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Viscoelasticity of hyaluronic acid-gelatin hydrogels for vocal fold tissue engineering

Siavash Kazemirad, PhD, Hossein K. Heris, PhD*, and Luc Mongeau, PhD

Biomechanics Research Laboratory, Department of Mechanical Engineering, McGill University, 817 Sherbrooke Street West, Montreal, Quebec, Canada H3A 0C3

Abstract

Cross-linked injectable hyaluronic acid-gelatin hydrogels have remarkable viscoelastic and biological properties for vocal fold tissue engineering. Patient-specific tuning of the viscoelastic properties of this injectable biomaterial could improve tissue regeneration. The frequency-dependent viscoelasticity of cross-linked hyaluronic acid-gelatin hydrogels was measured as a function of the concentration of hyaluronic acid, gelatin, and cross-linker. Synthetic extracellular matrix hydrogels were fabricated using thiol-modified hyaluronic acid and gelatin, and cross-linked by Poly(ethylene glycol)diacrylate. A recently developed characterization method based on Rayleigh wave propagation was used to quantify the frequency-dependent viscoelastic properties of these hydrogels, including shear storage and loss moduli, over a broad frequency range; i.e., from 40 to 4000 Hz. The viscoelastic properties of the hydrogels increased with frequency. The storage and loss moduli values and the rate of increase with frequency varied with the concentrations of the constituents. The range of the viscoelastic properties of the hydrogels was within that of human vocal fold tissue obtained from *in vivo* and *ex vivo* measurements. Frequency-dependent parametric relations were obtained using a linear least-squares regression. The results are useful to better fine-tune the storage and loss moduli of hyaluronic acid-gelatin hydrogels by varying the concentrations of the constituents for use in patient-specific treatments.

Keywords

Viscoelastic properties; Hyaluronan hydrogels; Vocal folds; Injectable biomaterials; Wave propagation method

INTRODUCTION

Many vocal fold disorders significantly affect the viscoelasticity of vocal fold tissue, and consequently hamper the self-sustained oscillations of the folds essential to voice production. One common example is vocal fold scarring, resulting from voice abuse or surgery. Scarring increases the overall stiffness of the tissue, presumably because of the appearance of high intensity cross-linked collagen fibers. Scarred vocal fold lamina propria is commonly treated using injectable biomaterials, such as hydrogels and commercial phonosurgical biomaterials, for vocal fold augmentation and repair.^{1,2} The frequency-

*Corresponding author: hossein.khadiviheris@mail.mcgill.ca, Tel: (+1) 514 560-6250.

dependent viscoelastic properties of injectable biomaterials must be measured to ensure their compatibility with the vibrating tissue throughout the frequency range of vocalization. The vocal fold tissue is a continuous medium, which possesses in principle many modes of vibration at distinct natural frequencies. Data obtained from electroglottography (EGG) and laser Doppler velocimetry of the human vocal folds during phonation has shown that there is significant vibration energy at frequencies up to 3 kHz for a fundamental frequency of 110 Hz.³ One can conclude that in singing, where the fundamental frequency reaches up to a few kHz⁴, higher harmonics of the vocal fold tissue vibration with corresponding frequencies up to several kHz might be existent.

Hyaluronic acid (HA) is a glycosaminoglycan. It is one of the main components of the extracellular matrix (ECM) of the human vocal fold lamina propria.^{5,6} It has been postulated that HA helps to maintain the viscosity and stiffness of the vocal fold tissue within an optimal range that eases phonation and helps the control of the fundamental frequency.⁷ Shear thinning of HA, i.e. the decrease in viscosity as the frequency of oscillation is increased, is assumed to play a role in wide bandwidth of phonation, especially in singing.^{7,8} In addition to HA's viscoelastic properties, its shock absorption and space filling characteristics make it to be a good injectable biomaterial for vocal fold tissue augmentation and repair.⁸ Clinical studies of vocal fold treatment using HA based injectable biomaterials yielded significant improvements in glottal closure and voice quality, as well as less resorption in comparison with collagen-based injectable biomaterials.^{9,10} Measurements of the viscoelastic properties of HA hydrogels have been performed.^{11–13} For example, Chan and Titze¹¹ measured the viscoelastic shear properties of HA gels at different concentrations, with and without fibronectin, at frequencies up to 15 Hz, and compared the results with those of the human vocal fold mucosa.

Gelatin (Ge), obtained from partially hydrolyzed collagen, is composed of a heterogeneous mixture of proteins.¹⁴ It is an excellent substrate for cell attachment, proliferation, and differentiation. Gelatin and HA have been cross-linked to fabricate synthetic ECM hydrogels for tissue engineering, three-dimensional cell culture, and wound repair.^{15,16} Cross-linked HA-Ge hydrogels have been produced for vocal fold tissue repair and tested on rabbit vocal folds that underwent biopsy bilaterally.^{17,18} The viscoelastic properties of the tissue were measured at frequencies up to 10 Hz, three weeks after biopsy and injection. The viscoelastic properties of the injected HA-Ge hydrogels, however, were not measured. The presented results suggested that a gelatin concentration of 5% provided a better wound-healing environment for tissue regeneration, which improved the tissue viscoelastic properties. The shear modulus of a variety of cross-linked HA-Ge hydrogels with different concentrations of HA, Ge, and cross-linker were measured using a shear rheometer at 1 Hz.¹⁹ The frequency-dependency of the viscoelastic properties of the fabricated hydrogels was not investigated.

Characterization of the viscoelastic properties of vocal fold injectable biomaterials has mostly been performed using parallel-plate rheometry.^{2,11,20–23} Based on the literature, methods developed for the measurement of the viscoelastic properties of these biomaterials are limited to frequencies below 250 Hz, thus do not cover the entire phonation frequency range. An experimental method for the characterization of frequency-dependent viscoelastic

properties of soft biomaterials based on Rayleigh (surface) wave propagation has recently been developed.^{24,25} The complex shear and elastic moduli of gelatin and silicone rubber samples were measured at frequencies up to 4 kHz, and verified through comparisons with results obtained using other independent methods.

In the present study, a variety of cross-linked HA-Ge hydrogels with tuned mechanical and biological properties were fabricated for use as synthetic ECM hydrogels in vocal fold tissue engineering. The storage and loss moduli of the synthesized hydrogels were measured over a broad frequency range; i.e., from 40 to 4000 Hz, using the Rayleigh wave propagation method.^{24,25} The effects of HA, Ge, and cross-linker concentrations on the frequency-dependent viscoelastic properties of these hydrogels were investigated. The obtained viscoelastic properties of the fabricated hydrogels were compared with those of human vocal tissue obtained from *ex vivo* and *in vivo* measurements and were found to be in good agreement.^{26,27} The viscoelastic properties of human vocal fold tissue can significantly vary from one subject to another, implying that no single injectable biomaterial works for any patient.²⁷ An appropriate injectable hydrogel with viscoelastic properties matched to those of any specific patient can be selected based on the data obtained in the present study. Important additional biological factors, such as cell attachment and proliferation, can also be varied through adjustments of the Ge concentration, considering its effect on the viscoelastic properties of the designed hydrogel.

MATERIALS AND METHODS

Materials

Thiolated HA (CMHA-S or Glycosil, Mn ~ 200kDa, thiolatation: 40% of carboxyl groups), thiolated Ge (Gtn- DTPH or Gelin-S, Mn ~ 25kDa, thiolatation: 40% of carboxyl groups), and polyethylene glycol diacrylate (PEGDA, mw ~ 3400g/mol) were generously donated by Glycosan Biosystems, Inc. (Salt Lake City, UT).

Hydrogel fabrication

Hydrogels were cast in a variety of compositions where a mixture of thiol-modified HA, thiol-modified Ge, and PEGDA cross-linker was prepared with various concentrations of these constituents. A schematic of thiol-modified HA and Ge cross-linked by PEGDA and the cross-linked network of HA-Ge hydrogels is shown in Figure 1. Samples were cured at room temperature after casting. Although 20 minutes was sufficient to ensure cross-linking, a 12-hours period was considered before performing the experiments to ensure a fully cured hydrogel and consistent mechanical properties. One of the samples, however, was tested 3 and also 12 hours after fabrication to investigate the cure time effect on hydrogel viscoelastic properties. Samples were kept under water during curing time, after the 20 minutes needed for initial cross-linking and hydrogel formation, to avoid dehydration.

Viscoelastic Characterization method

A characterization method based on Rayleigh wave propagation was used for the quantification of the frequency-dependent viscoelastic properties of the fabricated hydrogels.²⁵ Planar harmonic Rayleigh waves were produced on the surface of a sample

composed of a substrate with known material properties coated with a thin layer of the hydrogel to be characterized. A schematic of the fabricated layered sample and the Rayleigh wave propagation concept is shown in Figure 2. The substrate was made of a silicone rubber material, Ecoflex 10 Platinum Cure Silicone Rubber (Smooth-On, Inc), with a component ratio 1:1:3 (i.e., part A: part B: silicone thinner), by weight. The viscoelastic properties of the substrate material were measured using a previously validated method.²⁴ A transfer function method was used to obtain the complex Rayleigh wavenumbers at each excitation frequency through the phase and amplitude of the propagating waves measured at different locations along the propagation direction. An inverse wave propagation problem was solved; yielding a complex nonlinear dispersion equation. The complex shear modulus, $\hat{G} = G' + iG''$, where G' and G'' are the shear storage and loss moduli of the hydrogel were calculated at frequencies up to 4 kHz through the numerical solution of the dispersion equation using the measured Rayleigh wave speeds (wavenumbers) propagating on the sample surface.

A few drops of water were added to the four corners of the hydrogel samples far from the measurement area to account for minor dehydration during experiments. The hydrogel samples were weighed after the experiments and a negligible variation was observed in the samples weights, and thus an insignificant dehydration was inferred. The experiments were repeated at least five times for each hydrogel sample. The mean value and standard deviation of the results were calculated. The standard deviation values of the measured storage and loss moduli were less than 10 percent of the mean values. Thus, only the mean values are presented in the following sections.

RESULTS

The silicone rubber substrate and hydrogel specimens' dimensions and properties are given in Tables 1. The storage and loss moduli of the substrate in the frequency range from 40 Hz to 4 kHz are shown in Figure 3. As seen in this figure, a very soft silicone rubber material was used as the substrate to ensure the Rayleigh wave propagation in the top layer. The amplitude of the Rayleigh wave was not sufficiently large when there was a significant difference between the stiffness of the substrate and that of the top layer.

The storage and loss moduli and the loss factor of Sample 2, measured three and twelve hours after fabrication, are shown in the frequency range from 40 Hz to 4 kHz in Figure 4. The results are compared with those obtained from shear rheometry in the frequency range from 1 to 12 Hz.¹ As seen in this figure, the values obtained from the wave propagation method at high frequencies are consistent with those obtained from the rheometry at low frequencies. The storage and loss moduli of the hydrogel increased with the frequency throughout the frequency range of interest. The rate of increase in the moduli, however, changed in different frequency ranges. The greatest rate of increase occurred in the frequency range from 40 to 200 Hz where the loss factor, which is defined as the ratio of the loss modulus to the storage modulus, reached a local maximum value. This is qualitatively consistent with the theoretical expectations presented in the literature for the frequency-dependence of polymeric biomaterials.^{24,28,29} The storage and loss moduli values obtained twelve hours after fabrication were greater than those obtained only three hours after fabrication. The trends in the measured values were smoother, suggesting that the molecular

chains of the hydrogel were more uniformly cross-linked and entangled over the measurement area after twelve hours.

Figure 5 shows the storage and loss moduli of three hydrogels with different cross-linker concentrations, namely Samples 1, 2 and 3. The viscoelastic properties of human vocal tissue obtained from *ex vivo* and *in vivo* measurements are also shown in the same figure.^{26,27} The viscoelastic properties of the designed hydrogels were in the same range of those of human vocal fold tissue. More specifically, the storage moduli of Samples 1 and 2 were in good agreement with those from *ex vivo* and *in vivo* measurements, respectively, and the loss modulus of Sample 3 was the most similar to that from *in vivo* and *ex vivo* measurements. The storage and loss moduli of the hydrogels increased approximately linearly with an increase in the cross-linker concentration, while the trends of increase in values obtained for each hydrogel with frequency remained the same. By doubling the cross-linker concentration, the storage modulus of Sample 2 increased by a factor in the range between 1.54 and 2.02 compared to that of Sample 1 at different frequencies, with a mean value of 1.89. This factor was in the range between 1.47 and 2.53 with a mean value of 2.06 for the storage modulus of Sample 3 compared to that of Sample 2. This trend is in very good agreement with data presented by Vanderhooft *et al.*¹⁹ The average of increase rates in the storage modulus values by doubling the cross-linker concentration was reported to be 2.03 at 1 Hz in that study (please see Table 1 of reference 20). The loss modulus also increased by average factors of 2.19 and 2.81 for Samples 2 and 3 compared to Samples 1 and 2, respectively.

The storage and loss moduli of three hydrogels with different Ge concentrations are shown in Figure 6. Samples 4 and 5 were fabricated by the substitution of 10 and 20 percent of the HA content of Sample 2 with Ge, respectively. As seen in Fig. 6, the moduli of the hydrogels decreased by increasing the Ge concentration. This trend is in good agreement with data presented by Vanderhooft *et al.*¹⁹ Regardless of the decreases in the moduli, the storage modulus remained in a range similar to those obtained for human vocal fold tissue. The increase in Ge concentration resulted in a slight increase in the frequency at which the slope of storage and loss moduli trends changes. The differences between the moduli of hydrogels with different Ge concentrations were consequently smaller at high frequencies (above 400 Hz). For example, the storage and loss moduli of Sample 2 were 1.74 and 1.55 times greater than those of Sample 5 at low frequencies (between 40 and 400 Hz), respectively. These values were 1.53 and 1.21 at high frequencies (between 500 and 4000 Hz).

The frequency-dependency of storage and loss moduli of the fabricated hydrogels were parameterized using linear least-squares regressions. A power law relation between the frequency and the moduli was assumed. The regression parameters were obtained for the expressions:

$$G' = a f^b \Rightarrow \log G' = \log a + b \log f, \quad (1)$$

$$G'' = a f^b \Rightarrow \log G'' = \log a + b \log f, \quad (2)$$

where f is the frequency, and a and b are the curve fitting coefficients. These coefficients were separately calculated for the storage and loss moduli of each tested hydrogel, and are shown in Table 2. The coefficient of determination, R^2 , was greater than 0.95 for all cases. This confirms that the assumed power law relation was an ideal model to parameterize the data presented in this study.

For arbitrary concentrations of HA, Ge, and cross-linker, a predictive second-order polynomial curve fitting procedure was introduced to obtain the curve fitting parameters for a variety of hydrogels for two following cases: 1) HA hydrogels with fixed 0.5% thiolmodified HA and a variable concentration of the cross-linker (PEGDA); 2) HA-Ge hydrogels with initial concentrations of 0.5% thiol-modified HA and 0.5% PEGDA, in which a variable concentration of HA is replaced with Ge. The curve fitting parameters were obtained from the following relations:

$$a=a_1+a_2x+a_3x^2, \quad (3)$$

$$b=b_1+b_2x+b_3x^2, \quad (4)$$

in which a_i and b_i ($i = 1,2,3$) were calculated separately for the two mentioned cases using the curve fitting coefficients obtained for the tested hydrogels (Table 2) and given in Table 3. The variable x is the cross-linker concentration for the first case, and the concentration of HA replaced with Ge for the second case. The storage and loss moduli of an assumed case with 0.475% thiol-modified HA, 0.025% thiol-modified Ge, and 0.5% PEGDA; i.e., the second case where 0.025% of HA concentration is replaced with Ge, was obtained from Eqs. (1) to (4) and shown in Figure 6. As seen in this figure, the viscoelastic properties were accurately predicted by these parametric equations at frequencies higher than 100 Hz.

DISCUSSION

Among different available hydrogels and commercial phonosurgical biomaterials, HA-based hydrogels have clinically been shown to yield significant improvements in the quality of voice.^{9,10} In this study, cross-linked HA-Ge hydrogels for vocal fold tissue treatment were fabricated and characterized. The synthesis and characterization of a variety of HA-Ge hydrogels with viscoelastic properties similar to those of human vocal fold tissue provides physicians with the opportunity to choose the best injectable hydrogel for any specific patient.

The Rayleigh wave propagation method used in this study for the characterization of the viscoelastic properties of the fabricated hydrogels was compared and evaluated with two different characterization methods, namely longitudinal wave propagation and AFM-based indentation methods,²⁴ as well a characterization method based on surface wave propagation presented in an independent study.³⁰ The trends and values of the results obtained in this study were also in good agreement with those presented in the literature at low frequencies.^{1,19}

The viscoelastic properties of hydrogels are affected by various parameters such as the concentration, entanglement, and molecular weight of the constituents.¹⁹ In this study, we varied the cross-linker density and relative concentration of HA and Ge. Cross-link density, which is determined by the cross-linker concentration, is the most common parameter to tune the viscoelastic properties (more specifically the storage modulus) of designed HA-based hydrogels.¹² The storage and loss moduli of HA-Ge hydrogels tested in this study were increased approximately by a factor of two by doubling the cross-linker concentration suggesting a linear correlation. In most tissue engineering applications, the viscoelastic properties of the injectable biomaterial is matched with those of the native tissue for better structural and functional integrity with the host tissue. In addition to changes in the viscoelastic properties, the increase in cross-linker density decreases porosity and diffusivity of the network and ultimately affects the cellular behaviour such as cell migration in tissue engineering. Furthermore, higher cross-linking increases the *in vivo* residence time of the injectable biomaterial, which also affects the outcome of tissue engineering. On the other hand, the concentration of HA and Ge mainly affects the ligand-integrin mediated cellular response. For instance, HA molecules can reduce the inflammatory response of the host tissue after injection of the biomaterial and Ge increases the rate of cell adhesion to the matrix.¹ The variation of the mechanical properties with respect to the Ge concentration was assessed to better tune the viscoelastic properties where the addition of Ge is required to improve the cell adhesion characteristics of the injectable biomaterial.

The storage and loss moduli of tested hydrogels at high frequencies (few kHz) were one order of magnitude (10 times) greater than those at low frequencies. This important result emphasizes the importance of the characterization of *frequency-dependent* viscoelastic properties of the synthesized HA-Ge hydrogels with application for vocal fold tissue treatment. A non-invasive method has recently been developed for the *in vivo* measurements of the shear storage modulus of human vocal fold tissue during phonation.²⁷ This method can clinically be used to assess the viscoelastic properties of any specific patients' vocal fold tissue. Subsequently, an appropriate injectable HA-Ge hydrogel, with known viscoelastic properties using the frequency-dependent parametric relations provided here, Eqs. (1) to (4), can be fabricated based on the requirements for the treatment of the specific patient's vocal folds. This integrated methodology to characterize the frequency-dependent properties of any specific human vocal fold tissue and fabricate the optimum injectable hydrogel for each patient can potentially have broad clinical applications, especially for singers as the fundamental frequency of their vocal fold oscillation reaches high frequencies in singing.

HA-Ge hydrogels are macroscopically isotropic and homogeneous biomaterials. The Rayleigh wave propagation method also assumes an isotropic and homogeneous propagation media. However, the vocal fold tissue is anisotropic and inhomogeneous. There is currently no technique to measure patient-specific anisotropic properties of vocal fold tissue *in vivo* during phonation. Therefore, a knowledge of isotropic viscoelastic properties of vocal fold tissue and injectable biomaterials was assumed to be sufficient to fine tune the biomechanical and biochemical properties of the HA-Ge injectable hydrogels for the treatment of each specific human subjects' vocal fold tissue. Application of the wave propagation method to anisotropic materials may be the object of future studies.

CONCLUSION

Different cross-linked HA-Ge hydrogels with tuned mechanical and biological properties were fabricated for use as synthetic ECM biomaterials in vocal fold tissue engineering. The viscoelastic properties of the synthesized hydrogels were measured over a broad frequency range using a Rayleigh wave propagation method, and compared with those of human vocal fold tissue obtained from *in vivo* and *ex vivo* measurements. The results revealed that HA-Ge hydrogels with appropriate concentrations of HA, Ge, and the cross-linker have viscoelastic properties similar to those of human vocal fold tissue. More specifically, the values obtained for the storage and loss moduli of the fabricated hydrogels were in the same range of those of human vocal fold tissue, and also their frequency-dependency, i.e., the increase of the moduli with frequency, approximately resembled that of the tissue. Furthermore, the Ge content of these hydrogels adjusts their biochemical properties. Therefore, HA-Ge hydrogels are strong potential candidates for use as injectable biomaterials in vocal fold tissue treatment. The physician can choose one of the synthesized hydrogels presented in this study or fabricate a specific HA-based biomaterial with desirable biomechanical and biological properties required for the treatment of any specific patient's vocal fold tissue.

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Figure 1.

(a) Schematic of thiol-modified HA and Ge cross-linked by PEGDA.¹⁵ (b) Cross-linked network of HA-Ge hydrogels.¹

Figure 2.

Schematic of the fabricated layered sample and Rayleigh wave propagation concept. A linear actuator was used to produce Rayleigh waves on the surface of the sample. The sample is fixed to the superior surface of an optical table to minimize extraneous disturbances. The input vibrations of the actuator and the motion of the sample surface were measured using an accelerometer and a laser Doppler vibrometer, respectively.

Figure 3.

Storage and loss moduli versus frequency for the silicone rubber substrate with component ratio of 1:1:3 using a longitudinal wave propagation method in the frequency range from 40 Hz to 4 kHz. $\square$: storage modulus; $\circ$: loss modulus. - - - : regression for storage modulus; : regression for loss modulus. (Regressions are to guide the eye)

Figure 4.

(a) Storage and loss moduli and (b) loss modulus versus frequency for Sample 2 from the Rayleigh wave propagation method in the frequency range from 40 Hz to 4 kHz and those from the rheometry in the frequency range from 1 to 12 Hz. $\blacksquare$: storage modulus from the Rayleigh wave method, 12 hours after curing; $\bullet$: loss modulus from the Rayleigh wave method, 12 hours after curing; $\square$: storage modulus from the Rayleigh wave method, 3 hours after curing; $\circ$: loss modulus from the Rayleigh wave method, 3 hours after curing; $\blacksquare$: storage modulus from the rheometry; $\circ$: loss modulus from the rheometry; $\blacktriangle$: loss factor from the Rayleigh wave method, 12 hours after curing; $\triangle$: loss factor from the rheometry; : regression for storage modulus from the Rayleigh wave method, 12 hours after curing; - - - : regression for loss modulus from the Rayleigh wave method, 12 hours after curing; — : regression for loss factor. (Regressions are to guide the eye)

Figure 5.

(a) Storage modulus and (b) loss modulus versus frequency for three different hydrogels with different cross-linker concentrations in the frequency range from 40 Hz to 4 kHz. The moduli are compared with those of human vocal tissue obtained from *ex vivo* and *in vivo* measurements. Δ : Sample 1; $\square$: Sample 2; $\circ$: Sample 3; $\bullet$: the mean value of three male subject's vocal fold tissue from *in vivo* measurements²⁷; $\blacksquare$: the mean value of seven human vocal fold specimens obtained from *ex vivo* measurements.²⁶ : regression for Sample 1; - - - - : regression for Sample 2; — : regression for Sample 3. (Regressions are to guide the eye)

Figure 6.

(a) Storage modulus and (b) loss modulus versus frequency for three different hydrogels with different Ge concentrations in the frequency range from 40 Hz to 4 kHz. The moduli are compared with those of human vocal tissue obtained from *ex vivo* and *in vivo* measurements. $\square$: Sample 2; $\circ$: Sample 4; $\triangle$: Sample 5; - - - : regression for Sample 2; — : regression for Sample 4; : regression for Sample 5; : regression for an assumed case with 0.475% thiol-modified HA, 0.025% thiol-modified Ge, and 0.5% PEGDA.

Dimensions and properties of the silicone rubber substrate and hydrogel specimens. The acronym XL denotes the cross-linker. All the concentrations are given in weight per unit volume (w/v).

Table 1

Material	Length [10 ⁻³ m]	Width [10 ⁻³ m]	Height [10 ⁻³ m]	Mass (m) [10 ⁻³ kg]	Density ρ [kg/m ³]
Substrate: silicone rubber 1:1:3	76.2	38.1	38.1	112.2	1014
Sample 1: 0.5% HA + 0.25% XL	76.2	38.1	1.38	4.028	1007
Sample 2: 0.5% HA + 0.5% XL	76.2	38.1	1.38	4.040	1010
Sample 3: 0.5% HA + 1% XL	76.2	38.1	1.38	4.060	1015
Sample 4: 0.45% HA + 0.05% Ge + 0.5% XL	76.2	38.1	1.72	5.050	1010
Sample 5: 0.4% HA + 0.1% Ge + 0.5% XL	76.2	38.1	1.72	5.050	1010

The curve fitting and determination coefficients obtained from least-squares regressions to parameterize the storage and loss moduli of the fabricated hydrogels. All the concentrations are given in weight per unit volume (w/v).

Table 2

Material	Storage modulus $G' = a\omega^b$			Loss modulus $G'' = a\omega^b$		
	a	b	R^2	a	b	R^2
Sample 1: 0.5% HA + 0.25% XL	13.61	0.7034	0.9959	7.157	0.4610	0.9861
Sample 2: 0.5% HA + 0.5% XL	23.26	0.7182	0.9980	13.54	0.4839	0.9928
Sample 3: 0.5% HA + 1% XL	61.64	0.6824	0.9961	67.22	0.3956	0.9596
Sample 4: 0.45% HA + 0.05% Ge + 0.5% XL	19.61	0.7136	0.9960	12.24	0.4827	0.9857
Sample 5: 0.4% HA + 0.1% Ge + 0.5% XL	11.23	0.7601	0.9983	8.496	0.5206	0.9824

Table 3

The coefficients obtained from a second order polynomial curve fitting procedure for a variety of hydrogels for two general cases.

Curve fitting case	$a = a_1 + a_2x + a_3x^2$			$b = b_1 + b_2x + b_3x^2$		
	a_1	a_2	a_3	b_1	b_2	b_3
Storage modulus $G' = a f^b$						
Case 1	10.32	0.440	50.88	0.667	0.190	-0.174
Case 2	23.26	-25.70	-946.0	0.7182	-0.603	10.22
Loss modulus $G'' = a f^b$						
Case 1	14.41	-56.30	109.1	0.393	0.360	-0.358
Case 2	13.54	-1.56	-488.8	0.484	-0.415	7.820