

Microrheology of human synovial fluid of arthritis patients studied by diffusing wave spectroscopy

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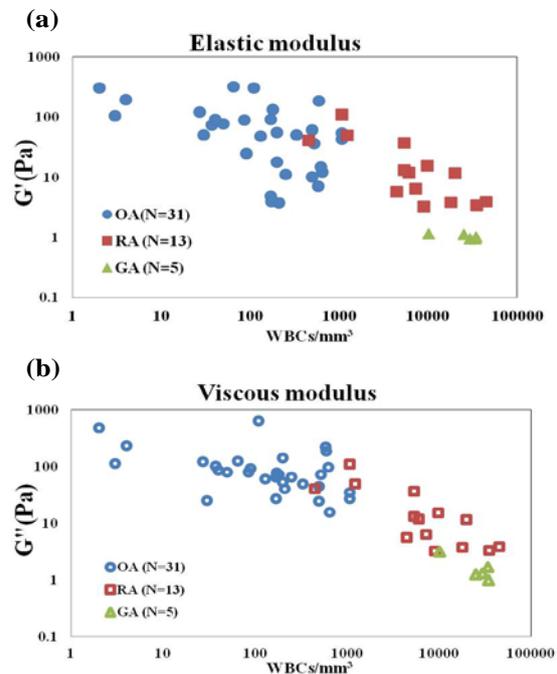
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The viscoelastic properties of synovial fluid (SF) are critical to its functions of lubrication and shock-absorption of joints in human body; a change in the viscoelastic properties, even of only a few percents, is often concomitant with arthritis. In this work, the elastic modulus $G'(f)$ and the viscous modulus $G''(f)$ of SF from patients suffering from three kinds of joint diseases, namely, osteoarthritis (OA), rheumatoid arthritis (RA), and gouty arthritis (GA), were determined as a function of frequency “ f ” (in the low frequency range from $f \sim 0.1$ Hz to 10Hz) by Diffusing Wave Spectroscopy (DWS) and correlated with the white blood cell (WBC) count. A strong correlation was observed, showing a higher WBC count corresponding to lower elastic and viscous moduli, G' and G'' ; further details depend on inflammatory vs. non-inflammatory, and on the severity of inflammation. Different types of arthritis lead to different degrees of decreasing viscoelasticity. Identical measurements were carried out with a commercial visco-supplementation (or artificial SF) to serve as reference. In general, the reduction in both G' and G'' was most severe in the case of GA and least severe in the case of OA. Besides, in all cases, the reduction in G' was more prominent than the reduction in G'' , indicating that in general, the deterioration in the elasticity of SF by inflammation is more severe than that in the viscosity. This simple method for quantitative physical characterization of synovial fluid may serve as a useful complementary metric to the conventional biochemical analysis in clinical diagnosis of arthritis.

1. Introduction

The viscoelastic properties of synovial fluid (SF) are critical to its functions of lubrication and shock-



Elastic and Viscous moduli (at 1Hz) of synovial fluids (at 27°C) from 49 arthritis patients vs. their white blood cell counts.

absorption of joints in human body; the viscoelastic properties depend strongly on the concentration and the molecular weight of hyaluronic acid (HA) [1], which is the key component of SF. The three-dimensional network structure of HA is critical to the unique viscoelastic

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property of SF. However, arthritis, the inflammation of joints, may reduce the molecular weight and/or the concentration of HA [1, 2], leading to a reduction in the viscoelasticity of SF, and causing damage in joints, and subsequently patient's pain and joint stiffness.

Among different kinds of arthritis, osteoarthritis (OA), rheumatoid arthritis (RA) and gouty arthritis (GA) are the three most prevalent diseases. OA is a non-inflammatory degenerative joint disease that is caused by over use of joints or aging, causing pain and disability to numerous people worldwide. RA is an autoimmune chronic inflammatory arthritis associated with the attack of autoantibody on the synovium and soft tissue, causing severe joint deformation and dysfunction. GA is a disease associated with uric acid crystal deposition which causes severe pain during acute attack and severe inflammation of joints. These diseases are diagnosed clinically by the symptoms and signs of patients as well as the X-ray image and serological studies such as rheumatic factors or serum uric acid levels. Current biochemical study of SF is also very important in distinguishing inflammatory arthritis vs. non-inflammatory arthritis, as well as to quantify the disease severity by the WBC count which often serves as an important indicator for the degree of inflammation.

Current SF analysis is typically based on a set of parameters and features including the white blood cell (WBC) count, viscosity (measured by capillary viscometer), Gram stain, fluid clarity, and examination via microscopy. Synovial fluid WBC count, obtained by either automatic cell counters or by manual counting via haematocytometric chambers, has been widely used in clinical settings to reflect the degree of inflammation present in the joint [3]. SF obtained from a normal joint has a low WBC count; i.e., normal SF is nearly acellular. Inflammatory and septic synovial fluids are characterized by increasing numbers of leukocytes. One study of SF from 40 asymptomatic, apparently normal adult knees, yielded an average WBC count of 35 cells/ μL , with none of the samples showing counts higher than 100 cells/ μL [4]. Previous studies [3, 5] also indicate that, cell counts of SF from asymptomatic patients, with established arthropathy and with clinically uninflamed knees, are well above the values found in normal joints. This has been observed in patients suffering from rheumatic arthritis, Systemic Lupus Erythematosus (SLE) arthropathy, or gouty arthritis. Specifically, patients suffering from OA (which is non-inflammatory) often show WBC counts of less than 2000 cells/ μL [6, 7]. For inflammatory joint diseases such as RA and GA, the WBC counts are higher, and could be as high as 100,000 cells/ μL under severe conditions. A systematic review in 2007 [8] of the predictive ability of diagnostic tests for septic arthritis in adults presenting with acute mono- or oligoarthritis also indicates that the likelihood of septic arthritis increases with increasing SF white cell count. Hence, higher WBC count has been generally regarded as one of the most im-

portant indicators to access the severity of inflammation in arthritis.

Measurements of the viscosity of SF by capillary viscometer in clinical laboratories often provide only qualitative information. Quantitative characterization of both the viscosity and the elasticity of SF may provide additional metrics for the diagnosis and the evaluation of arthritis patients.

Measurements of the viscoelastic properties of synovial fluid for possible complementary diagnosis of joint diseases have been reported in the literatures [5 - 10]. Earlier studies on the viscosity of synovial fluid from normal, osteoarthritic, and rheumatoid arthritic joints, measured by viscometers, indicated that the viscosity of normal synovial fluids is higher than that of the degenerative synovial fluids and inflammatory synovial fluids [9-12]. However, the elastic properties of synovial fluid from different arthritis (especially for gouty arthritis) have not been well characterized. For example, the viscoelastic properties of synovial fluid (from human knee joints) at different oscillation frequencies (f) had been measured by an oscillatory rheometer [13]. The structure of polymer (e.g., hyaluronic acid) plays an important role in the frequency dependence of viscoelasticity. In general, at low oscillation frequencies (typically for $f < 0.1\text{Hz}$), the polymer chains can release stress via deformation and disentanglement; hence, the polymer (such as SF and viscosupplementation in this case) behaves more like a viscous fluid (with the viscous modulus G'' greater than the elastic modulus G'). At higher oscillation frequencies, the polymer exhibits stronger elastic behavior (with $G' > G''$), because the polymer chains are not sufficiently agile to deform and disentangle in response to rapid change in stress [14].

In addition, it had been reported that for synovial fluid from patients with seronegative rheumatoid arthritis and seropositive arthritis and traumatic diseases, typical values of both G' and G'' are lower than the corresponding values for normal SF samples [14]. However, for a thorough elucidation of the relationship between viscoelasticity of synovial fluid and joint pathology, further studies will be required to identify detail biochemical origins contributing to the reduction in elasticity and viscosity of synovial fluid in arthritis patients.

In this paper, we present the correlation between viscoelastic property of SF and white blood cell (WBC) count to offer a complementary metric to the diagnosis of joint pathology. We quantify the viscoelasticity of SF in terms of the elastic modulus $G'(f)$ and the viscous modulus $G''(f)$ as a function of frequency (f), where the variable frequency (f) can be associated with the rate of stress resulting from the joint motion. Our clinical samples include SF from 49 patients suffering from one of the three common types of arthritis, osteoarthritis (OA), rheumatoid arthritis (RA) and gouty arthritis (GA). For comparison, we also measured $G'(f)$ and $G''(f)$ of a

commercial visco-supplementation (or artificial synovial fluid).

2. Experimental Procedures

2.1 Sample preparation

The SF samples were obtained with arthrocentesis under aseptic conditions, from the human knee joint of 49 patients, 31 diagnosed as suffering from OA, 13 from RA and 5 from GA. All samples came from Shuang Ho Hospital (IRB Statement and Approval Number: 201104003). For each patient, the white blood cell (WBC) count, obtained by standard commercial cytometric counting, was provided by the hospital laboratory. The viscoelastic properties of synovial fluid from patients of these three kinds of joint diseases (OA, RA & GA) were measured by a commercial micro-rheometer (Formulation SAS, Rheolaser Instruments) which requires a sample volume of approximately 1 ml.

For comparison and to serve as a reference, the viscoelastic properties of a commercial visco-supplementation (or artificial synovial fluid) containing 10mg/ml of sodium hyaluronate (NaHA) with molecular weight in the range of 600KDa to 1200KDa dissolved in physiological saline were also measured under identical condition. Currently, refilling visco-supplementation into the articulation to recover the viscoelasticity is among the safe and effective therapeutic treatments [15-17].

2.2 Diffusing wave spectroscopy

The commercial micro-rheometer (Formulation SAS, Rheolaser Instruments) used for the measurements of the viscoelasticity of our samples is based on Diffusing Wave Spectroscopy (DWS). For the specific model used in our experiments the upper frequency limit is approximately 10 Hz. Throughout our experiments, the lower frequency limit was chosen to be 0.1 Hz (corresponding to an integration time of 10 sec.), and the sample chamber was preheated to and maintained at 27°C (± 0.1 °C). For the experimental results reported in this paper, we repeated the experiment 20 times with each sample to obtain the mean values and the root mean square (RMS) variation; hence, the total time required for each sample is typically 3 to 4 minutes.

Diffusing Wave Spectroscopy (DWS) is a passive microrheology technique [18-23]. When a laser beam (wavelength = 650nm in our system) illuminates a fluid sample, the photons penetrating into the sample are backscattered by micro-objects, such as particles, droplets, fibers, etc., suspended in the fluid. A video camera is used to record the dynamic interference patterns of the backscattered waves, often known as ‘the speckle image’,

as is illustrated schematically in Fig. 1. The dark and the bright spots on the speckle image result from the destructive and the constructive interferences, respectively, among the backscattered waves. The scattering centers are unavoidably subjected to Brownian motion (due to thermal agitation) which induces light intensity fluctuation in the speckle image, and an overall dynamic deformation of the speckle pattern. Standard numerical algorithms have been established to deduce, from the dynamic speckle images, statistical parameters of the sample, such as the Relative Decorrelation (RDC) and the Mean Square Displacement (MSD) as a function of time. The elastic modulus $G'(f)$ and the viscous modulus $G''(f)$ of the sample as a function of frequency (f) can then be deduced from the MSD [20, 24]. $G'(f)$ and $G''(f)$ represent, respectively, the dynamic elastic response and the dynamic viscous response of the sample to mechanical stress.

Diffuse wave spectroscopy relies on light scattering from the sample. For synovial fluids, the endogenous scattering centers in the samples allow us to obtain repeatable data without the need to add any exogenous microbeads. Hence, we chose to take the data without adding any micro-beads. For the case of visco-supplementation, however, we do need to add microbeads into the sample solution to provide sufficient light scattering. For the data presented in Fig. 4 in the next section, we used polystyrene beads (diameter = 2.0 μ m) with a sparse concentration $\sim 0.002\%$ w/v. To check the dependence of the measured values of G' and G'' on the bead size, we measured $G'(f)$ and $G''(f)$ of visco-supplementation with 4 different sizes (diameter: 0.6 μ m, 1.0 μ m, 1.5 μ m, and 2.0 μ m) of microbeads; our results (data not shown) indicate that the size dependence is moderate. Specifically, at $f = 1$ Hz, the deviation due to different bead sizes is less than 28 % for G' and less than 17% for G'' .

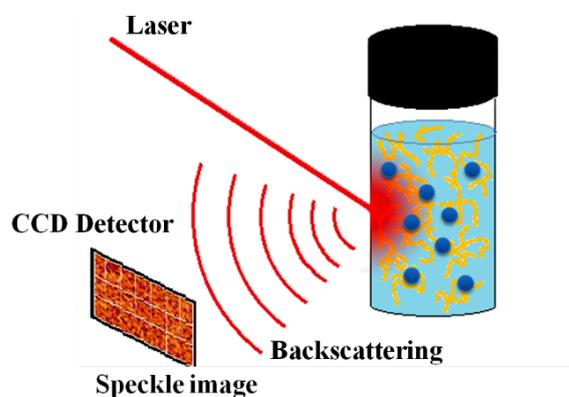


Figure 1 A schematic illustration of microrheology based on diffusing wave spectroscopy (DWS).

3. Experimental results and discussion

As a specific example, the frequency dependence of the dynamic elastic and viscous moduli, G' (f) and G'' (f) for synovial fluid from an osteoarthritis patient (with $\text{WBC} = 40/\text{mm}^3$), a rheumatoid arthritis patient (with $\text{WBC} = 5200/\text{mm}^3$), and a gouty arthritis patient (with $\text{WBC} = 10800/\text{mm}^3$) are shown in Fig. 2. All the data were taken with the sample chamber preheated to and maintained at 27°C ; each datum point represents an average over 20 repeated experiments and the root mean square (RMS) errors are indicated by the error bars.

In all three cases shown in Fig. 2, the viscous modulus G'' is higher than the elastic modulus G' in the low frequency regime (from $f = 0.1\text{Hz}$ to a few Hz), and the difference ($G'' - G'$) decreases as the frequency increases until the two cross-over. The crossing frequency " f_c " [defined by $G'(f_c) = G''(f_c)$], corresponds to the relaxation rate of the polymer chains; the inverse of the crossing frequency ($1/f_c$) can be interpreted as the polymer relaxation time (τ_r) [25]. A similar trend has been reported by Anadere et al [26] for near normal and rheumatoid arthritis fluid; The physical interpretation is that the SF behaves more like a viscous fluid at low frequencies to enhance its lubrication function in response to slow joint motion; in the higher frequency range, an increase in G' means a stiffer spring constant, and hence a higher resonance frequency. In other words, the undesirable resonance effect can be suppressed at relatively slow time scale. A comparison of the crossing frequency (f_c) for patients with osteoarthritis ($\text{WBC} = 40/\text{mm}^3$), rheumatoid arthritis ($\text{WBC} = 5200/\text{mm}^3$) and gouty arthritis ($\text{WBC} = 10800/\text{mm}^3$) indicates that the crossing frequency (f_c) increases (from $\sim 1\text{Hz}$ to 6Hz and to $>10\text{Hz}$) as the white blood cell count increases; in the case of gouty arthritis, the cross frequency, which is beyond the upper frequency limit of the instrument, was estimated by extrapolation of the experimental data.

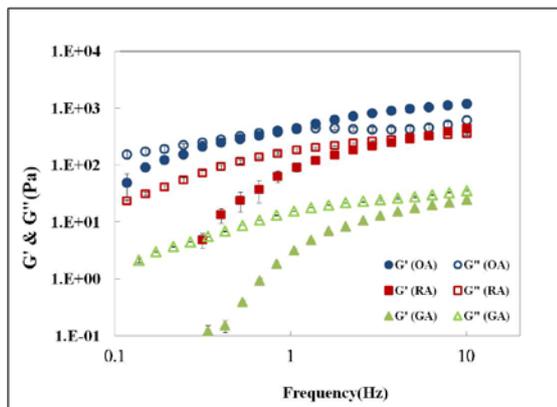


Figure 2 The elastic modulus $G'(f)$ and the viscous modulus $G''(f)$ as a function of frequency (f) for synovial fluid from os-

teoarthritis ($\text{WBC} = 40/\text{mm}^3$), rheumatoid arthritis ($\text{WBC} = 5200/\text{mm}^3$) and gouty arthritis ($\text{WBC} = 10800/\text{mm}^3$) patients: all the data were taken with the sample chamber preheated to and maintained at 27°C . Each datum point represents an average over 20 repeated experiments, and the root mean square (RMS) errors are represented by the error bars.

We observed a strong correlation of both the elastic modulus G' and the viscous modulus G'' with WBC counts as shown in Fig. 3 (for $f = 1\text{Hz}$) for SF samples from 49 arthritis patients, 31 diagnosed as suffering from OA, 13 from RA, and 5 from GA; both G' and G'' decrease as the WBC count increases.

The viscoelasticity of SF is mainly determined by the quality and the quantity of its constituent Hyaluronic Acid (HA) [27]. In diseased synovium, activated white blood cells induce reactive oxygen species (ROS), which de-polymerize hyaluronan, leading to a reduction in the concentration and/or the molecular weight of hyaluronan (HA) [28-30], and a consequential reduction in the viscoelasticity of SF. Since OA is a non-inflammatory degenerative joint disease, WBC count of patients suffering from OA is often smaller than those suffering from RA and GA; the inflammatory nature of both RA and GA leads to a much higher WBC count, and a significant decrease in both the elastic and the viscous moduli of SF.

The bar charts in Fig. 4 highlight the clear separation in the range of the values of G' and G'' (at 1Hz) and their ratio (G''/G') of SF samples taken from patients with different kinds of joint diseases (OA, RA, & GA). The number "N" represents the number of patients. For comparison and to serve as a reference, the experimental results for a commercial visco-supplementation (10mg/ml of NaHA, Mw $\sim 600\text{KDa}$ to 1200KDa), measured by the same method under identical condition, is also shown in Fig. 4 (in the leftmost triplet bars). The significant increment in G''/G' from left to right (the white bars in Fig. 4) indicates that in general, the deterioration in the elasticity of SF by inflammation is more severe than that in the viscosity.

To be more specific and for further classification, we have sub-categorized the white blood cells into neutrophil, lymphocyte, eosinophil, and monocyte; the percentage of these subclasses in the white blood cells (often known as the white blood cell differential count) from different types of arthritis patients is shown in Table 1. The reactive oxygen species (ROS) released by these different types of white blood cells are quite different. Neutrophils and Monocytes release $\text{O}_2^{\bullet-}$ [29, 31, 32] while lymphocytes release H_2O_2 [33]. In general, H_2O_2 is less reactive than $\text{O}_2^{\bullet-}$ [34]; hence, in arthritis with acute inflammation (such as in the case of GA) where neutrophils dominant, the HA is often destroyed more severely than in other cases, resulting in a much lower values of the viscoelasticity of SF as shown in Fig. 3 and Fig. 4.

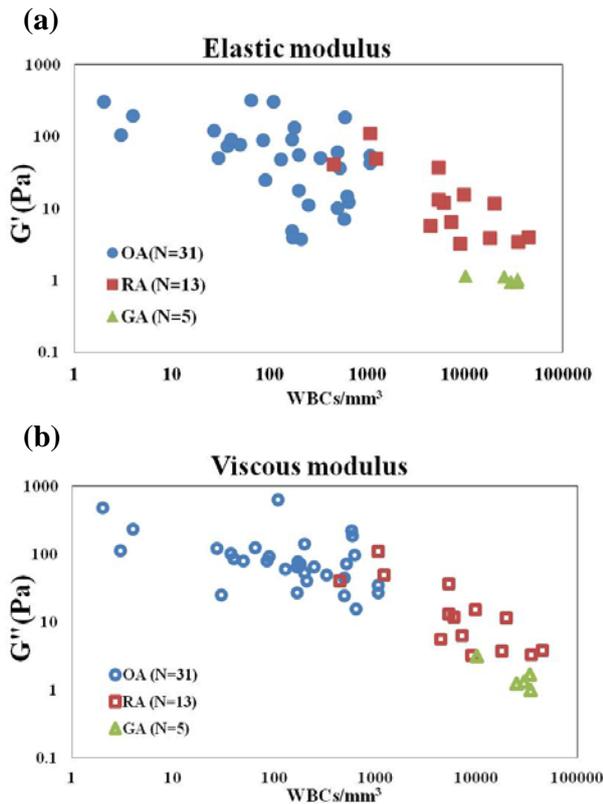


Figure 3 (a) Elastic modulus; (b) viscous modulus (both at 1 Hz) of synovial fluids (at 27°C) from 49 arthritis patients vs. their WBC counts.

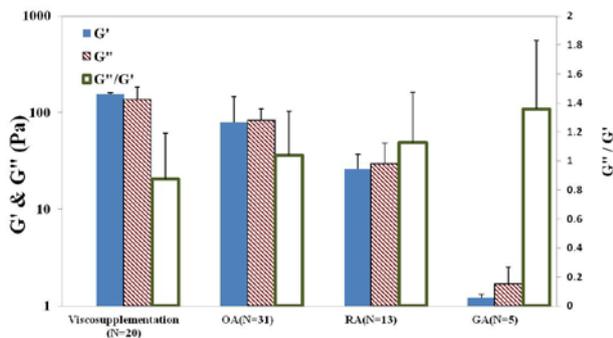


Figure 4 Bar charts to highlight the clear separation in the range of the values of the elastic modulus G' , the viscous modulus G'' , and the ratio G''/G' (all at 1Hz) of SF for patients with different kinds of joint diseases (OA, RA, & GA); “N” represents the number of patients. For comparison and to serve as a reference, the results for a commercial viscosupplementation (10mg/ml of NaHA, Mw = 600KDa ~ 1200KDa) are also shown in the left-most triplet bars.

Table1. Total and differential white blood cell counts in synovial fluid from arthritis patients, diagnosed as suffering from osteoarthritis (OA), rheumatoid Arthritis (RA) and gouty Arthritis (GA). The number “N” represents the number of patients in each category in our samples.

Group	WBC /mm ³	Neutrophil (%)	Lymphocyte (%)	Eosinophil (%)	Monocyte (%)	N
OA	206	5±5	84±13	0	9±9	31
RA	11728	78±39	10±9	0	12±9	13
GA	21240	87±49	6±4	0	3±2	5

Although both RA and GA belong to inflammatory arthritis, unlike RA which is a chronic deteriorating autoimmune disease, GA is usually characterized by acute attack episode. The samples taken from GA patients are usually collected while the patients are in their acute inflammatory phase, and the WBC count may decline if the joint fluid aspiration is not performed at the first moment (when the patients are still in their acute inflammatory phase). Besides, the chemical environment, including cytokine composition, may be different in acute and chronic arthritis such as GA and RA. All these factors are expected to jointly contribute to the result that even for the same WBC count, the values of G' and G'' are often significantly lower in the case of GA than in RA.

The general trend that the values of both G' and G'' descend according to the order (Normal > OA > RA > GA) is consistent with the results for viscosity measurement reported by Cooke et al. [35], for the cases of normal, OA and RA. However, in the case of synovial fluid from osteoarthritic knee joints, the absolute values of our results for G' and G'' (at 1 Hz) are approximately two orders of magnitude higher than the corresponding results measured by a conventional rheometer reported by M. Mensitieri et al. [36]. Since the samples were taken from different patients and since the WBC count or any other inflammatory markers were not given in Ref. 36, it is difficult to resolve the reason for this significant difference.

In clinical laboratories, quantitative measurements of the viscoelasticity of synovial fluid of arthritis patients are not among the standard procedures; the so call “String Test” (often measured by a capillary viscometer) [37], commonly practiced in clinical labs, provides only a qualitative estimate of the viscosity of the samples as either “high”, “medium”, or “low”, with no information at all on the elasticity. In this paper, we have measured the viscoelastic properties of synovial fluid samples and showed that they are highly correlated to the white blood count, which often serves as a standard indicator for the degree of inflammation. Hence, quantitative characterizations of the synovial fluid in terms of both the viscosity and the elasticity, described in the paper, provide a

viable complementary metric for the diagnosis of arthritis patients.

4. Summary and Conclusions

Viscoelastic properties of synovial fluid (SF) provide the functions of lubrication and shock-absorption of joints in human body; a change in the viscoelastic properties, even of only a few percents, is often concomitant with arthritis. In this paper, we report the correlation of viscoelasticity of synovial fluids of arthritis patients (including osteoarthritis, rheumatoid arthritis and gouty arthritis) to correlate with the white blood cell count (which often serves as an important indicator for the degree of inflammation in clinical diagnosis). The viscoelasticity of the samples was measured via diffusing wave spectroscopy, and characterized in terms of the elastic modulus $G'(f)$ and the viscous modulus $G''(f)$, as a function of frequency (f). For comparison, we also measured $G'(f)$ and $G''(f)$ of a commercial visco-supplementation (or artificial synovial fluid).

Our results clearly indicate that (1) as the white blood cell count increases, both G' and G'' decrease; (2) the average values of G' and G'' of SF from patients suffering from osteoarthritis (OA), rheumatoid arthritis (RA) and gouty arthritis (GA) follow a descending order with $OA > RA > GA$; (3) the crossing frequency (f_c) increases (from ~ 1 Hz to 6 Hz and to >10 Hz) with increasing degree of inflammation in the same order (of OA, RA, and GA); (4) in general, the inflammation reduce the elasticity of SF much more than its viscosity. This simple method for quantitative physical characterization of synovial fluid may serve as a useful complementary metric to the conventional biochemical analysis in clinical diagnosis of arthritis.

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