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Viscoelastic Evaluation of Different Hyaluronic Acid Based Fillers Using Vibrational Optical Coherence Tomography

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Abstract

Hylauronic acid (HA) is used as a viscoelastic in Ophthalmology during cataract surgery based on its high viscosity at rest, its ability to shear thin and dissipate energy during phacoemulsification. However, these properties of HA solutions would make them susceptible to migration when used as dermal filler materials. In this study, we apply a new technique termed vibrational optical coherence tomography (VOCT) to compare the physical properties of different HA solutions and fillers used in facial aesthetics. Results presented in this study suggest that HA solutions and HA dermal fillers have markedly different physical properties. HA solutions are highly viscoelastic with high % viscous losses while fillers tend to have lower viscous energy dissipation properties. Clinical observations suggest that the high loss fillers are injected more superficially in the face where tension and internal and external forces are more likely minimized giving tissue of the hands and lips more volume and allowing more natural movement. In contrast, the lower loss gels that are used to lift tissue, generally have a higher G', and are injected deeper into the face where injection and internal forces are likely to be higher. It is concluded that HA filler gel design can be optimized by use of VOCT to evaluate the % viscous energy loss both *in vitro* and *in vivo.*

Keywords

Hyaluronic Acid, Injectable Filler, Dermal Filler, Plastic Surgery, Cosmetic

Surgery, Viscoelastic, Mechanical Properties, Facial Surgery, Optical Coherence Tomography, Vibrational Optical Coherence Tomography

1. Introduction

Hyaluronic acid (HA) also known as hyaluronan, is polysaccharide composed of repeating β-1-4-linked D-glucuronic acid and β-1-3-linked N-acetyl-D-glucosamine disaccharide units [1]. The various names of HA reflect the properties of the molecule under various conditions. At neutral pH, HA exists as a polyelectrolyte with associated cations, frequently as a sodium salt; therefore, the name sodium hyaluronate. The name was later amended to "hyaluronate" in reference to its salt form or "hyaluronan," a term used to encompass all forms of the molecule [1].

HA is found ubiquitously in the ECM of all vertebrate tissues including blood, synovial fluid, vitreous body, pericellular matrix, cytoplasm, and nucleus. Its use as a viscoelastic in Ophthalmology during cataract surgery is based on the molecule's ability to shear thin and thereby absorb energy during phacoemulsification [2]. This property is a result of the reversible hydrogen bonding that occurs between side chains of the molecule [2]. However, to limit its ability to shear thin and flow under applied stress requires the formation of covalent crosslinks between HA chains.

Hyaluronic acid (HA) injectable dermal fillers (DFs) have become the most popular agents for soft tissue contouring and volumizing. HA fillers are reported to be characterized by ideal properties such as biocompatibility, biodegradability, and versatility. These filler properties enable HA DFs to dominate and revolutionize the filler market with numerous products differing in HA sourcing, degree of crosslinking, concentration, hardness, cohesiveness, and consistency. The inclusion or lack of inclusion of an anesthetic, indications for use, and longevity of correction are other variables examined in designing DFs [3] [4]. However, there are potential complications that can arise from use of these materials [5] [6].

The rheological and mechanical properties of HA fillers have been reviewed recently [4]. HA fillers have been developed that are both viscous solutions and solution-gel mixtures containing uncrosslinked and crosslinked macromolecules. HA fillers are crosslinked using a number of different crosslinking agents. The HA concentration of fillers ranges from 5.5 to 28 mg/ml, % crosslinking from 0 to 20%, shear elastic modulus from 100 to 1800 Pa, and particle size from 0 to over 1050 micrometers [4]. Observations of the physical properties of HA solutions and gels are important parameters that control the behavior of fillers in clinical use. However, currently, there are no tests available to study the physical properties of fillers both before and after implantation. We have developed a new technique termed vibrational optical coherence tomography (VOCT) that

can be used to evaluate HA filler properties both *in vitro* and *in vivo*.

The purpose of this paper is to introduce the use of VOCT to evaluate the physical properties of HA fillers before clinical use. VOCT has been used to evaluate the properties of skin and scar tissue *in vivo* [7]-[14], it can also be used to evaluate the mechanical behavior of skin after filler injections. Characterization of filler viscoelastic properties is needed to determine the desired clinical outcome; how the starting HA filler properties influence the quality of the outcome is a question that needs to be answered.

2. Materials and Methods

VOCT is new technique that measures the resonant frequency and viscous losses of solutions of macromolecules and gels. Unlike measurement of solution viscosity or viscoelasticity using a viscometer, this technique provides measures of both cohesive as well as viscous energy dissipation of materials when a mechanical vibration is applied. This method can be used both *in vitro* and *in vivo* unlike a viscometer which can only be used *in vitro* [7]-[14].

Commercial samples of pure HA with molecular weights of 5 k and 1.8 M were obtained from Lifecore Biomedical, LLC. (Chaska, Mn). Samples of dermal fillers listed in **Table 1** were obtained from Allergan Inc. (Dublin, Ire). A drop containing between 0.1 and 0.2 ml of each sample was placed on a glass slide for examination. The slide was the placed on a rigid frame that had a hole cut out so that the sound was applied to the slide from below. The sample volume did not influence the measurement of resonant frequency. Samples were tested at 22˚C by applying a sinusoidal sound wave from a speaker placed beneath the sample as discussed previously [7]-[14]. The frame, speaker, and glass slide resonant frequencies were measured in the absence of the samples. Sample weighted displacements were corrected for any resonant frequencies of the speaker and support

Table 1. HA formulations studied using vibrational optical coherence tomography. The % viscous loss is reported at 30 Hz for the HAs studied.

Sample	Conc (mg/ml)	Resonant Freq (Hz)	Viscous Loss (%)	Clinical Use
Restylane D	20	150	27	md/deep
Restylane Lft	20	140	27	md/deep
Voluma XC	20	150	25	md/deep
Vollure XC	17.5	150	35	sup/md/deep
Restylane L	20	150	35	sup/md/deep
Juvederm U	24	140 - 180	40	md/sup
Restylane S	20	180	40	sup
HA (5 k)	20	140	60	N/A
HA (1.8 M)	20	220	60	N/A

Note: the standard deviation of resonant frequency measurements is about 6 Hz while that for % viscosity measurements is about 10% of the % reported. Abbreviations: sp = superficial, md = mid, N/A = not applicable, Freq = resonant frequency, Conc = concentration.

materials, or from line voltage variations.

Transverse forces were applied to the sample by positioning an acoustic loudspeaker (Intervox S225RA-40) beneath the sample. A function generator (Agilent) was used to drive the speaker with sinusoidal waveforms at varying amplitudes and frequencies. The resonant frequency was determined as the frequency at which the maximum displacement was observed [7]-[14].

Transverse sample displacement was measured by spectral-domain optical coherence tomography (SD-OCT), a non-contact, interferometric technique [7]-[14]. The resonant frequency of each sample was initially estimated at a single point by measuring the transverse displacement resulting from sinusoidal driving frequencies ranging from 20 Hz to 500 Hz, in steps of 50 Hz. Once the region where the maximum displacement was identified, smaller steps of 10 Hz were used to more accurately identify the peak frequency and the actual resonant frequency, f_n.

Measurement of Elastic and Viscous Behaviors

The elastic and viscous components of the viscoelastic behavior were obtained from measurements made from the driving frequency peak as described previously [7]-[14]. The elastic component was obtained from the peak height while the viscous component was obtained by dividing the change in frequency at the half height of the peak (*i.e.* 3 db down from maximum peak in power spectrum) by the driving frequency. This method is known as the half-height bandwidth method discussed by Paul Macioce (http://www.roush.com/wp-content/uploads/2015/09/Insight.pdf). The viscous loss in percent for each sample was tabulated as a function of the applied sound frequency.

3. Results

Weighted displacement versus frequency plots for droplets of pure HA with molecular weights 5 k and 1.8 M are shown in **Figure 1**. Major peaks are shown at frequencies of 140 Hz (Mw = 5 k) and 220 Hz (Mw = 1.8 M). The % viscous contribution to the viscoelastic behavior is shown in **Figure 2**. Both pure HA samples, independent of molecular weight, have viscous losses that are about 60% at 30 Hz suggesting that these materials are more viscous than elastic at low frequencies. At frequencies above 200 Hz, these HAs have % viscous losses that approach 10% and behave almost purely elastically. In the absence of crosslinks, HA molecules are highly mobile and do not remain in place when an applied force is exerted on their surfaces.

Weighted displacement measurements versus frequency for representative dermal fillers listed in **Table 1** are shown in **Figure 3** and the % loss behavior for typical dermal fillers examined in this study are shown in **Figure 4**. The weighted displacement versus resonant frequency curves for all the filler materials exhibit resonant frequencies that center around 150 Hz. The percent loss behaviors of the fillers fall into the two groups the group with losses from about 35% to

40% at 20 Hz (group A) and those with losses of about 25% (group B) at 20 Hz. At high frequencies the % loss decreases to about 5% for all the dermal filler samples studied. At high frequencies all fillers behave almost purely elastic. Note the gel dermal fillers that are used in cosmetic surgery are more elastic (less

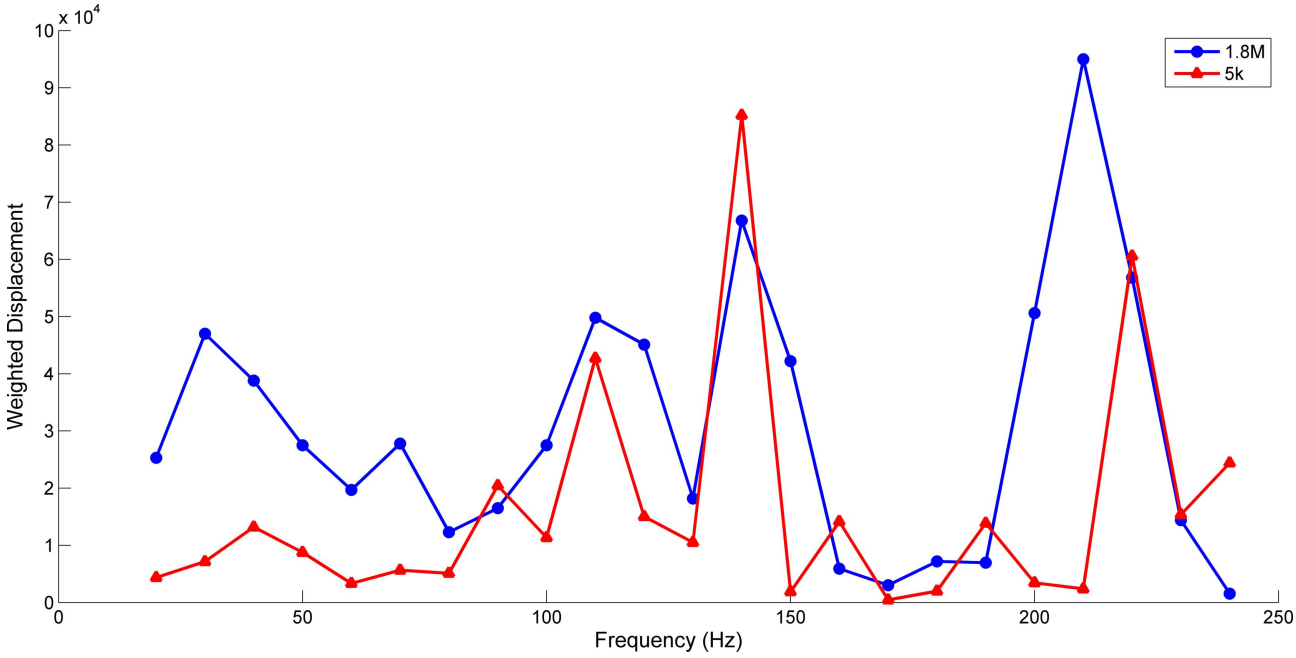

Figure 1. Plot of weighted displacement versus frequency for a drop of purified HA solution with molecular weights of 5 K and 1.8 M. The major peaks are seen at 140 Hz (Mw = 5 k) and 220 Hz (Mw = 1.5 M) for a drop of solution on a glass slide. Note the non-major peaks arise from multiple vibrations associated with within the droplet that occur during energy dissipation.

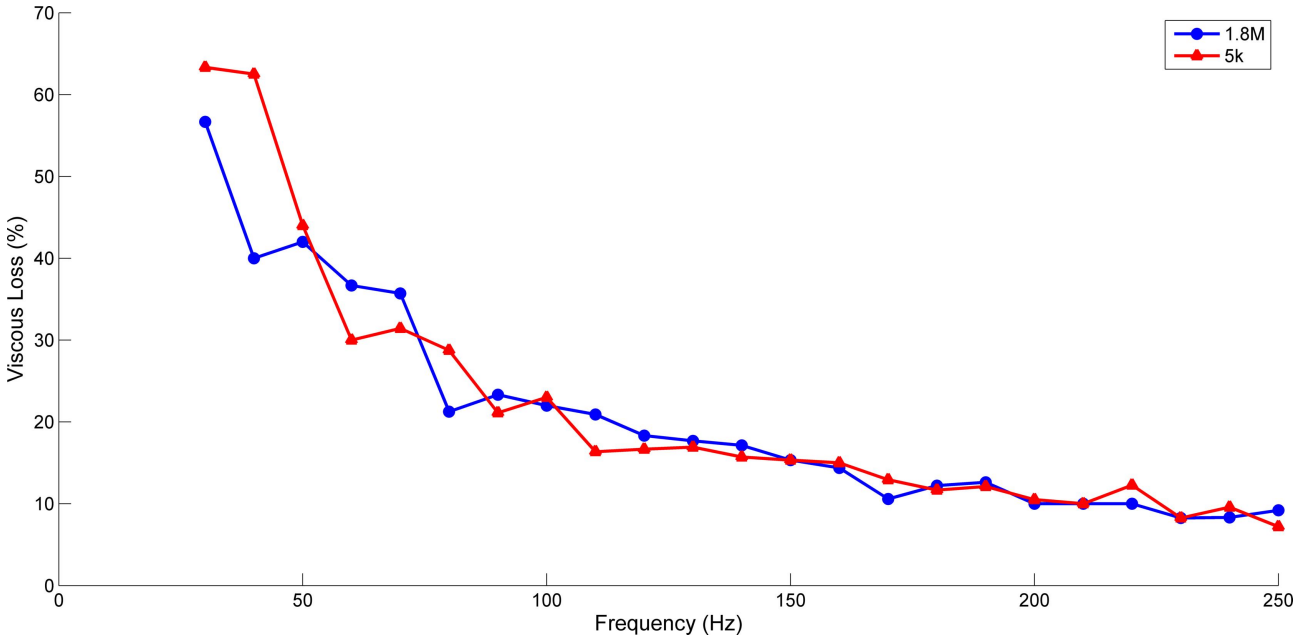

Figure 2. % Viscous loss as a function of frequency for a drop of purified HA solutions. Note the % viscous loss of low (5 k) and high (1.8 M) molecular weight HA fractions are very high at low frequency (60%) and approach a value of 10% at high frequencies. At low frequencies HA solutions are viscous liquids that dissipate energy by shear thinning.

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