Effect of Hyaluronic Acid Molecular Weight on the Rheology of Synovial Fluid Analogues

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Introduction: Lubricant composition and in particular the presence of proteins [1] and hyaluronic acid [2], plays an important role in the tribology of joint replacements. Hyaluronic acid [HA] is the glycosaminoglycan responsible for the unique rheological characteristics of both normal and periprosthetic fluid [3, 4, 5]. Bovine calf serum (BCS), which exhibits Newtonian behaviour [5, 6] is the lubricant currently used in wear testing of orthopaedic implants. Recent studies have investigated the addition of HA to BCS [6, 7]. The concentration of HA is typically 0.9 mg/mL at revision total knee arthroplasty (TKA) and 1.3 mg/mL at primary TKA [3]. However, molecular weight is a variable. Molecular weight is known to change the rheological behaviour of other polymer systems [8]. The objective of the present study is to examine the effect of hyaluronic acid molecular weight on the rheology of HA-BCS solutions in both oscillatory and steady shear modes of deformation.

Methods: BCS was prepared by the following additions to the 500 mL stock BCS (HyClone™ Laboratories, Logan, UT): 20 mL of 0.2 micron filtered 15.4% EDTA solution (VWR Canlab™, Mississauga, ON) as a chelating agent to calcium, 5 mL of hydrated Fungizone and 3 mL of Penicillin Streptomycin (Invitrogen™ Canada Inc., Burlington, ON) as antimicrobial agents. BCS with protein concentration of 34 mg/mL (which is relevant to concentrations found in fluid at revision arthroplasty [3]) was obtained by dilution with 0.2 micron filtered deionized and distilled water. HA of two molecular weights was obtained. HA1 (M_w= 1.0 X 10^6), (Fisher Scientific™, Whitby, ON) and HA2 (M_w= 2.48 X 10^6), (Genzyme™, Cambridge, MA), were used to make 4 HA-BCS solutions: 2 (1 mg/mL HA) and 2 (4 mg/mL HA). Rheological measurements were conducted with a TA Instruments™ AR 2000™ Rheometer. Steady shear measurements were conducted using two fixtures: 2° 60 mm stainless steel cone and plate and a 0.5° 60 mm acrylic cone and plate. Oscillatory measurements were conducted using a 0.5° 60 mm acrylic cone and plate fixture. Three trials were obtained for each solution for each measurement at 37 °C and the average value of viscosity and dynamic moduli was calculated for each shear rate/frequency. Repeatable data were obtained with a maximum deviation of 5% in viscosity and 15% in dynamic moduli which is within rheometer accuracy.

Results and Discussion: The steady shear viscosity of the HA-BCS solutions is plotted in Figure 1. Data for the 1mg/mL HA2 solution was obtained previously [7]. The viscosity increases with concentration, especially at low shear rates. For HA of the same concentration, the viscosity of solutions with HA2 is higher than that of solutions with HA1. The viscosity at low shear rates and extent of shear thinning is also more pronounced in solutions with HA2. Higher molecular weight HA in solution allows for more molecular entanglements that lead to an increase in resistance to flow, and hence an increase in viscosity. The behaviour of HA in the BCS medium is typical of polymer solutions.

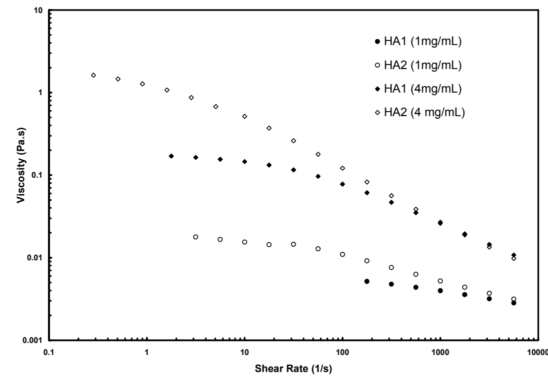

Figure 1. Steady shear viscosity of HA-BCS solutions.

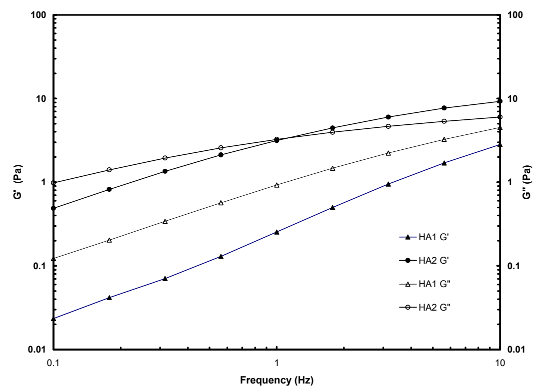

Figure 2. Dynamic moduli of HA-BCS solutions with 4mg/mL HA concentration

In Figure 2, cross-over between the dynamic moduli does not occur for the HA1 solution within the examined frequency range. With the higher molecular weight HA, molecular entanglements allow for the formation of a network structure; viscous behaviour is observed at low frequencies (G">G') and elastic behaviour is observed at high frequencies (G'>G"). The frequency at which this transition occurs is 1.14 Hz.

Conclusions: At the same concentration in BCS, HA2 (M_w= 2.48 X 10^6) solutions have higher viscosity than those of HA1 (M_w= 1.0 X 10^6). Furthermore, cross-over of the dynamic moduli is not exhibited in the HA1 solution compared to the HA2 solution at the same concentration. This suggests that it is insufficient to design synovial fluid analogues without also considering the molecular weight of HA in solution.

References: [1] Liao Y et al. J Biomed Mater Res. 1999; 48: 465-473. [2] Aurora A et al. Trans ORS 51. 2005; 0167. [3] Mazzucco D et al. Biomaterials. 2004; 25: 4433-4445. [4] Krause WE et al. Biomacromolecules. 2001; 2: 65-69. [5] Mazzucco D et al. J Orthop Res. 2002; 20: 1157-1163. [6] Tanner SL et al. Trans Soc Biomat 29. 2003; 108. [7] Fam H et al. Trans Can Soc Biomat 24. 2005; 23-24. [8] Ambrosio L et al. Pure Appl. Chem. 1999; A36(7&8): 991-1000.