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RHEOLOGICAL STUDY OF A NOVEL VISCOSUPPLEMENT

Michael Chernos, Dana Grecov, Tassos Anastassiades, Ezra Kwok
University of British Columbia, Queen's University

INTRODUCTION

Synovial fluid is a naturally produced joint lubricant in the human body consisting of proteins, lipids and hyaluronic acid [1]. Healthy synovial fluid protects human joints by lubricating the articulating surfaces and absorbing shock within a joint. While lesser components in synovial fluid such as lubricin have been demonstrated to affect joint lubrication, hyaluronic acid is primarily responsible for the bulk rheological properties that make it an effective lubricant [2][3][4][5].

With the onset of osteoarthritis, a common degenerative joint disease, the molecular weight and concentration of hyaluronic acid are found to decrease [6]. With the progression of osteoarthritis, lubricating and shock absorption properties of synovial fluid degrade causing cartilage wear and in severe cases, bone on bone contact [7]. Additionally, as the molecular weight of hyaluronic acid decreases, an increase in inflammatory response is also observed [8].

Viscosupplementation has been developed as a treatment for osteoarthritis in weight bearing joints that involves replacing or supplementing diseased synovial fluid with a bio-compatible fluid that is shear thinning and viscoelastic. The supplement is injected directly into the intra-articular joint cavity. Viscosupplements typically consist of hyaluronic acid that has been formulated to minimize friction and improve shock absorption within the joint [9][4].

The hyaluronic acid derivatives studied were developed by Dr. Tassos Anastassiades while studying the inflammatory response to low molecular weight hyaluronic acid and N-acetyl moieties. Of the five compounds studied, the butyrylated derivative was found to exhibit anti-inflammatory properties [8]. Therefore there exists the potential to improve current viscosupplementation therapy by enhancing osteoarthritic synovial fluid viscoelastic properties while simultaneously reducing joint inflammation.

METHODS AND MATERIALS

Sample preparation

A parent hyaluronic acid derived from *streptococcus equi* along with four, low molecular weight derivatives were studied and compared to existing viscosupplements as a means for assessing validity as a treatment for osteoarthritis. The hyaluronic acid derivatives with the sample number used to refer to each compound and the preparation method are presented in Table 1. It should be noted the deacetylation process causes bond cleavage, resulting in the decrease in molecular weight from parent to derivative compounds. The average molecular weight of the parent compound is approximately 1500-1800 kDa, while the derivative molecular weights fall within the range of 30-214 kDa [8].

Table 1: Hyaluronic acid derivatives, preparation method and corresponding sample numbers.

	Compound	Preparation
Sample 1	Hyaluronic Acid	-
Sample 2	DHA	Deacetylated hyaluronic acid*
Sample 3	AHA-1	Reacetylated DHA*
Sample 4	AHA-2	Reacetylated DHA**
Sample 5	BHA	Butyrylated DHA

* deacetylated via hydrazinolysis

** deacetylated with NaOH

Each of the above compounds was diluted to a concentration of 5 mg/mL in phosphate buffer solution (pH 7.2) and mixed at room temperature for 3 hours. Rheological tests were conducted immediately following the mixing procedure.

Experimental Methods

A Malvern Kinexus Ultra rheometer with a 50 mm, 1 degree cone and plate geometry was used for all rheological measurements. A sample volume of 0.58 mL was required to load the rheometer. All tests were conducted at 37 ^{o}C to replicate physiological temperature.

Three replicate runs were conducted for each test. The final results presented are the average of all three runs completed.

Shear rheology tests

All samples were pre-sheared at 0.1 Pa for 1 minute followed by a zero shear rest period of 2 minutes to remove any loading effects and shear history. Shear viscosity was then measured on the shear rate range of 0.01 to 1000 s^{-1}. Measurements had best repeatability on the range of 0.1 to 1000 s^{-1}.

Shear viscosity results were fitted with the Carreau-Yassuda model, which has previously been used to model shear rheology of synovial fluid and hyaluronic acid [8]. The Carreau-Yassuda model is given by Equation 1.

$$\frac{\eta - \eta_\infty}{\eta_0 - \eta_\infty} = \left[1 + (\lambda\dot{\gamma})^a\right]^{\frac{1-n}{a}} \qquad (1)$$

Where η is the sample viscosity, η_0 is the zero shear viscosity, η_∞ is the infinite viscosity, λ is the sample critical point at which shear thinning begins, $\dot{\gamma}$ is shear rate, a is a constant relating to the width of the shear thinning region, and n is a constant describing the degree of shear thinning.

Viscoelastic Tests

Prior to oscillatory measurements, the same 1 minute pre-shear and rest period were applied to the sample as before the shear measurements.

Strain amplitude sweep measurements were conducted at 1 Hz to identify the linear viscoelastic region for each sample. 5 % strain was found to fall within the linear viscoelastic region for all samples.

A final 1 minute pre-shear and 2 minute rest period was applied before conducting frequency amplitude sweep measurements at 5% strain over the frequency range of 0.05 to 30 Hz. For lower viscosity samples, accurate viscoelastic measurements could only be obtained on the frequency range of 1 to 10 Hz.

Time Dependence

Time dependent behavior was investigated by measuring sample viscosity as a function of time at a constant shear rate of 0.05 s^{-1} for 30 min.

RESULTS AND DISCUSSION

Shear Rheology

As expected, all samples displayed shear-thinning behaviour. Only Sample 1 displayed a zero shear plateau over the investigated range of shear rates, while all samples tended to converge on similar infinite shear viscosities of approximately 0.002-0.003 Pa s. The degree of shear thinning was similar for all compounds. Table 2 summarizes the Carreau-Yassuda model fit parameters for all samples.

Table 2: Carreau-Yassuda model fit parameters for all samples investigated.

	a	***n***	***λ***	***η₀***	***η∞***
Sample 1	1.02	0.530	0.03	0.08	0.003
Sample 2	55.00	-0.101	99.70	32.00	0.002
Sample 3	20.00	-0.044	8.36	0.70	0.002
Sample 4	10.62	0.026	110.60	0.50	0.003
Sample 5	4.68	-0.030	122.20	1.20	0.002

From Table 2 it can be observed that Sample 2 has the greatest initial viscosity and exhibits shear thinning behavior over a larger range of shear rates than the other samples based on the large *a* value. Zero shear values presented in Table 2 tend to be lower than values reported for viscosupplements elsewhere, however the compounds in this study were investigated at lower concentrations of hyaluronic acid [11][6].

Overall, the shear viscosity properties of the investigated samples reflect behaviour observed in common viscosupplements such as Synvisc, Monovisc, Orthovisc and Suplasyn, suggesting the samples under investigation may have suitable shear rheology for use as a viscosupplement. Although the viscosity tends to be much lower in the investigated samples, this could be modified by increasing the concentration of hyaluronic acid or the degree of crosslinking [1][6][11].

Viscoelastic Properties

Due to the low viscosity of the samples investigated, the oscillatory behaviour of the samples could only be studied over the frequency range of 1 to 10 Hz. Throughout this range, samples 2 and 3 display purely gel behaviour while samples 1,4, and 5 display a crossover from liquid to gel behaviour. These results, along with the magnitude of the storage and loss moduli at walking frequency are summarized in Table 3.

Table 3: Viscoelastic properties of investigated samples at crossover and 2.5 Hz.

	f_c (Hz)	G_c (Pa)	$G'_{2.5}$ (Pa)	$G''_{2.5}$ (Pa)	Behaviour
Sample 1	10.0	2.20	0.19	1.04	Liquid & Gel
Sample 2	-	-	0.58	0.17	Gel
Sample 3	-	-	1.18	0.24	Gel
Sample 4	4.2	0.20	0.052	0.15	Liquid & Gel
Sample 5	2.6	0.080	0.013	0.061	Liquid & Gel

Sample 1 is observed to have similar viscoelastic properties to the low molecular weight derivatives.

For comparison, the dynamic moduli of three commercial viscosupplements are presented in Table 4. VS 1 is a non-cross linked high molecular weight, VS 2 is a non-cross-linked low molecular weight and VS 3 is cross-linked [8].

Table 4: Viscosupplement dynamic moduli at 37 ^{o}C [8].

	G_c (Pa)	$G'_{2.5}$ (Pa)	$G''_{2.5}$ (Pa)	Behaviour
VS 1	0.398	111.2	61.48	Liquid & Gel
VS 2	-	3.36	10.78	Gel
VS 3	-	118.1	22.46	Gel

As observed in Table 4, the dynamic moduli of commercial viscosupplements tend to be significantly higher than for the samples investigated in this study. This is in large part due to the difference in hyaluronic acid concentration and molecular weight [1][11]. VS 1, for example, has a molecular weight on the order of 6000-7000 kDa and an hyaluronic acid concentration of 8 mg/mL [6]. Other viscosupplements such as Monovisc, contain hyaluronic acid at concentrations as high as 20 mg/mL [6].

It would be feasible to adjust the viscoelastic properties to more closely resemble existing viscosupplements by scaling up the concentration of hyaluronic acid or by adjusting the degree of cross-linking [12]. It is therefore believed that Sample 5 could be used as a viscosupplement in the treatment of mild to moderate osteoarthritis and while providing adequate viscoelastic properties, offer the advantage of anti-inflammatory properties to reduce joint swelling.

Time dependent behavior

Sample 2 was found to have time dependent behavior at low shear rates. Simple shear viscosity is plotted as a function of time in Figure 1.

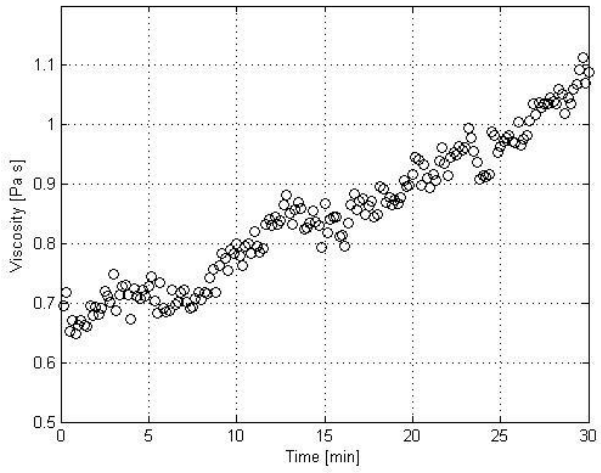

Figure 1: Viscosity of sample 5 is plotted as a function of time at a constant shear rate of 0.05 s^{-1}.

Time dependent behavior for sample 2 is observed at a shear rate of 0.05 s^{-1}. The moderate rheopexy observed at low shear rates

for sample 2 is also observed in synovial fluid [10]. This property is useful in situations where an individual may be standing for long periods of time, allowing the viscosupplement to increase in viscosity to help prevent contact at the articular surfaces in a knee joint.

Suitability as a Viscosupplement

Before use as a viscosupplement, it would be necessary to increase the viscosity and dynamic moduli. It is believed that this would be feasible to achieve simply by increasing the concentration of hyaluronic acid or by increasing the degree of crosslinking.

With improved rheological properties, it is believed that in conjunction with anti-inflammatory properties the Sample 5 would make an effective viscosupplement.

CONCLUSIONS

The hyaluronic acid derivatives investigated in this study exhibited typical viscosupplement behaviour, with clear shear thinning and viscoelastic properties. Of the four derivatives, sample 2 exhibited the highest viscosity and dynamic moduli. Sample 5 displayed lower viscosity and viscoelasticity, however it would be of particular interest to investigate improving these parameters as it has been demonstrated to possess strong anti-inflammatory activity making it perhaps the most suitable candidate for viscosupplementation [8].

Commercial viscosupplements had significantly greater viscosities and dynamic moduli. This difference is attributed to a difference in hyaluronic acid molecular weight and concentration. It is believed that the viscoelastic properties of the samples studied could be increased by increasing the hyaluronic acid concentration or by increasing the degree of crosslinking.

Based on the observed rheological behaviour and potential to enhance viscoelastic properties by increasing hyaluronic acid concentration, it is believed that the novel, anti-inflammatory compounds investigated would make an effective viscosupplement in treating mild to moderate osteoarthritis in weight bearing joints.

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