Rheological Study of Healthy and Pathological Synovial Fluid after Surgery for Repair of Rupture of the Cranial Cruciate Ligament of Dogs

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ABSTRACT

The results of the present research showed that the healthy synovial fluid (SF) exhibits small viscosity changes as a function of the temperature and satisfactory viscoelastic characteristics. On the contrary, in pathological conditions (e.g. cases of rupture of the cranial cruciate ligament), measured viscosity for the same shear rate region was found drastically decreased, i.e. between 0.1 Pas and 0.03 Pas.

INTRODUCTION

The synovial fluid rheology can influence the tribology of the joints either of healthy individuals, or of individuals with pathological problems. For instance, there is enough evidence that the rheological behaviour of synovial fluid changes at the appearance of arthritis or osteoarthritis, exhibiting decreased viscoelasticity[1,2]. The function of the joints is strongly related to the condition of the SF, composed of proteins and Hyaluronic Acid (HA). The SF is a derivative of blood serum via a natural process of osmosis in joint cavity. The normal SF is substantially transparent and lightly yellow-coloured. It has the 1/3 of the proteins concentration of blood and contains only the proteins of small molecular weight, such as albumin. A crucial parameter of effective operation of healthy synovial fluid is considered to be the hyaluronic acid, which is a polysaccharide[3]. In the joint, HA serves various important functions, such as viscoelasticity to the SF and boundary lubrication of the intra-articular soft tissues. The molecular weight of HA changes with the condition of the joint, i.e. healthy or pathological. The mean molecular weight ($\overline{Mw}$) of the HA in the SF ranges from 6.3×10^6 up to 7.6×10^6 Da, while the concentration of HA, in mg per ml of SF, ranges from 1.5-2 mg/ml SF for the lower limit, up to 3.5-4 mg/ml SF for the highest measured concentrations[4].

In the following Fig. 1 the chemical structure of the basic repeated unit of HA is given.

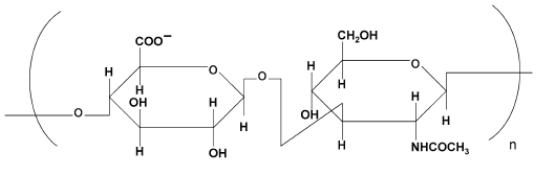

Figure 1. The chemical structure of HA[5].

Theories propose lubrication action of SF via a thin film mechanism between the cartilages in joint (ranging from 10 to 50

μm). When the surfaces of a joint are in contact (1 nm to 100 nm), a mechanism of HA adsorption on a monolayer level, that functions as absorber to the movements and pressure, is adopted[6,7]. However, the correlation of SF rheology to contained substances, such as the protein albumin, it has been recently attempted[5]. Oates et al. (2006)[5] pointed out the important role that this particular protein plays, by a spontaneous aggregation at a state of rest of the joint and a consequent structure created with the HA of SF.

In addition, a method of injecting solutions of HA of various $\overline{Mw}$ in pathological joints it has been adopted as a supplementary treatment method, the so-called *viscosupplementation*[1,2]. To this respect, it has been reported that the extent of the viscoelastic behaviour of the SF after the application of the injection, is directly related to the extent of the branched polymerization of the initial HA[8,9].

Rupture of the cranial cruciate ligament is a common orthopaedic injury encountered in dogs; therefore, synovial fluid samples obtained from injured joints, as well as normal synovial fluid of adult dogs, were examined with respect to their rheological properties.

The aim of the present research was to evaluate qualitatively and quantitatively where it was possible, the rheological properties of synovial fluid from adult dogs, of different age and pathological situations. The research included rheological characterization of solutions of hyaluronic acid as well as complete rheological characterization of synovial fluid from dogs, i.e. viscosity measurements at various shear rates, measurements of the G′, G′′, and η'.

EXPERIMENTAL–MATERIALS

The measurements of the present work were carried out with the rheometers AR-G2 and CarriMed CSL100 by TA Inst. Acrylic cone and metallic parallel disks geometries were used, having diameters of 1.5 cm and 1 cm respectively. The cone angle was 1^o and the gap between the cone and the plate of sample placement was 125 μm. This geometry was used for the rheometer CarriMed CSL100. In the geometry of parallel disks the gap was up to 150 μm, mainly due to the small quantities of SF samples. The parallel disks geometry was used for the measurements with the AR-G2 rheoemeter.

The measurements were carried out at two temperatures, 25 ^{o}C and 38 ^{o}C, i.e. the ambient temperature and that of the animal's body. The temperature of the samples controlled with an accuracy of ± 0.1 ^{o}C, by the Peltier system of the rheometers, on the plate on which the samples were placed. In all measurements a cylindrical cover made by plexiglas was placed over the samples, with humid absorbent material, in order to create a closed, saturated volume round the sample and to prevent evaporation of the sample.

The SF samples were obtained with arthrocentisis under aseptic conditions, from the knee joint of healthy dogs and from clinical cases of dogs with rupture of cranial cruciate ligament. 21G needles attached to 2.5 or 5 ml syringes were also used to obtain the SF samples. During the procedure all animals were under sedation or general anaesthesia. Furthermore, the rheological behaviour of a commercial product that is approved for intra-articular injection, namely Hyalart (Fidia, Italy) of 1% HA w/v, in corresponding temperatures 25 ^{o}C and 38 ^{o}C, was also examined.

RESULTS–DISCUSSION

The measurements initially were carried out with the HA solution of Hyalart. The results reveal the non-Newtonian behaviour for the product and its shear thinning response was relatively small. In the low shear region (i.e. < 1 s^{-1}) a decrease of 40% is recorded, from 25 ^{o}C to 37 ^{o}C. Thus, the product Hyalart shows significant temperature dependent rheological

behaviour. The corresponding results are shown in the following Fig. 2.

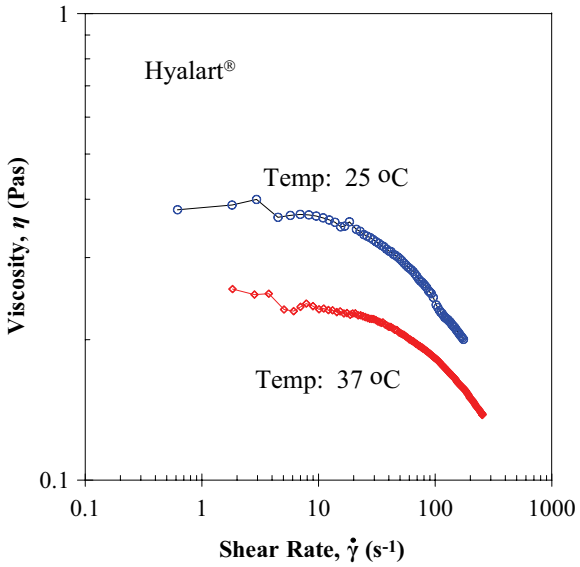

Figure 2. Viscosity curves for the Hyalart.

Moreover, concerning the viscoelastic response, the viscous response was more important than the elastic, and as a result the storage modulus G´´ was found to be higher than the elastic modulus G´. These results are presented in the following Fig.3.

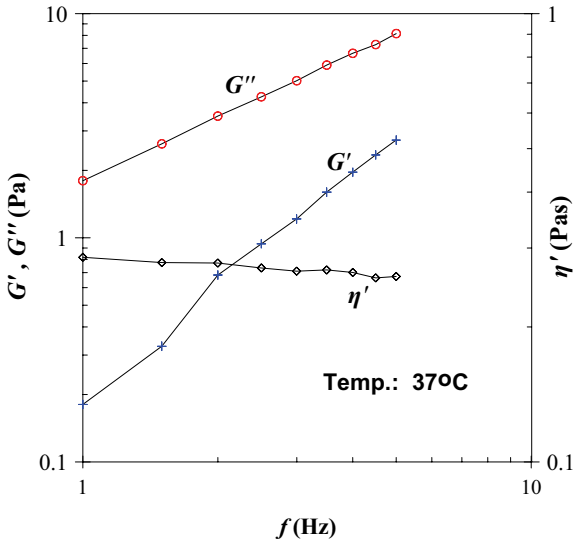

Figure 3. Viscoelastic behaviour of the Hyalart for strain value 0.01 ($\gamma\% = 1\%$).

The viscoelastic response of the normal SF was quite different, as it appears in Fig. 4; the storage modulus G´´ was found to be lower than the elastic modulus G´ about an order of magnitude. The measurements were carried out in the normal animal's temperature, i.e. 38 ^{o}C.

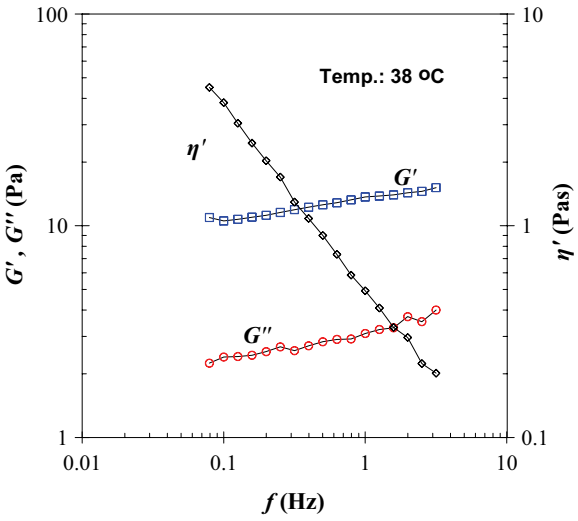

Figure 4. Viscoelastic behaviour of the normal SF for strain value 0.01 ($\gamma\% = 1\%$).

To this respect, the response of the SF under dynamic measurements is in accordance with previous studies on SF rheology[5,8,10].

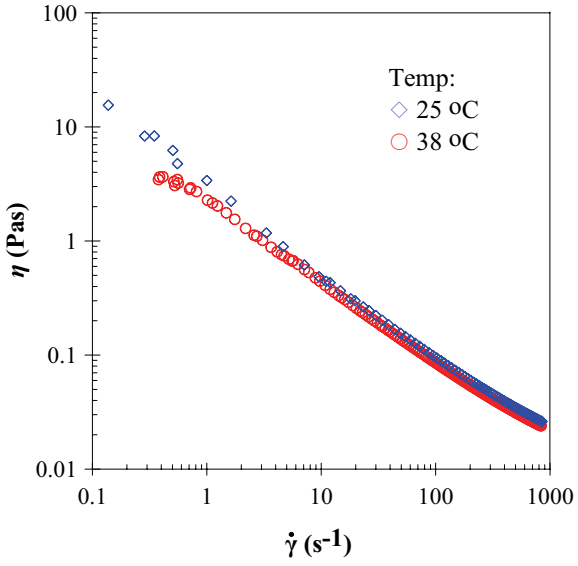

Figure 5. Temperature effect on the viscosity of the normal SF.

The results of Fig. 5 were obtained with the rheometer CarriMed CSL100; this is the reason that the lower achieved shear rate was about 0.1 s^{-1} (since this rheometer, in principal, is a controlled stress one). This figure shows that the normal SF exhibits practically a non-temperature dependent behaviour. This is an important observation since locally the knee joint may present temperature deviations from the average animal's body temperature.

In a next step the study was focused on the time needed for the SF to retrieve its properties after a surgery for treatment of cranial cruciate ligament, which is a common orthopaedic injury in dogs.

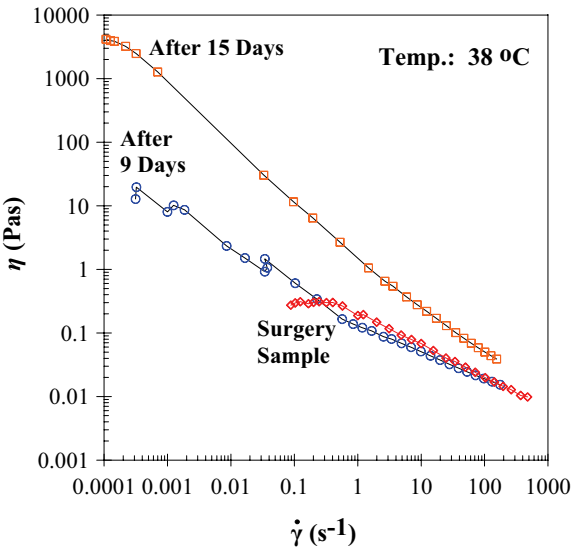

Figure 6. Comparison of the viscosity curves of the SF during the post-surgery period.

Fig. 6 shows the viscosity curves for samples obtained during the surgery, after one week and finally after two weeks. The results were obtained by using the rheometer AR-G2. The lower achieved shear rate in this case was about 10^{-4} s^{-1}. Although, in all cases the non-Newtonian behaviour is present, only the curve corresponding to the sample of the two weeks post-surgery period converge to the behaviour of the normal SF. This means that the required time to convalesce the SF its properties (e.g. shear thinning behaviour) is about two weeks.

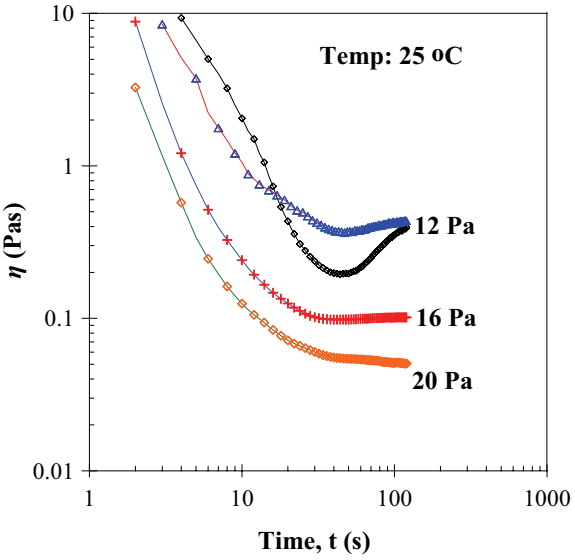

Figure 7. Comparison of the effect of constant applied stress on a normal SF sample.

Finally, application of a constant shear stress on the samples was performed in order to evaluate the time needed for the SF to adjust its response to the stress conditions. The time for the medium stresses applied to these sets of measurements was essentially the same and it was about less than a minute. It must be pointed out that for the lower stresses an unexpected overshoot was recorded; for this set of measurements this stress value corresponds to 12 Pa. This observation could be attributed to a structural rearrangement of the HA chains, to which the proteins of small molecular weight (e.g. albumin) contribute significantly.

CONCLUSIONS

The results of the present investigation showed that the normal synovial fluid exhibits small viscosity changes as a function of temperature and satisfactory viscoelastic characteristics. The storage modulus G′ was found to be an order of magnitude higher than the loss modulus G′′,

for the same applied frequencies. By increasing shear rate, $\dot{\gamma}$, from 0.1 s^{-1} up to the value of 10^3 s^{-1}, non-Newtonian viscosity values are decreased by two orders of magnitude, from 5 Pas to 0.05 Pas. Steady stress measurements revealed that the synovial fluid viscosity requires 40 s to 50 s to reach steady plateau values.

On the contrary, in pathological conditions of rupture of the cranial cruciate ligament, measured viscosity for the same region of $\dot{\gamma}$ was found drastically decreased, i.e. between 0.1 Pas and 0.03 Pas. After two weeks period from the surgery the rheological behaviour of the post-treatment SF converge to the normal one. On the other hand, at the post-treatment period of one week the SF exhibits behaviour of a non-Newtonian diluted biopolymer. Finally, the present results could constitute a base of comparison for corresponding human pathological situations.

ACKNOWLEDGMENTS

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