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Dynamic tensile properties of bovine periodontal ligament: A nonlinear viscoelastic model

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ABSTRACT

As a support to the tooth, the mechanical response of the periodontal ligament (PDL) is complex. Like other connective tissues, the PDL exhibits non-linear and time-dependent behavior. The viscoelasticity of the PDL plays a significant role in low and high loading rates. Little information, however, is available on the short-term viscoelastic behavior of the PDL. Also, due to the highly non-linear stress–strain response, it was hypothesized that the dynamic viscoelastic properties of the PDL would be greatly dependent on the preload. Therefore, the present study was designed to explore the dynamic tensile properties of the bovine PDL as a function of loading frequency and preload. The in vitro dynamic tensile tests were performed over a wide range of frequencies (0.01–100 Hz) with dynamic force amplitude of 1 N and different preloads of 3, 5 and 10 N. The generalized Maxwell model was utilized to describe the non-linear viscoelastic behavior of the PDL. The low loss factor of the bovine PDL, measured between 0.04 and 0.08, indicates low energy dissipation due to the high content of collagen fibers. Moreover, the influence of viscous components in the linear region of the stress–strain curve (10 N preload) was lower than those of the toe region (3 N preload). The data reported in this study could be used in developing accurate computational models of the PDL.

1. Introduction

As the main connector of tooth and jaw bone, the periodontal ligament (PDL) absorbs occlusal forces and evenly distributes them into the alveolar bone (Berkovitz et al., 1995; Picton, 1989). The PDL is also responsible for bone remodeling during orthodontic tooth movement (Chen et al., 2014; Papadopoulou et al., 2013; Slomka et al., 2008). Thus, understanding functions of PDL is necessary to characterize its mechanical behavior (Natali et al., 2004; Picton, 1989). Although the PDL possesses lower stiffness compared to bone and tooth, its mechanical response to high and low loading rates is distinct. The PDL exhibits rigid behavior in high loading rates (Komatsu, 2010).

PDL is a compact fibrous tissue connecting the tooth to the surrounding bone. It's rich in type I collagen fibers which are embedded in a hydrated ground substance of proteoglycans and glycoproteins (Sloan and Carter, 1995). Structurally, collagen fibers resist tensile loads and ground substance tolerates compressive loads. Thus, the PDL responds differently to tensile and compressive loads (Atkinson and Ralph, 1977; Dorow et al., 2003). Furthermore, the composite structure of the PDL leads to a non-linear (hyperelastic) and time-dependent (viscous) mechanical behavior (Komatsu, 2010).

The viscoelastic behavior of the PDL has been examined with stress relaxation and creep tests using quasi-static experimental setups (Komatsu et al., 2007a; Shibata et al., 2006). Besides the importance of the PDL's creep behavior in orthodontic treatments, the creep tests may confront some difficulties in application of constant forces (Romanyk et al., 2013). Experimental findings have shown that the stress relaxation response of the PDL depends on the applied strain, indicating a non-linear viscoelastic behavior (Komatsu et al., 2007a). Nevertheless, the majority of the viscoelastic models of the PDL were presented using Fung's (1993) quasi-linear viscoelastic (QLV) formulation (Natali et al., 2004; Toms et al., 2002). The QLV formulation is a simplified version of the non-linear viscoelastic model wherein the stress relaxation behavior is independent of the applied strain; hence, it is unable to describe the non-linear viscous phenomena (Davis and De Vita (2012); Provenzano et al., 2002; Troyer and Puttlitz, 2012). Although those experiments have provided valuable insights into the long-term response of the PDL, its short-term response has been disregarded. The long- and short-term behaviors of the PDL, respectively, represent its response to low, e.g., orthodontic treatments, and high e.g., mastication or impact, loading rates (Bergomi et al., 2010).

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Since the PDL is subjected to dynamic loads (tensile, compressive and shear) in a wide range of frequencies (Bergomi et al., 2010), it is necessary to examine its dynamic viscoelastic properties in order to establish a profound understanding of PDL's short- and long-term responses (Sanctuary et al., 2005; Tanaka et al., 2007). For instance, Tanaka et al. (2007) investigated the dynamic shear properties in porcine PDL and observed the influences of the amplitude and frequency of loading on the storage and loss moduli.

In addition to the frequency, the dynamic mechanical properties of the PDL may depend on preload due to its non-linear stress–strain behavior. Increasing the strain magnitude from the toe to the linear region of the stress–strain curve increases the tensile stiffness of the PDL; hence, it was hypothesized that the complex modulus would be augmented by any increase in preload. Lomakin et al. (2014) studied the effects of pre-stress on dynamic tensile behavior of the temporomandibular joint disc and found that greater amounts of pre-stress increase the storage modulus and decrease the loss factor.

To determine the short- and long-term tensile responses of the PDL and its correlation with the preload, the present study followed two main goals: (1) to investigate the dynamic tensile properties of the PDL over a wide range of frequencies (0.01–100 Hz) with three different preloads by the dynamic mechanical analyzer (DMA), and (2) to develop a non-linear viscoelastic phenomenological model for subsequent computational analysis of the PDL in impact, mastication or orthodontics simulations.

2. Materials and methods

2.1. Sample preparation

Similar to the technique described by Bergomi et al. (2010), a cattle bovine mandible (from 3 to 5 years old) was taken from the local slaughterhouse within 3 h of the animal's death. The rationale for choosing the bovine PDL is its structural resemblance to the human PDL (Berkovitz, 1990). Then, it was brought to the laboratory within 30 min in a refrigerated container at 5 °C for subsequent sectioning. After removing the peripheral soft tissues from the teeth, right and left first molar dental blocks surrounded by jaw bone were separated. The blocks were sectioned transversely across the longitudinal axis of the molar tooth with a bone saw in the presence of normal saline solution. From each molar, four transverse sections of 2 mm thick were obtained. Afterwards, bar-shaped samples were extracted from these sections with $2\times4\times8$ mm dimensions. Depending on the depth of the transverse section, 3–5 bar-shaped samples were obtained from each section (Fig. 1). The bar-shaped samples were cut so that the dominant PDL collagen fibers were almost aligned along the length of the samples, i.e., parallel to the direction of mechanical testing. The entire cutting process took about 2 h. Immediately after preparation, all samples were stored at -20 °C for up to a week. On the day of testing, the bar-shaped samples were removed from the freezer and thawed to room temperature. A total of 12 defect-free samples were selected for DMA tests. The images of superior and inferior surfaces of each bar-shaped sample were taken with an optical microscope to determine the average initial length of PDL.

2.2. Test machine

The dynamic tensile tests of the bar-shaped samples were performed using DMA apparatus (TTDMA, Triton Technology, UK). The applied force in the DMA is calculated based on the input signal to the electro-magnet coil in the driver. The displacement is measured with a linear variable differential transformer (LVDT). The machine has a displacement resolution of 10 nm and a load reading of 0.25 mN. The bone and tooth components of each bar-shaped sample were held by the apparatus clamps.

2.3. Test procedure

Since assessing zero displacement point was proven to be inaccurate (Lomakin et al., 2014), loading profile was chosen to be "force-controlled" rather than "displacement-controlled" in this study.

2.3.1. Preconditioning

Preconditioning of biological tissues prior to testing is important as it returns the structural collagen fibers to physiological conditions (Viidik, 1990). In this study, in vitro preconditioning was performed on the samples by applying 10 cycles (1 Hz frequency, 5 N amplitude). The majority of earlier studies have used 1 Hz frequency (Bergomi et al., 2010; Sanctuary et al., 2005) and displacement amplitude of 35% of PDL's initial length (Bergomi et al., 2010; Shibata et al., 2006) in their preconditioning phase. This displacement level was almost equal to 5 N in our preliminary PDL rupture tests. Furthermore, the number of preconditioning cycles was selected based on preliminary mechanical tests. The results became repeatable after about 10 cycles. This number has also been reported in the literature from 10 to 30 cycles (Pini et al., 2004; Sanctuary et al., 2005).

2.3.2. Dynamic mechanical analysis

After preconditioning, each bar-shaped sample was subjected to sinusoidal tensile loads with an amplitude of 1 N and a preload of 3, 5 or 10 N. Each specimen was tested in 16 discrete frequencies ranging from 0.01 to 100 Hz. It has been shown that the frequency order does not change the results (Barnes et al., 2015). At each frequency, 5 tensile cycles were applied (Fig. 2a and b). Bergomi (2008) also stated that 3–5 cycles are sufficient to reach stable tensile response. Four samples per preload were tested. The rationale for the selection of these preloads was based on preliminary examinations of the stress–strain curves of the PDL indicated that 3 N preload lays in the toe region, 5 N preload in the boundary between the toe and the linear region, and 10 N preload in the linear region (Fig. 2a). Complex modulus (E^*), storage modulus (E'), loss modulus (E'') and loss factor ($\tan\delta$) were reported as functions of frequency and preload.

2.3.3. Analysis of data

During the test, tensile sinusoidal stress $\sigma-\sigma_m=\Delta\sigma\sin(\omega t)$ was applied on the samples with σ_m, $\Delta\sigma$ and ω denoting mean stress (zero amplitude stress in dynamic loadings), stress oscillation amplitude and angular frequency, respectively. The resulting sinusoidal strain was described as $\varepsilon-\varepsilon_m=\Delta\varepsilon\sin(\omega t-\delta)$ where ε_m, $\Delta\varepsilon$ and δ represent mean strain, strain oscillation amplitude and phase angle, respectively. In the above-mentioned relationships, $\Delta\sigma$ was calculated by dividing the force oscillation amplitude (ΔF) by the initial cross-sectional area of each

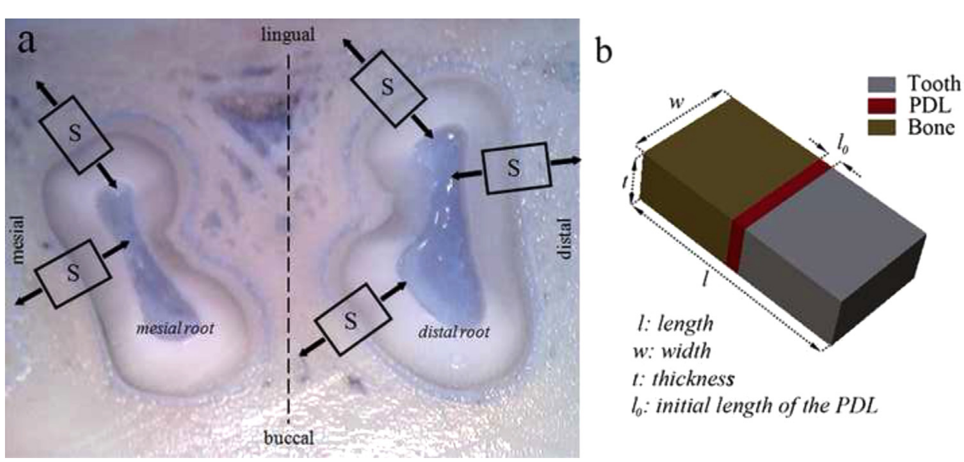

Fig. 1. (a) Photograph of a typical transverse section of the left molar and anatomical locations of the bar-shaped samples (S). Arrows aligned with the tension axis; (b) schematic view of the main sample composed of bone, PDL and tooth.