

Characterization of Human Dental Pulp Tissue Under Oscillatory Shear and Compression

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Availability of material as well as biological properties of native tissues is critical for biomaterial design and synthesis for regenerative engineering. Until recently, selection of biomaterials and biomolecule carriers for dental pulp regeneration has been done randomly or based on experience mainly due to the absence of benchmark data for dental pulp tissue. This study, for the first time, characterizes the linear viscoelastic material functions and compressive properties of human dental pulp tissue harvested from wisdom teeth, under oscillatory shear and compression. The results revealed a gel-like behavior of the pulp tissue over the frequency range of 0.1–100 rps. Uniaxial compression tests generated peak normal stress and compressive modulus values of 39.1 ± 20.4 kPa and 5.5 ± 2.8 kPa, respectively. Taken collectively, the linear viscoelastic and uniaxial compressive properties of the human dental pulp tissue reported here should enable the better tailoring of biomaterials or biomolecule carriers to be employed in dental pulp regeneration. [DOI: 10.1115/1.4033437]

Keywords: tooth, pulp, regeneration, viscoelastic, compression, biomaterial

1 Introduction

Dental pulp is a soft connective tissue containing blood vessels and nerves to sustain its own physiological functions and those of the adjacent tissues. It is also a residence for cells of different types, including odontoblasts populated at the dentin surface and fibroblasts distributed throughout the extracellular matrix (ECM) of the pulp. The ECM is also rich in terms of collagens (collagens type 1: 56%, type 3: 41%, type 5: 2%) and noncollagenous (chondroitin 4- and 6-sulfate: 60%, dermatan sulfate: 34%, keratan sulfate: 2%, and glycosaminoglycans as proteoglycans) [1], distributed within the interstitial fluid. These organic constituents together with the cells regulate the structure–function relationship of the pulp tissue, collagen type I presumably contributing to its viscoelastic [2] and biomechanical properties, such as stiffness and strength [3], and proteoglycans mostly adding to its viscoelasticity [1]. The pulp tissue is a relatively nonmineralized soft tissue, and lack of mineralization in pulp might be attributed to the absence of specific molecules which are present in the adjacent mineralized dentin tissue. Prior investigations [1] suggest that markers of mineralization such as dentin sialoprotein, dentin phosphoprotein, dentin matrix protein-1, and osteocalcin are predominantly expressed by odontoblasts.

Regeneration potential of dental pulp will certainly avoid complications associated with the endodontically treated tooth. Endodontically treated tooth leads to considerable structural deformations due to removal of part of enamel, dentin, and pulp. Such deformations may result in tooth fracture and trauma as the postoperative tooth becomes deceased and brittle. Because of the lost pulpal sensation and inability of the tooth to detect microbial challenges, it can also be more susceptible to re-infections [4]. Since a regenerated tooth is expected to function similar to its native counterpart, selection of appropriate biomaterials plays a significant role in this process. However, lack of knowledge about the material properties of human dental pulp and high costs

associated with the optimization studies render the process of biomaterial selection difficult. In this context, viscoelastic and biomechanical characterization of the native dental pulp tissue is regarded as an essential element of biomaterial development.

Our group earlier tested and reported the viscoelastic properties of dental pulp tissue harvested from miniature-pig. Although it was the first report of its kind in regard to characterization of dental pulp tissue, literature is still deficient in data related with human dental pulp tissue. This study, therefore, aims at filling this gap by characterizing human dental pulp tissue under oscillatory shear and compression to determine its material properties.

Biomaterials for tooth regeneration are expected to provide a homelike environment for cells, be biocompatible and biodegradable, allow functionality for a variety of cells, be clinically applicable, and possess multiple structural characteristics due to well-orchestrated hierarchical structures and functions of dental tissues. However, research studies have shown that the interactions between the cells and their niche are closely related to physicochemical properties of the scaffolding materials [5,6]. In this regard, biomaterial selection, design, and development for regenerative dentistry require the understanding of viscoelastic material functions and biomechanical behavior of the native pulp tissue. Such properties can provide useful tools to be utilized for the selection and tailoring of biomaterials to act as scaffolds for tissue engineering of dental pulp.

Here, with the aim of establishing a benchmark data for biomaterials to be used in dental pulp regeneration, we characterize and report the linear viscoelastic properties and uniaxial compression behavior of human pulp tissue.

2 Materials and Methods

2.1 Dental Pulp Harvesting. Pulp tissue was obtained from the wisdom tooth of human patients (multiple donor, $n = 3$) following a protocol approved by the noninterventional Clinical Research Ethics Board of Hacettepe University. Briefly, after the application of local anesthesia, the tooth was extracted and stored in 3% penicillin–streptomycin solution. The crown was removed

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Manuscript received February 15, 2016; final manuscript received April 19, 2016; published online May 2, 2016. Assoc. Editor: Michael Detamore.

from the dentin–cementum junction while immersed in sterile phosphate-buffered saline (PBS), and the fresh pulp tissue was removed as an intact tissue by gently pulling the tissue out using forceps. Test specimens were then formed by punching cylindrical disks of approximately 3.2 mm diameter, soaked into PBS, kept refrigerated, and characterized within 24 hrs (Fig. 1). The specimens were obtained from the coronal pulp occupying the crown of the tooth because only the coronal zone has sufficiently large area to cover the entire disk area of the rheometer.

2.2 Characterization of Viscoelastic Material Functions.

The samples were characterized in compression and oscillatory shear using a Discovery Hybrid Rheometer (TA Instruments, New Castle, DE). The experimental setup used in this study was similar to that employed earlier for the characterization of native miniature-pig dental pulp tissue and cartilage tissue [7,8]. Briefly, the specimen was inserted between the custom-made disk (3.2 mm in diameter) and the bottom platform of the rheometer, where the disk is also immersed in PBS solution kept at 37°C to prevent drying of the specimens during the experiments (Fig. 1). The upper disk either oscillates in the clockwise and counter-clockwise directions or translates in the downward direction at a constant velocity and is also connected to the torque and normal force transducer. The PBS containing custom-made environmental chamber consisted of a Petri dish attached to the lower platform (Fig. 1(b)).

2.2.1 Small-Amplitude Oscillatory Shear. In small amplitude oscillatory shear, the shear strain oscillates as a function of time as $\gamma = \gamma_0 \sin(\omega t)$, where γ_0 is shear strain amplitude (i.e., $\theta D/h$, where θ is the angular displacement, D is the disk diameter, and h is the gap in between the two disks), ω is the oscillation frequency, and t is the time. The shear stress, τ , response to the oscillatory deformation consists of two components related to the energy stored and energy dissipated as heat, i.e., $\tau = G'(\omega) \gamma_0 \sin(\omega t) + G''(\omega) \gamma_0 \cos(\omega t)$, where $G'(\omega)$ is the shear storage modulus and $G''(\omega)$ is the shear loss modulus. The ratio of $G''(\omega)$ to $G'(\omega)$ is $\tan \delta$. The oscillatory shearing needs to be carried out in the linear viscoelastic region at which the moduli are independent of the strain amplitude. The strain amplitude sweeps indicated that up to the strain amplitude of 1%, the oscillatory shear deformation of the native tissue took place in the linear region. Therefore, the dynamic properties $G'(\omega)$ and $G''(\omega)$ were characterized as a function of frequency in the range of 0.1–100 rps at 1% strain. Sweeping the frequencies enables the characterization of the linear viscoelastic response of the tissue over a range of time scales. In general, at relatively short characteristic times of deformation,

the elastic response is underscored, while the viscous flow behavior is emphasized at longer characteristic times. Our earlier time sweep studies performed on articular cartilage and pig dental pulp tissue revealed that the samples were stable within the time scale of the experiments (typically less than 20 min of shearing for each sample).

2.2.2 Biomechanical Characterization Upon Compression. Uniaxial compression of the native tissues was performed in the strain range of 0–10% at a constant compression rate of 0.05 mm/min. This strain level was previously used in biomechanical characterizations of native and engineered cartilage tissues as well as miniature-pig dental pulp tissues [7,8]. Here, the specimens were initially squeezed using a normal force of approximately 0.03 N to obtain full contact, and the compression test was then started. In a related set of experiments, the normal stress relaxation behavior of the specimens to the compressive loading was characterized upon application of step strains within 3–4 s.

2.3 Statistics. The experiments were designed not to have more than one group, which is the human dental pulp tissue only. Therefore, no statistical analysis of the data was needed. The values reported were all in the form of average \pm STD.

3 Results

3.1 Strain-Sweep Test. The dynamic material functions, namely, the storage modulus (G'), the loss modulus (G''), and $\tan \delta$ (G''/G') of the human pulp tissue were measured over the strain magnitude range of 1–100% at 1 rps frequency. It was observed that the storage and loss moduli, as well as $\tan \delta$ values remained independent of the strain amplitude over the range of strain amplitudes studied, and thus in this strain amplitude range, human dental pulp tissue exhibited linear viscoelastic behavior (Fig. 2(a)).

3.2 Frequency Dependence of the Linear Viscoelastic Material Functions. The frequency-sweep experiments were performed in the frequency range of 0.1–100 rps at the strain amplitude of 1%. As noted earlier, at this strain amplitude, the human pulp tissue exhibited linear viscoelastic behavior. The frequency dependencies of the storage modulus (G'), loss modulus (G''), and $\tan \delta$ (G''/G') are shown in Figs. 2(b)–2(d). Results show that storage modulus values are greater than the loss modulus values (relatively small $\tan \delta$ values, i.e., between 0.1 and 0.5) and the moduli are nearly independent of frequency and exhibit parallel behavior (Fig. 2).

Therefore, the results of the frequency-sweep experiments (Figs. 2(b)–2(d)) revealed that the human pulp tissue exhibits storage modulus, G' , values between 2000 and 7000 Pa and loss modulus, G'' , values of around 1000 Pa.

3.3 Relaxation Behavior Following Uniaxial Compression. Figure 3(a) shows the response of the specimens of the human pulp tissue upon 20% compression applied for the duration of 3–4 s. The decline in stress after imposing a nominal strain determines the time-dependent relaxation behavior.

As shown in Fig. 3(a), the normal stress decreased with time at a similar rate it was initially applied. The relaxation behavior of the samples was characterized by a steady decrease in the normal stress as a function of time up to about 1000 s, after which the normal stress becomes negligibly small. Specifically, normal stress reached a value of 0.26 ± 1.48 kPa in 1035 s. The peak stress upon 20% compression in 3.8 s was determined to be 102.0 ± 39.6 (Fig. 3(a)).

3.4 Compressive Stress Versus Strain Behavior. The compressive stress–strain behavior of the human pulp tissue is shown in Fig. 3(b). The Young's modulus, peak normal stress, and the toughness (area under the stress–strain curve) of the pulp tissue

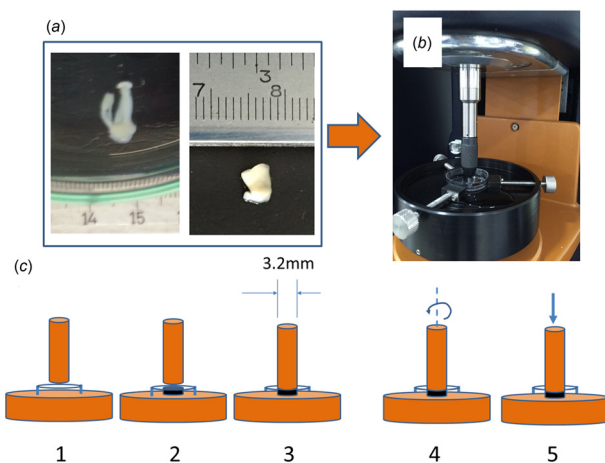


Fig. 1 Harvesting and characterization of human dental pulp tissue obtained from wisdom tooth ($n = 3$). Sampling of dental pulp (a); rheological characterization in PBS using a custom-made hydration chamber (b) under shear and compression (c). In (a), each space in scale bar is 1 mm.

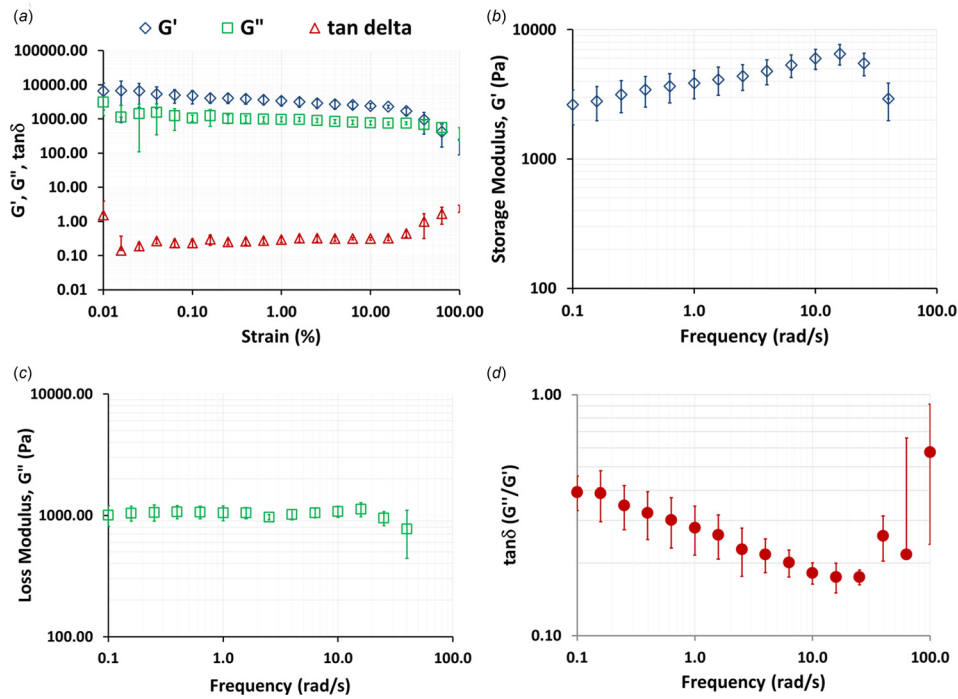


Fig. 2 Rheological characterization. Storage modulus, loss modulus, and $\tan\delta$ versus strain amplitude behavior of native pulp tissue at 1 rps and 37 °C (a). Frequency dependence of the storage modulus (b), the loss modulus (c), and $\tan\delta$ (d) of the native human dental pulp tissue at 1% strain and 37 °C. Error bars represent standard deviation, $n = 3$.

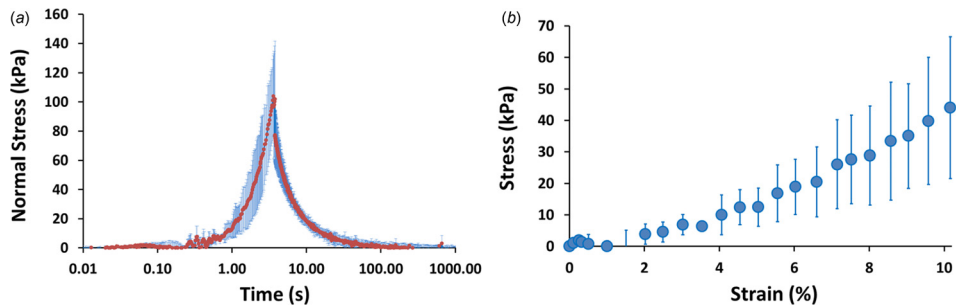


Fig. 3 Compression stress–relaxation response of the native pulp tissue over 1000 s upon 20% compression at 0.05 mm/s (a) and compression response of the native pulp tissue at 10% strain (b), $n = 3$. In A, the solid line represents average data and the dispersions are standard deviations. Error bars represent standard deviations.

Table 1 Biomechanical properties of the human pulp tissue. Data reported as AVG \pm STD.

Young's modulus (kPa)	Peak stress (kPa)	Area (toughness) (kPa)
5.5 \pm 2.8	39.1 \pm 20.4	139.1 \pm 75.1

determined at 10% strain are presented in Table 1. Specifically, at 10% strain, the Young's modulus, peak normal stress, and toughness values were 5.5 \pm 2.8 kPa, 39.1 \pm 20.4 kPa, and 139.1 \pm 75.1 kPa, respectively.

4 Discussion

Up to date, cell transplantation was the major strategy for dental pulp regeneration [9–11], where selection of cell-seeding material is typically based on the ease of handling or optimization. Efforts to match the biomechanical properties as well as viscoelastic

material functions of the biomaterial with those of the native dental pulp tissue have been lacking up to now. Having seen such a deficiency in the relevant literature, our group earlier measured these properties for the miniature-pig dental pulp, which was the first study of this kind [8]. To add to the existing pool of information related to dental pulp regeneration, this study, for the first time, offers viscoelasticity data for the human dental pulp and suggests that a more representative biomaterial can be selected by making use of the findings obtained from viscoelastic characterization of human dental pulp. Therefore, the findings reported here should set a benchmark in the development of clinically feasible biomaterials for human pulp regeneration.

The native pulp tissues obtained from the wisdom tooth of human subjects were characterized in terms of their rheological properties under oscillatory shear and biomechanical properties under compression. The strain-sweep experiments demonstrated that the storage modulus, G' , the loss modulus, G'' , as well as $\tan\delta$ values for the specimens were not affected by the strain amplitude up to 1% strain and that they all exhibited linear viscoelastic

behavior within this range. The wall-slip effect is a characteristic of materials exhibiting gel-like behavior [12]. It is clear from strain-sweep experiments (Fig. 2) that a negligible degree of wall-slip is observed up to 1% strain level, yet wall-slip becomes more influential afterward. This behavior is an integral part of such materials, and the data involving wall-slip effect may be corrected to obtain the true shear at the wall of the disks if needed. Generally, presence of proteoglycan molecules present in most of the soft connective tissues leads to surface lubrication, which eventually causes wall-slip effect in rheological characterization [7]. Such common behavior of soft biological tissues can also be thought to play significant roles here for human dental pulp tissue. Our prior tests performed on the native articular cartilage demonstrated such behavior [7]. However, one of our earlier studies performed on miniature-pig dental pulp tissue did not require such characterization based on the relatively low proteoglycan amount, e.g., 0.43% of dry weight [8], of the dental pulp tissue. In this study, using similar analogy, wall-slip analysis has been neglected.

The results of the frequency-sweep tests suggest that the viscoelastic behavior of the human pulp tissue exhibits features that are typical of the behavior of microgels. The existence of gel-like behavior is indicated by small values of $\tan \delta$, typically in the range 0.1–0.5 in this study [7,13,14]. The dental pulp tissue consists of cells and ECM components such as collagen fibers, proteoglycans, and water and electrolytes. These interact to form porous composite fiber-reinforced organic solid matrix, which eventually contribute to the viscoelastic behavior of the pulp tissue. In this regard, the proteoglycans are networks with the ability to store deformational energy [15]. The cross-linked fibrous collagens, on the other hand, provide tensile stiffness and strength to the tissue [16]. Overall, collagens and proteoglycans and their interactions with cells and other ECM components in an environment filled with the interstitial fluid can be said to determine the viscoelastic behavior of dental pulp tissue [17,18].

The ECM of dental pulp tissue is rich in terms of collagen (type 1: 56%, type 3: 41%, type 5: 2%) proteins [1]. The composition and type of collagens present in the pulp tissue resemble those contained in the tendon tissue in that they both contain type 1 and 3 collagens and that type 1 content is much higher than type 3 [19]. Therefore, the cross-link formation within the fibrillar structure of collagen in pulp tissue may be explained by the events taking place during cross-link formation in tendon tissue. In tendon tissue, the trivalent intermolecular pyridinoline cross-links and lysyl pyridinoline stabilize the fibrillar structure of collagen and control the mechanical properties of the tissue. Enzymatically formed covalent immature cross-links are then converted to mature trivalent cross-links with tissue maturation [20]. It is accepted that cross-link density in collagenous tissues is considerably higher in older individuals [21], possibly leading to age-dependent changes in biomechanical properties. At the single collagen fibril level, it is proposed that molecular rearrangement of collagen molecules and water molecules provide the mechanism for viscoelastic behavior. Shen et al. [2] postulate that when a fibril is stressed, (i) the collagen molecules may unwind, straighten, or slide with respect to one another, or (ii) the interstitial fluid molecules may rotate, translate within the fibril, or be ejected from the fibril. This results in the rearrangement of the network that could create a back stress in the fibril. Upon removal of the applied stress, this back stress leads to the rearrangement of collagen molecules and water molecules within the fibril, which eventually determines the collagen viscoelasticity.

It is emphasized here that the permanent junction points formed by the cross-linked collagen fibers/fibrils may have led to a nearly frequency-independent behavior in the human pulp tissue. The $\tan \delta$ values changed in the range 0.1–0.5 in the 1–100 rps range, indicating viscoelastic behavior, yet did not reach the purely elastic behavior point.

Compression–relaxation tests performed to evaluate the time-dependent behavior of the human pulp tissue also support the strong role of viscoelasticity. A Newtonian fluid behavior suggests

that the stress applied on a subject would decrease instantaneously upon removal of the deformation. For a purely elastic material, the stress associated with a continuously maintained strain would remain constant. In a viscoelastic behavior, on the other hand, time-dependent stress–relaxation occurs. For the human dental pulp tissue, characterized in this study, the viscoelasticity played roles to define its relaxation behavior.

As noted earlier, our group performed similar tests on dental pulp tissues harvested from miniature-pig [8]. Comparison of human dental pulp tissue with that of the miniature-pig in terms of storage modulus and loss modulus revealed one order of magnitude difference between these two species (values for human dental pulp are greater). In addition, $\tan \delta$ values dispersed in a similar neighborhood (0.1–0.5 for miniature-pig, 0.1–0.7 for human subjects). Nevertheless, it should be noted that the miniature-pig study involved mandibular canine, while this study employed wisdom teeth. In this study, we did not use human canine because it is almost impossible to find a reason to extract healthy canine from human subjects, while wisdom tooth is readily available. Also, a human canine would not give sufficiently large area to be squeezed properly within the disks of the rheometer. However, we strongly believe that a comparison between the species should involve one-to-one comparison whenever possible.

The stress–relaxation path for human and miniature-pig dental pulp tissues followed similar behavior. Both materials relaxed almost completely, indicating a viscoelastic fluid type of relaxation behavior. It took approximately 1000 s for both materials to relax back, with a residual stress of 0.26 ± 1.48 kPa for human pulp and 0.23 ± 0.09 kPa for miniature-pig.

Compressive biomechanical tests of human and miniature-pig dental pulp tissues also generated different results. For instance, the peak stresses at 10% compression at a rate of 0.05 mm/min were 39.1 ± 20.4 and 7.3 ± 3.0 kPa for human subjects and miniature-pig subjects, respectively. Similarly, human dental pulp tissue had higher Young's Modulus than that of miniature-pig (5.5 ± 2.8 for human and 0.8 ± 0.4 for miniature-pig). As seen in the standard deviation values, the data collected during the experiments are quite dispersed. The high uncertainty in these experiments may be attributed to the age of the donors (ranging between 16 and 25 yrs). As pointed out earlier, the cross-link density in collagenous tissues is considerably higher in older individuals that can possibly lead to changes in biomechanical properties.

It is also important to elaborate on potential group of biomaterials that can be designed using the data generated in this study. In fact, our group earlier characterized both rheologically and biomechanically three of the hydrogels (namely, agarose, alginate, and collagen) widely utilized in regenerative engineering approaches [8]. Comparisons of the linear viscoelastic material functions of the human pulp tissue of this study with those of the three hydrogels revealed that at the constant gelation agent concentration of 2%, the dynamic properties, i.e., storage and loss moduli and the $\tan \delta$, of the agarose- and alginate-based gels were similar to those of the human pulp tissue. Under uniaxial compression, the peak normal stresses and compressive moduli of the human pulp tissue exhibited significantly higher compressive properties than those of the hydrogels.

5 Conclusions

In this study, linear viscoelastic properties and uniaxial compressive properties of human dental pulp tissue were investigated. Findings suggest that the human dental pulp tissue exhibits characteristics of a viscoelastic material and possesses a gel-like behavior. These properties should shed a light for the investigators interested in clinically relevant biomaterial development for dental pulp tissue.

Acknowledgment

We are grateful to Dr. Hacer Aksel from Hacettepe University, Ankara, Turkey, for providing us human dental pulp tissues. We

are also thankful to Can Samiloglu and Hasan Kasikci from Likrom, Istanbul, Turkey, for their help with the tools for the characterization.

Nomenclature

- D = disk diameter
 $G'(\omega)$ = shear storage modulus
 $G''(\omega)$ = shear loss modulus
 h = gap in between the two disks
 N = normal force
 Pa = unit for shear stress and normal stress
 t = time
 $\tan\delta$ = ratio of shear loss modulus to shear storage modulus
 γ = shear strain amplitude
 γ_0 = zero shear strain amplitude defined as $\theta D/h$
 θ = angular displacement
 τ = shear stress defined as $G'(\omega) \gamma_0 \sin(\omega t) + G''(\omega) \gamma_0 \cos(\omega t)$
 ω = oscillation frequency

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