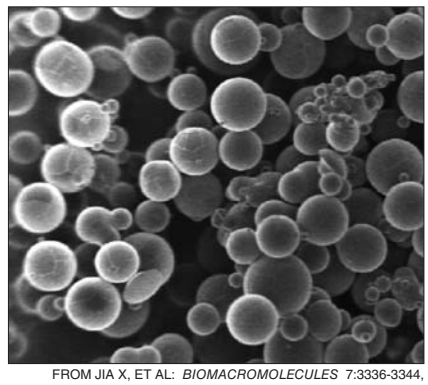

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Fig. 3. Scanning electron micrograph of HA-based microspheres.

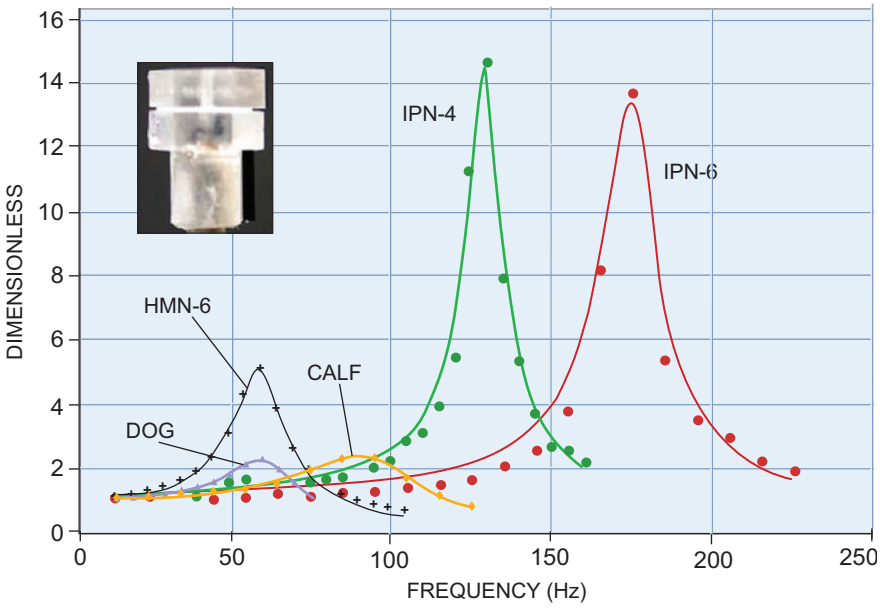

Fig. 4. Frequency dependence of amplification factors for three synthetic materials HMN-6, IPN-4, and IPN-6 and lamina propria samples from a dog larynx and calf larynx. Best-fit models are shown as solid curves, with experimental results shown as symbols. The sample dimensions (height h and radius a) and the viscoelastic moduli G_1 and δ that provide the best fit between model predictions and experimental results for the respective materials are:

HMN-6: h = 0.87 mm, a = 2.68 mm, G_1 = 244 Pa, δ = 0.389 radians

IPN-4: h = 0.997 mm, a = 2.54 mm, G_1 = 3230 Pa, δ = 0.132 radians

IPN-6: h = 0.969 mm, a = 2.51 mm, G_1 = 6066 Pa, δ = 0.14 radians

Dog LP: h = 0.134 mm, a = 2.182 mm, G_1 = 1027 Pa, δ = 0.92 radians

Calf LP: h = 0.19 mm, a = 2.07 mm, G_1 = 440 Pa, δ = 0.88 radians

Therefore, the mechanical response of lamina propria at phonation frequencies needs to be viewed as involving the propagation of stress waves through the sample.

A first stress-wave approach to determine the viscoelastic properties of candidate replacement materials involved the propagation of longitudinal waves along the axis of cylindrical rods of photocrosslinked hydrogels.[8] An acoustic shaker was used to subject the base of the rod to an oscillatory vertical motion. The motion of the free, top end of the rod was monitored using a laser-Doppler vibrometer.

From the measured amplification factor (the amplitude of the velocity at the top of the rod divided by the amplitude of the velocity at the shaker surface) and the phase shift between the top and bottom ends of the rod, a viscoelastic wave propagation solution was used to determine the frequency-dependent viscoelastic moduli for the hydrogel. While this method could extend the measurement of viscoelastic properties into the range of phonation frequencies, it could not be used for the lamina propria because the lamina propria is too thin to allow the preparation of slender cylindrical specimens.

We have recently overcome this difficulty by using torsional waves instead of longitudinal waves to evaluate thin cylindrical samples with thicknesses compatible with those of SLP.[9] A thin cylindrical sample of a soft material is placed between two hexagonal plates [Fig. 4, inset]. The bottom plate is rotated back and forth by means of a galvanometer at frequencies up to 2500 Hz.

To ensure that the shear strains in the sample are sufficiently small for linear viscoelasticity to be a satisfactory approximation, the imposed rotations are kept less than ±0.2°. The rotations of the top and bottom plates are monitored by an optical lever technique in which laser beams, reflected off aluminized faces of the hexagonal plates, are passed through a spherical lens, a cylindrical lens, and a butterfly-shaped mask before being captured by photodiode detectors. The spherical lens expands the beam and the cylindrical lens focuses the beam into a vertical sheet of light at the mask. Because of the mask's shape, the output of each photodiode is proportional to the angular rotation of the respective plate.

The rotation of the bottom plate is driven by a computer-controlled frequency generator that steps through a sequence of frequencies. At each frequency, the average amplitude of the rotation of each plate is obtained. The experimentally determined amplification factor is obtained as the ratio of the amplitude of the rotation of the top plate to that of the bottom plate. Calibration differences between the recorded outputs for the two plates are accounted for by adjusting the experimentally determined amplification factor to approach the required value of unity as the frequency goes to zero.

Amplification factors obtained for each frequency are compared with those predicted by a simulation of the experiment based on a torsional wave solution for a linear viscoelastic material.

The viscoelastic description of the material is expressed in terms of a storage modulus $Gf(?)$, that characterizes the elastic shearing stiffness of the material, and a loss angle $\delta(f)$, that characterizes the damping of the material in the sense that $\tan \delta(f)$ is proportional to the energy dissipated per cycle divided by the maximum energy stored during the cycle.

The output signal shows a peak in the amplification factor at a particular frequency. For each test, the frequency at which the peak occurs

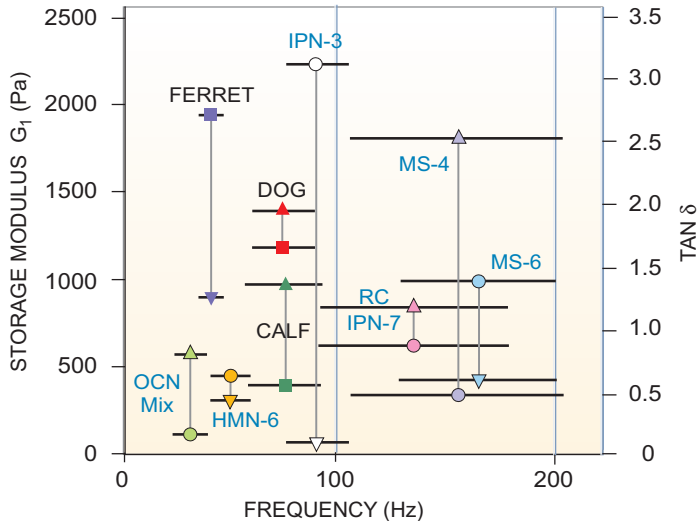

Fig. 5. Inferred storage moduli G_1 and the loss measure $tan\,\delta$ for the shearing response of lamina propria from several animals and candidate replacement materials. The width of the horizontal lines indicates the frequency range over which the best-fit model provides very good agreement with the measured amplification factors.

The storage moduli (*squares, circles*) and the $tan\,\delta$ values (*triangles*) for the replacement materials span the range covered by the lamina propria of natural tissue in the animals investigated.

determines a storage modulus G_1. The amplitude and breadth of the peak determine a loss angle δ.

From the results shown in Figure 4, it is evident that the linear viscoelastic wave analysis provides a remarkably good fit to the observed frequency dependence of the amplification factor over a range of frequencies near the peak. The height h and radius a of the sample (required for wave analysis) are obtained from digital images of the sample in place between the two hexagonal plates [Fig. 4, inset].

Inferred storage moduli G_1 and the loss measure $tan\,\delta$ were determined for lamina propria from several animals and for several candidate replacement materials, as shown in Figure 5.

The soft HA-based hydrogel microspheres, which can be synthesized with two levels of crosslinking to adjust independently the degradation rate and the viscoelastic properties, appear to have considerable potential for vocal fold generation. The wide ranges of storage moduli and $tan\,\delta$ values shown for these materials in Figure 5 are comparable to the ranges measured for lamina propria of the animals investigated.

Thus, it appears to be possible to develop synthetic materials that have the viscoelastic properties of natural lamina propria. However, matching the viscoelastic response of the natural lamina propria, while ensuring that the material biodegrades sufficiently slowly for clinical applications, remains a challenge. At present, the HA-based microsphere networks look *most* promising in providing both the low stiffness and the slow degradation that are required. As a future possibility, we are studying biomimetic polymers that can be used either as an injectable remedy for the treatment of vocal fold scarring or as scaffolds for vocal fold tissue engineering.

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