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MICRO-RHEOLOGY OF BIOLOGICAL AND BIO-ARTIFICIAL FLUIDS

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ABSTRACT

Microrheology is a quite new, but interesting technique in the biological field. This non-destructive measuring methods infers elastic and viscous flow parameters from the motion of embedded tracer particles using the generalized Stokes-Einstein relation. It is offering access from 1 to 10.000 Hz with small sample volumes (µl) and cellular-scale resolution. This study has been focusing on the viscosity of different biological fluids like cerebrospinal fluid (CSF, human), synovial fluid (SF, dog, horse) and artificial CSF and SF.

The viscoelastic behavior of the studied samples is largely attributable to their protein content. To test this, artificial cerebrospinal fluid (CSF) and synovial fluid (SF) as well as their individual components were prepared and analyzed, using the same parameters as for corresponding biological samples. To validate the method, measurements were compared with macro-rheological data and demonstrated that the results from both approaches are complementary[1,2,6,8].

INTRODUCTION

Microrheology studies the mechanical properties of viscous materials in a microscopic behavior. Due to the low sample amount and the non- destructive measuring technique, this method is quite interesting for biological probes. Compared with conventional rheology, microrheology can cover a broader frequency range from 1 to 10000 Hz. It has been shown that microrheological measurements agree very well with conventional double gap measurements. For this reason, this technique is perfect for biological samples due to the fact that it is often difficult to get enough sample volume for conventional measurements (e.g. tears, synovial fluid). This method can be divided in two different measuring types, i.e. the active and the passive. In active microrheology particles are driven by an external force, for example a magnetic field, whereas the passive form uses well defined and freely diffusing tracer particles exhibishing Brownian motion, driven by thermal energy. This mobility is measured to obtain the viscosity and the structure of the fluid. The mean square displacement (MSD) is an important parameter in microrheology for quantitative interpreting of the viscosity and sheer modulus of

the sample. In Newtonian fluids, the slope of the MSD directly yields the diffusion coefficient and thus the viscosity via the Stokes–Einstein relation. In viscoelastic samples, the MSD enabling determination of the frequency-dependent modulus from passive tracer motion [1,8,12].

In this study, artificial cerebrospinal fluid (CSF)[3,4,5,7,14], artificial synovial fluid (SF)[13], human cerebrospinal fluid, and synovial samples from dogs and horses were used [3,4,5]. It should be noted that the synovial fluid samples from dogs were collected from animals with joint problems, whereas the horse samples were taken from unaffected joints post mortem.

MATERIALS AND METHODS

Human CSF samples were collected from patients suffering from hydrocephalus, as part of the standard medical therapy.

Synovial fluid samples from dogs were collected from "Tierklinik Sattledt" as excess material during necessary treatments or examinations.

Synovial fluid sample from horses were collected from "Tierklinik Tillysburg" post mortem. As the horses showed no abnormalities in their gait or signs of discomfort, it was assumed that there were no pathological changes in the synovial fluid; however, no clinical examinations were performed.

Artificial SF

TABLE 1: Composition of artificial SF, with a concentration of 3 g/L in total[13].

Substance	Amount
Ig G	20 mg
Hyaluronic Acid, 1440 kDA	5,6 mg
Human Albumin (20%)	0,2 ml
Ringersolution	1,8 ml

Artificial CSF

TABLE 2: Composition of artificial CSF, with a concentration of 19 g/L in total[14].

Substance	Amount
Glucose	69,8 mg
Lactate	18,2 mg
Human Albumin	180 µl
Ringersolution	up to 100 ml in total

Collection

All fluids were collected under sterile conditions, and the samples were stored in sterile sample containers at 4 °C until measurement.

Conventional Rheometry

All samples were measured using the double-gap method with an Anton Paar rheometer (Anton Paar, MCR 702, Graz, Austria) at 37 °C with a double gap geometry (DG26.7/T200/SS).

Microrheology

For passive microrheology measurements, a sample volume of 1 mL was spiked with 30 µL of tracer particles (0.1 µm, Polystyrene, Sigma-Aldrich) in a plastic microcuvette and homogenized. Measurements were performed on the Malvern Zetasizer Nano Plus with a 621 nm laser at a 173° backscattering angle at 37 °C.

Conventional Rheology

The measurements were performed on an Anton Paar Rheometer 702, with the double gap method also at 37 °C[9,10,11].

High Frequency Rheology

An electromechanical quartz crystal tuning fork (dimensions of 6x1,5x0,35mm³) vibrates at a frequency of 25 - 28 kHz with amplitudes in the nm range. Fluid forces acting on the vibrating element change the resonance characteristics of the electrical frequency response. From these changes the sheer moduli are determined. The exact sheer rates are non-unform und unknown,

but low. Also, the vibration frequency cannot be adjusted. However, this method is most sensitive to changes of rheological fluid properties and features additional benefits including low sample volume, fast temperature control, low cleaning effort, high robustness, and low fouling due to conformal parylene coating of the sensor.

RESULTS AND CONCLUSION

Comparing microrheological measurements of hyaluronic acid and humane albumin (**Fig. 1**) shows stronger interactions in humane albumin compared to pure hyaluronic acid, which suggests the formation of a protein network in the human albumin. All measurements are performed at body temperature for better comparison of the results.

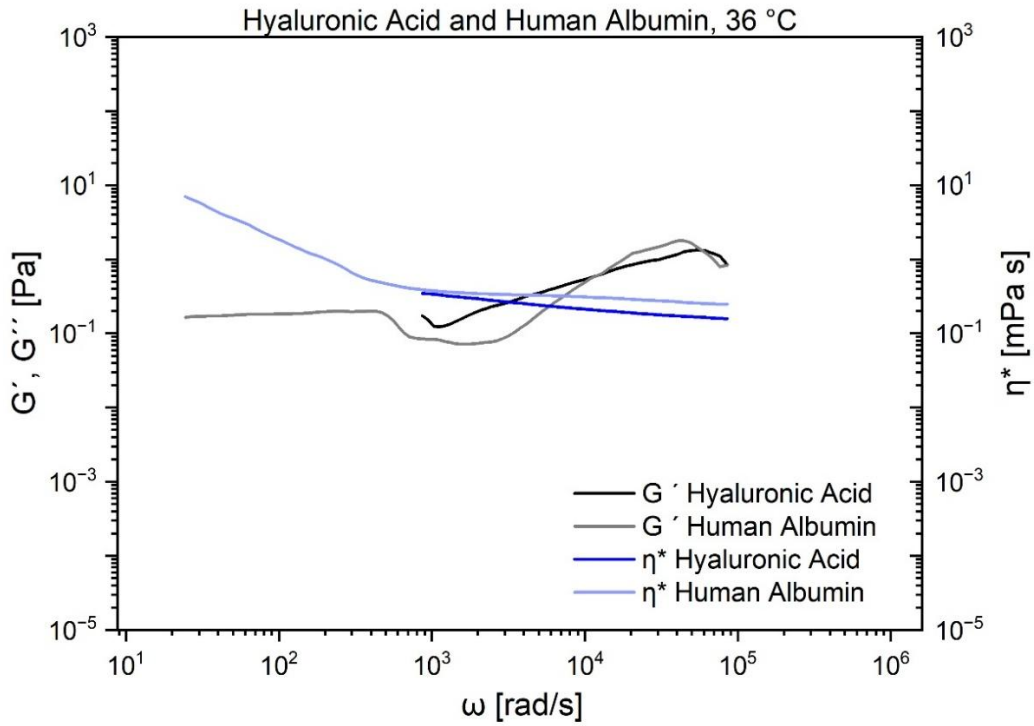

FIGURE 1: Storage modulus and viscosity of hyalurionic acid and humane albumin at 36 °C.

In **Fig. 2**, artificial synovial fluid was compared with the synovial fluid of a horse and a dog. It can be shown that the storage modulus and viscosity of the artificial synovial fluid behave similarly to those of the horse. In contrast, the values for the dog's synovial fluid are lower. One possible reason is that the dog suffered from joint disorder, whereas the horse showed no abnormalities in its joint apparatus.

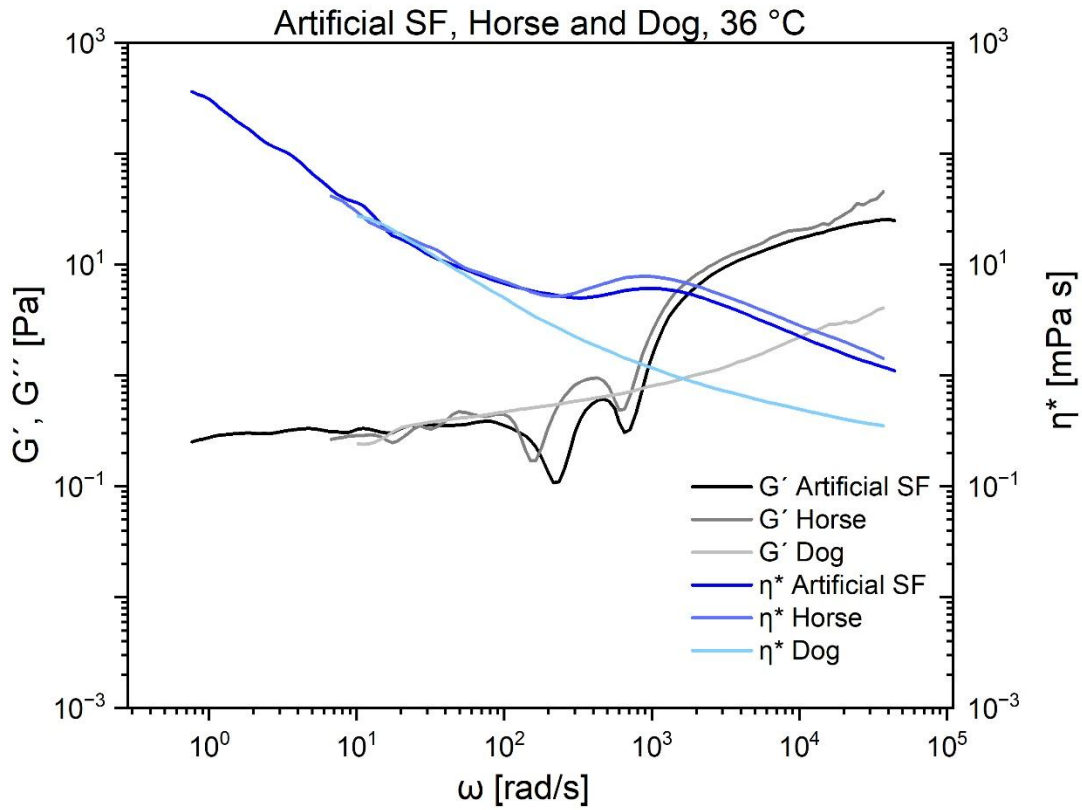

FIGURE 2: Storage modulus and viscosity of artificial SF and SF from horse and dog at 36 °C.

Fig. 3 shows a comparison between human CSF and artificial CSF. The storage modulus and viscosity values of the human samples are very similar, whereas the artificially prepared CSF falls within a much higher frequency range, and its viscosity is below the storage modulus.

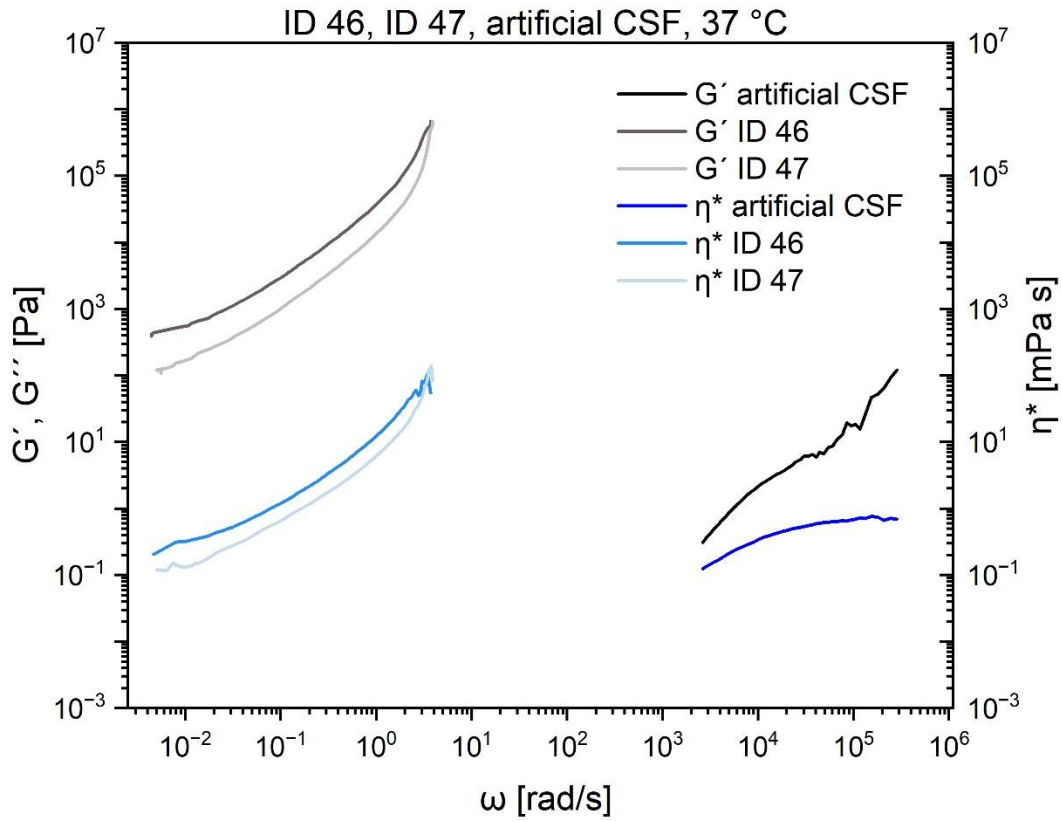

FIGURE 3: Storage modulus and viscosity of artificial CSF, ID 46 and ID 47 at 37 °C.

In **Fig. 4**, the integration of conventional rheology with microrheology is shown. On the right, a red dot marks a measurement with a high-frequency sensor. It was demonstrated that all three measurement methods agree very well and can be effectively combined. In other words, microrheological measurements on biological samples with small sample volumes can provide a good prediction for the frequency range of conventional measurements [5].

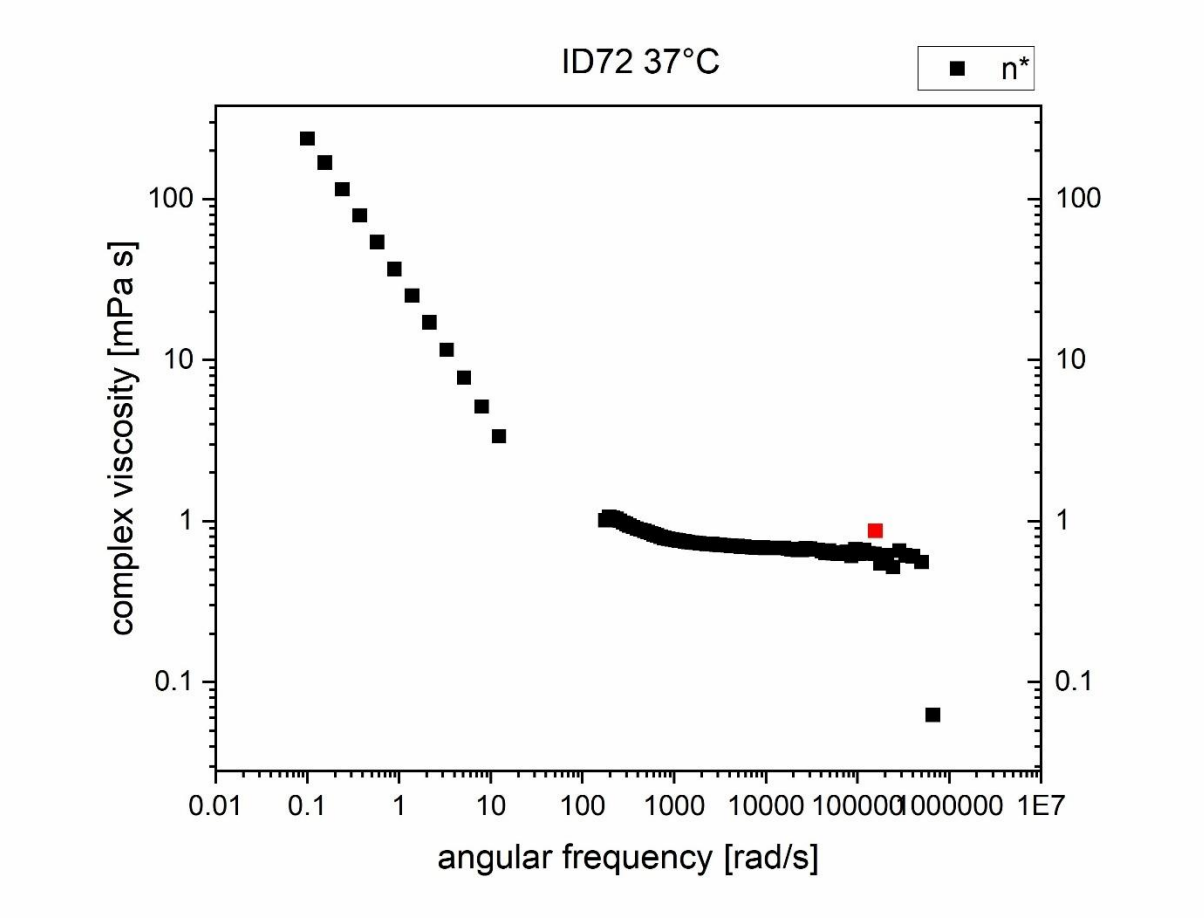

FIGURE 4: Comparison of Micro- (right hand side) and Macrorheology (left hand side) and a red sensor point of ID 72 at 37 °C.

In summary, it can be shown that biological samples from humans and animals exhibit a network structure that is less pronounced in artificially prepared solutions. These viscous properties are important for joint lubrication in the case of synovial fluid. In patients with hydrocephalus, the viscosity of the CSF might become an important clinical parameter for deciding whether a shunt will be necessary after intraventricular or subarachnoid hemorrhage. Initial experiments with a high-frequency sensor showed that this very fast method also yields values similar to those from microrheological investigations. Further samples need to be examined and compared with laboratory parameters, and the artificial solutions may need to be adjusted to achieve better viscosity values.

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This study involving human samples was approved by the Ethics Commitee, Faculty of Medicine, Johannes Kepler University Linz, MED Campus I, ADMBuilding 7th Floor, Krankenhausstaße 5, 4020 Linz (approval No. 1025/2022) on 1 July 2022. This study was conducted in accordance with local legislation and institutional requirements. The participants provided written informed consent to participate in this study.