Original Article

Viscoelastic properties of human periodontal ligament: Effects of the loading frequency and location

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ABSTRACT

Objectives: To determine the viscoelastic properties of the human periodontal ligament (PDL) using dynamic mechanical analysis (DMA).

Materials and Methods: This study was carried out on three human maxillary jaw segments containing six upper central incisors and four lateral incisors. DMA was used to investigate the mechanical response of the human PDL. Dynamic sinusoidal loading was carried out with an amplitude of 3 N and frequencies between 0.5 Hz and 10 Hz. All samples were grouped by tooth positions and longitudinal locations.

Results: An increase of oscillation frequency resulted in marked changes in the storage and loss moduli of the PDL. The storage modulus ranged from 0.808 MPa to 7.274 MPa, and the loss modulus varied from 0.087 MPa to 0.891 MPa. The tan δ , representing the ratio between viscosity and elasticity, remained constant with frequency. The trends for storage and loss moduli were described by exponential fits. The dynamic moduli of the central incisor were higher than those of the lateral incisor. The PDL samples from the gingival third of the root showed lower storage and loss moduli than those from the middle third of the root.

Conclusions: Human PDL is viscoelastic through the range of frequencies tested: 0.5–10 Hz. The viscoelastic relationship changed with respect to frequency, tooth position, and root level. (*Angle Orthod.* 2019;89:480–487.)

KEY WORDS: Viscoelasticity; Periodontal ligament; Dynamic mechanical analysis

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INTRODUCTION

The periodontal ligament (PDL) serves as a connective tissue structure that links teeth to the alveolar bone. The PDL consists of collagen fibers with complex orientation and a ground substance of fluid, proteoglycans, and glycoproteins.¹ The unique composition and graded structure of the PDL leads to special mechanical properties.^{2,3}

In past decades, numerous studies have been devoted to determining the mechanical properties of the PDL. Since collagen fiber is a viscoelastic material,⁴ more studies have focused on the time-dependent mechanical behavior of the PDL using the creep⁵ and stress-relaxation tests.⁶ The experimental results confirmed that the PDL exhibited a nonlinear viscoelastic behavior⁷ and provided valuable information on quasi-static response of the PDL. However, under conditions such as mastication and orthodontic tooth movement, the PDL is subjected to dynamic loading, which has been rarely investigated⁷ in human

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PDL. Therefore, it is necessary to investigate the dynamic viscoelastic properties of the human PDL to obtain insight into the deformation mechanism of the PDL during mastication, impact, and orthodontic treatment.

Since the 1990s, dynamic mechanical analysis (DMA) has been used to investigate the viscoelastic properties of biological tissue, such as lens,⁸ articular cartilage,⁹ cardiovascular tissue,¹⁰ bladder tissue,¹¹ and so on. The frequency dependency of the storage (E') and loss (E") moduli of various biomaterials was obtained from DMA testing. One advantage of DMA for characterizing the mechanical properties of soft tissue is that relatively small sizes of specimens are required, since it is difficult to obtain large PDL specimens. Therefore, DMA was used in the present study to characterize the dynamic viscoelastic behavior of the human PDL.

The aim of this study was to measure the viscoelastic properties of the human PDL using DMA. The experiments were performed using the frequency creep method to test, specifically, whether the properties change with tooth position and longitudinal location. The data, in terms of the storage and loss moduli, were analyzed to evaluate the influence of loading frequency and location of the PDL on the viscoelastic behavior of the PDL.

MATERIALS AND METHODS

Sample Preparation

This study was reviewed and approved by the Institutional Review Board of Nanjing Medical University (No. [2015]169). Human maxillary incisor PDL samples were used for DMA. Three human maxillary jaw segments from three fresh corpses (male, 31-52 vears old, dentally and periodontally healthy), containing six upper central incisors and four lateral incisors, were brought to the laboratory and stored in a freezing container. Soft tissues were removed from the maxilla by a surgical scalpel. Each specimen with incisor and bone was sectioned out individually with a bone saw. Each bone-PDL-cementum complex was attached to a fixture and embedded in wax. Transverse sections 2mm thick were cut perpendicularly to the root longitudinal axis using a rotating blade saw (Isomet Low Speed Saw, Buehler, Lake Bluff, III) with the speed of 500 r/min. Copious normal saline irrigation was applied to specimens during cutting to prevent heat-induced denaturation of the tissue. From each root, two experimental samples were obtained from the cervical portion, and another two were obtained from the middle portion. Then, bar-shaped samples were extracted from these sections with $8 \times 6 \times 2$ mm dimensions using a dental low-speed hand piece (Figure 1a-c),

Test Machine

The DMA procedure was applied on the bar-shaped samples using a Pyris Diamond Dynamic Mechanical Analyzer (Perkin Elmer, Waltham, Mass). The machine has a force resolution of 0.2 mN and a displacement resolution of 10 nm. Custom-designed fixtures were used to enable the testing of samples. The fixtures were composed of two vertical grips for the tensile testing and produced tension on the top and bottom of the specimen. In the tensile tests, fixtures were designed to clamp the bony and dental part of each specimen firmly along the axis of transverse sections (Figure 1d). The PDL samples were covered by normal saline so that the specimens did not dehydrate during testing.

Test Procedure

Before testing, the specimens were defrosted at room temperature and soaked in normal saline solution. Since the displacement-control mode was shown to be inaccurate in DMA,13 the force-control mode of the loading profile was used in the present study. Each sample was subjected to sinusoidal tensile loading with a force amplitude of 3 N, resulting in an approximate strain of 0.2. Therefore, the maximum strain of the PDL was set to be 0.3. This strain amplitude was applied to minimize the effects of diversity in the thickness of the specimen and to protect the PDL from fracture failure. Each sample was tested under five frequencies (0.5, 1, 2, 5, and 10 Hz) for 15 minutes each. In the preliminary experiment of dynamic loading, it was confirmed that the experimental data became repeatable after about five cycles, which was consistent with the experimental results reported in the literature.⁷ The preconditioning in earlier studies¹⁴ was reported using 1 Hz and a strain of 0.25.

Micro computed tomography (vivaCT 80, SCANCO Medical, Bassersdorf, Switzerland) scanning was used in the preliminary test on human anterior teeth with a standard acquisition protocol (55 kVp, 145 μ A, 8 W, and 16- μ m voxel size). The results showed that the thickness of the PDL in the buccal and palatal part varied more than that in the mesial and distal part. Insufficient bone quantity was found in the buccal alveolus. As a result, it was hard to fabricate a proper buccal or palatal specimen, which was able to be firmly fixed to the dynamic mechanical analyzer. For this



Figure 1. (a) Cervical and middle part of the root. (b) Transverse sections of the specimen composed of bone, PDL, and tooth. (c) Schematic view of the rectangular specimen. (d) Tensile DMA setup for human PDL.

(1)

(2)

reason, the present study concentrated on the mesial and distal part of the human maxillary incisor PDL.

The stress σ and strain ε are a function of time t in the

 $\sigma(t) = \sigma_0 \sin \omega t$

 $\varepsilon(t) = \varepsilon_0 \sin(\omega t - \delta)$

where σ_0 and ε_0 are the stress and strain amplitude,

Data Analysis

DMA test:

respectively. ω and δ denote the frequency of the loading and the phase angle, respectively.

The dynamic tensile parameters were determined by the equations¹⁵:

$$\boldsymbol{E}^{*} = \boldsymbol{E}' + \boldsymbol{i}\boldsymbol{E}'' \tag{3}$$

$$i = \sqrt{-1} \tag{4}$$

$$E' = |E^*| \cos \delta \tag{5}$$

$$E'' = |E^*| \sin \delta \tag{6}$$

$$E^*| = \sqrt{E'2 + E''2}$$
(7)

$$\tan \delta = E''/E' \tag{8}$$

with E', E'', and $\tan \delta$ representing the storage modulus, loss modulus, and loss tangent, respectively.

Experimental data from the last five cycles for each frequency were used to determine the viscoelastic properties of the PDL. All specimens were grouped by tooth positions (the central and lateral incisors) and longitudinal locations (the cervical and middle regions). The differences between each group were compared by one-way analysis of variance. The apical specimens were not used in this study because the apical fibers are in the radial direction. All experimental data were analyzed using the software IBM SPSS Statistics version 20 (SPSS Inc, Chicago, III). The 95% confidence intervals were also generated using SPSS.

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Table 1. Specimens	Individu from T	ial Information for high the second sec	or the 14 Huma egments	n Incisor PDL		
Specimen	Age	Tooth	Vertical	Horizontal		
No.		Position	Location	Location		
1	1965	Central incisor	Cervical region	Distal region		
2	1965	Lateral incisor	Middle region	Mesial region		
3	1965	Central incisor	Middle region	Distal region		
4	1965	Central incisor	Middle region	Mesial region		
5	1971	Central incisor	Cervical region	Distal region		
6	1971	Central incisor	Cervical region	Mesial region		
7 8 9	1971 1971 1971	Central incisor Central incisor	Middle region Middle region	Mesial region Distal region		
10	1986	Lateral incisor	Middle region	Distal region		
11	1986	Central incisor	Middle region	Mesial region		
12	1986	Central incisor	Cervical region	Distal region		
13	1986	Central incisor	Cervical region	Mesial region		
14	1986	Lateral incisor	Middle region	Mesial region		

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Figure 2. Storage (E') and loss (E") modulus against frequency for all samples. Error bars denote standard deviations.

The *P* value was calculated to examine the significance of the fitting curve for storage and loss moduli. *P* values less than .05 indicated that the fitting curve of the relationship was significant.

RESULTS

Figure 2 displays the experimental results of the DMA tests with the frequency between 0.5 and 10 Hz. The value of storage and loss moduli ranged from 0.808 MPa to 7.274 MPa from and 0.087 MPa to 0.891 MPa, respectively. No significant difference was observed for the effect of the frequency on the storage and loss moduli in the frequency (P > .05). In other words, the tan δ remained almost constant within this frequency range.

The variation of storage modulus between 0.1 Hz and 10 Hz could be described by an exponential function as

$$E' = 2.712e^{0.079f}$$
 for $0.1 < \omega < 10$ (9)

The curve fits showed strong a correlation with R^2 values of .782 and greater (most between .95 and 1; Table 2), which were statistically significant (P < .05).

Each sample exhibited the same trend for loss modulus, which was described by an exponential fit.

$$E'' = 0.361 e^{0.106f} \text{ for } 0.1 < \omega < 10 \tag{10}$$

These exponential fits also showed a strong correlation with R^2 values of .699 and greater (most between .95 and 1; Table 2), which were also statistically significant (Figure 3). The storage and loss moduli showed significant differences between the middle and cervical group from the same root (Figure 4; Table 3). More specifically, the cervical group showed the lower storage and loss moduli, on average of 2.32 MPa and 0.348 MPa, respectively. The

dynamic moduli of the middle group were 4.00 MPa (storage modulus) and 0.58 MPa (loss modulus).

There were obvious differences in the storage and loss moduli between the middle group of central and lateral incisors from the same maxilla (P < .05; Figure 5; Table 4). The mean values of dynamic moduli in central incisors were greater than those in the lateral incisors. Differences among those in the cervical group were not compared because of the small sample size (n = 5).

DISCUSSION

This was the first study to investigate the dynamic tensile properties of human PDL using DMA. Previous studies found that human chewing frequency varied between 0.95 and 2.17 Hz.¹⁶ In terms of simulating occlusal load under physiological or traumatic conditions, the present study used DMA to interpret the

Table 2. Curve Fit for Storage and Loss Moduli of Each Specimen^a

Specimen	Sto (E	rage Mod ^[7]) Curve F <i>E' = Ae^s</i>	ulus =it,	Lo (E	Loss Modulus (E'') Curve Fit, $E'' = Ce^{D'}$				
No.	А	В	R²	С	D	R²			
1	2.975	0.083	0.996	0.722	0.089	0.996			
2	3.914	0.091	0.997	0.425	0.128	0.997			
3	8.973	0.034	0.961	1.533	0.036	0.743			
4	2.594	0.064	0.782	0.420	0.083	0.699			
5	2.310	0.115	0.977	0.345	0.100	0.989			
6	5.896	0.088	0.974	0.611	0.128	0.916			
7	0.866	0.070	0.981	0.126	0.063	0.847			
8	5.354	0.249	0.996	1.338	0.324	0.969			
9	4.624	0.072	0.973	0.386	0.147	0.995			
10	3.244	0.079	0.980	0.577	0.072	0.988			
11	1.340	0.045	0.975	0.096	0.143	0.998			
12	1.928	0.098	0.990	0.195	0.196	0.999			
13	4.844	0.054	0.948	0.375	0.108	0.993			
14	1.149	0.085	0.991	0.148	0.108	0.997			

^a All coefficients were found to be statistically significant (P < 0.05).



Figure 3. Curve fit of storage (a) and loss (b) modulus for all human PDL specimens. Data point represents the average values of the specimens, respectively.



Figure 4. The storage (a) and loss (b) modulus of the central and lateral incisors in the middle region of the root.

Table 3. Storage and loss Moduli, tan \delta, for the Cervical and Middle Region of the Central Incisors from Each Sample

	Sample 1 (1965)				Sample 2 (1971)				Sample 3 (1986)			
	Cervical Group		Middle Group		Cervical Group		Middle Group		Cervical Group		Middle Group	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
<i>E</i> ′, MPa	3.478	0.823	4.044	0.895	1.862	0.731	6.347	0.622	1.390	0.070	2.779	0.743
<i>E</i> ″, MPa tan∂	0.462 0.138	0.059 0.027	0.694 0.184	0.104 0.061	0.351 0.183	0.175 0.053	0.683 0.107	0.103 0.007	0.109 0.078	0.017 0.008	0.422 0.143	0.198 0.035

Table 4. Storage and loss Moduli, $tan \delta$, for the Middle Region of the Central and Lateral Incisors from Each Sample

	Sample 1 (1965)					Sample 2 (1971)				Sample 3 (1986)			
	Central Incisor		Lateral Incisor		Central Incisor		Lateral Incisor		Central Incisor		Lateral Incisor		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
E', MPa	4.044	0.895	2.418	0.256	6.347	0.625	4.900	0.405	2.789	0.105	1.234	0.023	
E'' , MPa tan δ	0.693 0.184	0.104 0.061	0.468 0.194	0.048 0.003	0.683 0.107	0.103 0.007	0.438 0.089	0.072 0.008	0.422 0.143	0.028 0.005	0.163 0.132	0.004 0.025	



Figure 5. Differences in the storage (a) and loss (b) moduli between the cervical and middle groups of the incisor PDL. The dynamic modulus of other middle samples is higher than that of cervical samples.

dynamic tensile properties of the human PDL in a frequency range of 0.1 and 10 Hz. The results of the present study were consistent with those reported before,^{10,11} in which the measured storage and loss moduli increased as a function of frequency, but tan δ showed small variation with the frequency. In comparison to the dynamic shear properties of porcine PDL,¹⁷ the dynamic modulus in the present study was higher at comparable frequencies. Oskui and Hashemi7 investigated the time and preload dependency of the bovine PDL using DMA. The tensile moduli of the present study were smaller than those of the bovine PDL. The storage modulus reported previously varied from 5 MPa to 20 MPa. The loss modulus of the bovine PDL ranged between 0.5 MPa and 1.4 MPa. Some studies found that the structure of bovine PDL was not markedly different from human PDL. The differences between bovine and human PDL may be attributed to the larger size of bovine teeth and that the masticatory pattern between bovines and humans is different. Considering the histologic and morphologic disparities, it is essential to study the dynamic tensile properties of human PDL to discern the difference and relation of mechanical properties between human and bovine PDL. This discrepancy may also be caused by the different dimensions of experimental species and testing protocols.

The storage and loss moduli represent the ability to store and dissipate energy, respectively. Because of the extremely high stiffnesses of the tooth and bone, the data from the apparatus can represent the dynamic tensile properties of the PDL. The experimental data showed that the storage modulus increased distinctly in the range of 0.1 to 5 Hz and remained almost constant between 5 Hz and 10 Hz. The increasing value of the storage modulus with the load frequency, which represents the elastic stiffness of material, illustrated that the stiffness of the PDL is correlated with the loading frequency. The loss modulus mostly maintained between the range of 0–1 MPa. A significant correlation between the frequency and the dynamic modulus has been reported in porcine bladder¹¹ and bovine articular cartilage.¹⁸ The ratio of the storage and loss moduli, tan δ , remained constant with the load frequency, which indicated that E' and E'' varied proportionally. The mean phase angles between stress and strain were in the range of 15° to 27°, which revealed that human PDL was viscoelastic throughout the full testing frequencies.

The PDL is composed of cells and an extracellular matrix, such as collagen fibers and ground substance. The ground substance is the major component of the ligament and consists of 70% fluid and 30% noncollagenous matrix, including proteoglycans and glycoproteins, which play the viscous role in the PDL.14 The collagen fibers, which are mainly composed of collagen I and III in the PDL, are arranged in definite and distinct bundles and are responsible for nonlinear elastic properties.⁴ The increasing storage modulus with the frequency implies the stiffening of the collagen with higher strain rates. The viscosity of the ground substance increases with the loading frequency in the range between 0.1 and 5 Hz as well. It has been reported that mechanical force can induce fibronectin and collagen synthesis.19 Conclusively, the PDL of central incisors contains more collagen fibers, which leads to its higher stiffness.

In the longitudinal group, the middle group PDL samples exhibited greater storage and loss moduli, indicating this group showed more stiffness and viscosity. In other words, the middle region of the root may demonstrate more resistance against loading. The

arrangement and volume fraction of collagen fibers have effects on tensile properties. The possible explanation for this result may be the different composition of the PDL in the longitudinal axis. It was observed that the middle region consisted of oblique PDL fibers (OF) and the cervical PDL was composed of horizontal fibers (HF).²⁰ The different volume fraction of collagen fibers and elastin between OF and HF may be the main reason for the difference in viscoelastic properties in the cervical and middle region of the root. It should be noted that the preparation of specimens might cause damage to the PDL fibers. It has been reported that about 80% of the oblique fibers in the PDL within the transverse section are intact in the mesial root of the rat molar.²¹

The findings of this study may be helpful in orthodontic treatment. The storage and loss moduli of central incisors was larger than those of lateral incisors, which demonstrated that the central incisor PDL was stiffer and exhibited higher viscosity than that of the lateral incisors. Therefore, the central incisors need larger orthodontic force than the lateral incisors to accomplish orthodontic tooth movement, which is coincident with clinical observation: the central incisors would have less instantaneous movement under occlusal loading. It should be considered that the PDL of central incisors could dissipate more energy and be more resistant to loading. In addition to the PDL proportion when choosing anchorage teeth, the stiffness and viscosity of the PDL should be considered. The PDL in the middle-root region exhibited less deformation under loading because of its stiffness. Hence, the center of rotation may be in the middle region of the root when a single force is applied to a tooth at the bracket level. In addition, the viscoelastic properties of the PDL are important for finite element simulation, which could be used to simulate tooth movement during orthodontic treatment.

One possible limitation of this study was that the PDL response under compressive or long-term loading was not studied. In physiological conditions, teeth are anchored by the PDL in the socket. In the present study, the flow of fluid and the influence of blood were not considered. The vasculature may influence the mechanical properties of the PDL. The results of this study reflected only the mechanical response of the bone-PDL-tooth complex, not the whole intact PDL.

CONCLUSIONS

 This study illustrated that the human PDL is viscoelastic in the range of the frequency tested: 0.5–10 Hz. The viscoelastic properties depend on the loading frequency. This dependency can be described by an exponential function. The tooth position and longitudinal location may influence the viscoelasticity of the PDL.

• The results explain some characteristics observed in orthodontic treatment and provide viscoelastic properties of the human PDL for the finite element simulation of orthodontic treatment.

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