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# The time-dependent biomechanical behaviour of the periodontal ligament—an *in vitro* experimental study in minipig mandibular two-rooted premolars

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SUMMARY The aim of the present work was to evaluate the biomechanical behaviour of the periodontal ligament (PDL) with respect to force development with different controlled loading velocities. For this purpose, an *in vitro* experimental study was performed on 18 minipig jaw segments. Displacements with variable increasing loading time were applied to one premolar crown of each jaw segment into the linguobuccal direction through a force sensor provided by a specialized biomechanical set-up. The predefined displacement values to be achieved were 0.1 and 0.2 mm. Each of the given displacement increments was applied on the specimens with a linear displacement increase employing the following time spans: 5, 10, 20, 30, 60, 120, 300, 450, and 600 seconds. Force values were measured during load application to register force/displacement diagrams and after the maximum displacement was reached force decay was monitored for a period of 600 seconds. Force/time curves for each tooth were plotted according to the data obtained. Diagrams of the maximum force values obtained from these plots and the force at the end of each measurement were extracted for all teeth. Forces at the point when maximum displacement was reached ranged from 0.5 to 2.5 N for the 0.1 mm activation and showed extreme variation with the specimens. The factor of volume and surface area of the individual roots were evaluated and found not to be responsible for these deviations. A comparable behaviour was recorded for the 0.2 mm deflection, however, on a higher force level. The results show that the force development at different displacement velocities is complex and dominated by the PDL biomechanical characteristics.

## Introduction

The periodontal ligament (PDL) is a highly specialized connective tissue that fills the space between the tooth root and its alveolus. It consists of a fibrous stroma in a gel of ground substance containing cells, blood vessels, and nerves. The PDL stabilizes the tooth and supplies nutrients and repairs. It also harbours a variety of cell population that is able to stimulate periodontal regeneration meaning the formation of new bone, cementum, and connective tissue attachment (Berkovitz, 1990).

The PDL is the most deformable tissue in the periodontal system allowing for tooth mobility under functional loads. The protective function involves the transmission of occlusive and masticatory forces to the supporting structures of the tooth (Natali *et al.*, 2004). Due to the much lower stiffness of the PDL in comparison to the surrounding alveolar bone, the PDL plays a major role with respect to the initial tooth mobility (ITM), which is affected by the different components building up the PDL (Mühlemann, 1960). Apart from the biomechanical processes, during bone remodelling, the understanding of biomechanical phenomena accompanying the initial tooth displacement is the first step towards this direction (Cronau *et al.*, 2006).

When the force is released, the tooth returns to its original position. The return is caused by the restoring forces of the surrounding bone and soft tissue as well as the refilling of blood vessels and interstitual fluid (Wills *et al.*, 1972). After a period of fast restoration, a slow phase follows until the tooth returns to its original position.

However, the PDL also affects the long-term movement and its strain alterations regulate the cellular activity in the periodontal space involved in the alveolar bone remodelling processes (Roberts *et al.*, 2004). If the tooth deflection is maintained for a very long time, the stresses and strains in the PDL will trigger bone remodelling processes and result in a permanent change in the position of the tooth.

The viscoelastic behaviour of the PDL is attributed to the combination of fluid and elastic elements in its structure. The biomechanical characteristics of this tissue also involve non-linearity and time dependency and additionally, loading history influences its behaviour (Maurel *et al.*, 1998; Pini *et al.*, 2002).

The aim of the study was to evaluate the biomechanical time-dependent behaviour of the PDL in relation to tooth displacement with controlled loading velocities. Developed forces were measured while predefined maximum displacements at numerous controlled time spans were achieved. We assume to gain sufficient and validated data to better define a constitutive law of the PDL, especially including the time-dependant behaviour of the PDL for use in numerical finite element studies of force/displacement characteristics of teeth.

## Material and methods

The ITM was measured on a total of 18 deciduous premolars of 3- to 12-month old minipigs. Pigs are particularly suitable as experimental animals as they are omnivorous just like humans, have a similar masticatory cycle and also go through a phase of primary teeth with a development similar to the human one (Weaver *et al.*, 1962; Herring, 1976). Deciduous teeth were selected in order to collect the specimens from young healthy animals as the material properties which concern our study do not exhibit variations among deciduous and permanent teeth as long as they are taken from the same animal species (Bourauel *et al.*, 2006; Natali *et al.*, 2007; Slomka *et al.*, 2008).

Fresh pig mandibles were collected immediately after the animals were killed and were directly transferred to a refrigerated container until freezing to −28°C in order to avoid alterations of their mechanical properties (Thomas and Gresham, 1963). They were subsequently radiographed in order to choose teeth with no root resorption radiographically observed (Figure 1). The second and third premolars of the fresh pig jaw segments were chosen as their root geometries resemble the root shape of human M1 and M2. After the selection of the suitable premolar, the mandible was segmented. Each jaw segment consisted of the premolar, its PDL intact, and surrounding alveolar bone. The specimen width was determined using as guiding lines for the segmentation the half of the mesiodistal width of the crown of the adjacent teeth. The height was determined with respect to the anatomy of the mandible placing the borders of the segment always inside the cancellous bone region and involving as much cancellous bone region as possible. Attention was paid so that the PDL of the tooth to be examined would not be injured and no contact points between adjacent teeth would exist. The jaw segments were kept frozen under −28°C.

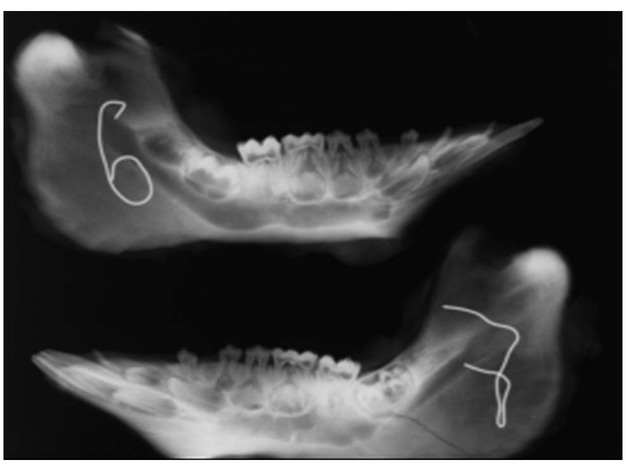


**Figure 1** All mandibles selected were marked and radiographed to examine the suitability of the premolars used in the experimental set-up.

Each time the measurement took place, the jaw segments were placed in envelopes soaked with 0.9 per cent NaCl to avoid drying out and alteration of the biomechanical properties of the PDL and the bone. The lower portion of each bone segment was placed in a metal carrier and embedded in resin (Technovit 4004, Kulzer, Germany). The preparation embedded in resin to less than the lower third of the bone height was provided with a hollow in the centre of the cervical third of the tooth crown on its lingual side (Figure 2A). The specimens were embedded such that the point of force application on the tooth's crown was always similar with respect to the distance of the centre of resistance (CR) of the respective tooth. The position of the CR was determined from the x-rays and was assumed to be at 40 per cent of the root length down to the apex, seen from the alveolar crest.

The measured displacements are combined displacements resulting from tooth deformation, PDL deformation, bone deformation, and resin deformation. We decided to assume rigid constrains of the bone base as from the material parameters, especially of the PDL and the bone, and the overall geometry of the specimens, the deflection of the resin is far less than 1 per cent of the tooth. Thus, we assume to basically measure tooth deflection and bone deformation, which will be separated in finite element simulations.

The measurements were carried out at room temperature using the biomechanical Hexapod Measurement System (HexMeS, Figure 2B; Keilig *et al.*, 2004). The set-up consists of a hexapod positioning system (Hexapod M850, Physik Instrumente, Karlsruhe, Germany), a high-resolution three-dimensional (3D) force torque transducer (FTS Nano 12/120, Schunck, Lauffen/Neckar), and a 3D optical displacement measurement system (3 cameras JAI CV-M1; Stemmer Imaging, Puchheim, Germany). Loads can be applied via a fine tip to the specimen (Figure 2B and 2C) by moving the Hexapod with the force sensor mounted on it. Specimens were positioned in the experimental set-up at a 90 degrees angle to the power arm of the force/torque sensor (Figure 2C). A lingually positioned tip was used to transmit pure continuously increasing displacements into the linguobuccal direction at the position of the hollow on the tooth's crown. The type of movement simulated was uncontrolled tipping.

Subsequent linear increasing displacements from 0.0 to 0.1 mm and 0.0–0.2 mm, respectively, within predefined activation times of 5, 10, 20, 30, 60, 120, 300, 450, and

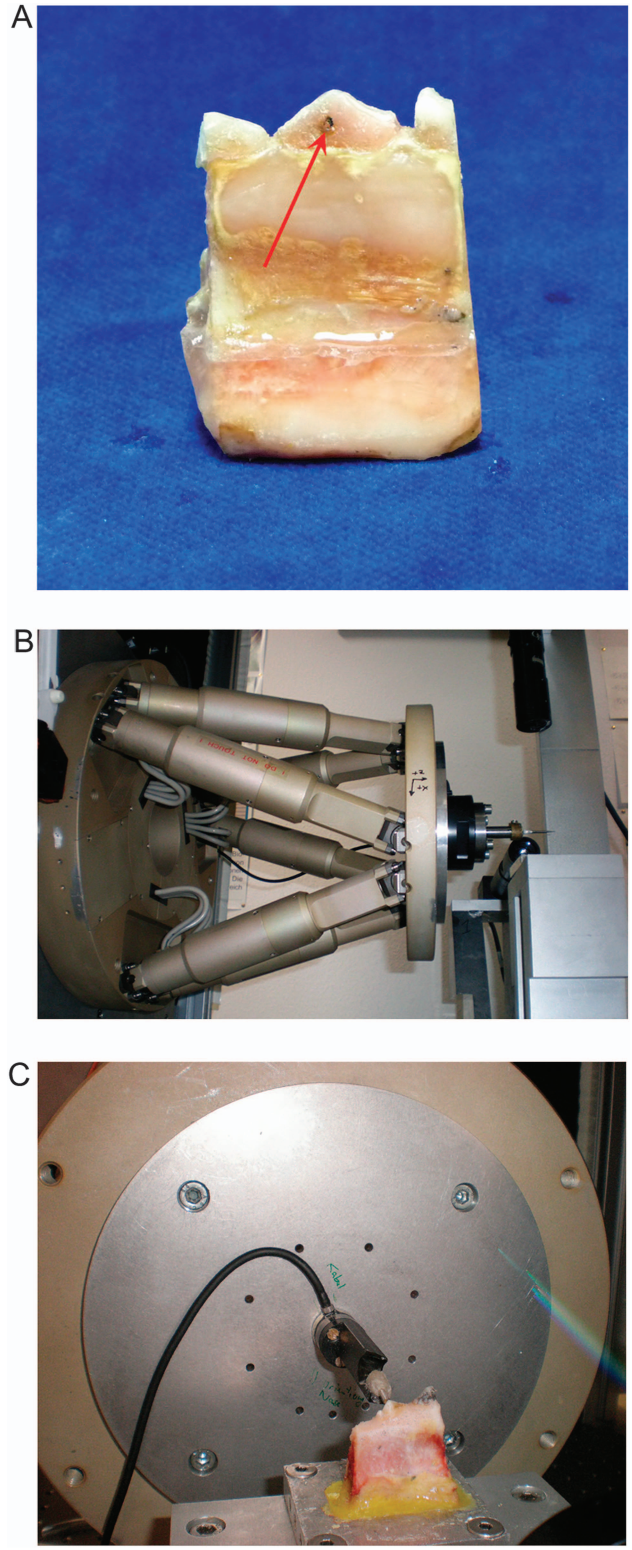


**Figure 2** (A) The specimens used were carefully segmented and separated from the jaw. Each jaw segment consisted of the premolar, its PDL intact, and surrounding alveolar bone. Forces were applied to the centre of the crown (arrow). (B) The experimental study was conducted using the Hexapod Measurement System, a biomechanical set-up especially designed to measure force/deflection characteristics of different dental materials and devices. (C) A specimen mounted in the set-up.

600 seconds were applied to the premolar crowns. Resulting forces were measured using the 3D force torque sensor during the loading phase up to maximum displacement as well as for a period of 600 seconds after reaching full deflection of 0.1 and 0.2 mm, respectively. Following displacement release, a decay time of 15 minutes was introduced between two measurements to allow a complete relaxation of the PDL in order to return to its original position and fully relaxed state. Each set of measurements for every specimen took about 9 hours to be completed. A total measurement cycle for each specimen took place in 1 day every time. After the procedure, the specimens were restored in the refrigerator under −28°C in order to maintain the biomechanical properties of the tissues comprising the specimen for future micro computerized tomography scanning and numerical analysis.

Time/force diagrams for each tooth and each time/deflection combination were generated based on the values measured. Maximum developed forces at maximum deflection as well as forces after relaxation were obtained. The intraosseous root volume and root surface of each premolar were also calculated using the formula of an idealized paraboloid geometry (Bronstein *et al.*, 2007) after estimating root length and diameter through the existing radiographs in order to determine whether serious deviations in root geometry existed that could influence the results. Root length and diameter were determined using the x-ray scans and a Spearman rank correlation test was performed to test the significance of root geometric parameters.

## Results

The applied displacement and the corresponding measured force acting on the tooth with regard to time is shown in Figure 3 for a selected specimen and displacements of 0.1 (Figure 3A) and 0.2 mm (Figure 3B) with an activation time of 20 seconds. The initial part of the loading curves is the displacement/force increase within the first 20 seconds until the total displacements of 0.1 (Figure 3A) and 0.2 mm (Figure 3B) were reached. Subsequently, the displacements were kept constant and the force decay was registered over a total of 600 seconds. The maximum force was clearly higher for the 0.2 mm displacement (2.85 N compared to 0.61 N). A comparable behaviour was registered for all the other specimens, however, with extreme variations in the maximum forces.

Figure 4A depicts the maximum forces for the individual specimens at a deflection of 0.1 mm and the different loading times; Figure 4B is the diagram of the respective forces after the full relaxation period of 600 seconds. Each curve and data point represents the force of an individual specimen. Maximum forces vary from 0.45 to 2.50 N (loading time: 5 seconds) and decrease to 0.05–0.60 N at a loading time of 600 seconds. Load magnitudes rise dramatically when the applied displacement is increased to 0.2 mm (not shown).

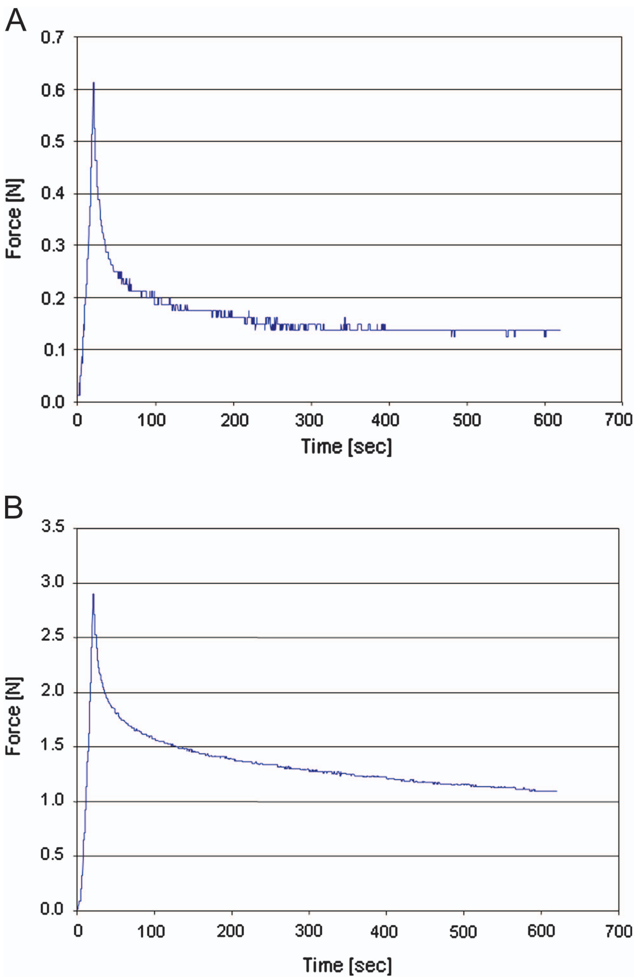


**Figure 3** (A) Force/time diagram of a load acting on specimen number 6 for a displacement of 0.1 mm applied within 20 seconds. After the peak force is reached, the force decay is registered of further 600 seconds. (B) Same specimen as in Figure 3A with an applied displacement of 0.2 mm at same loading rate of 20 seconds. The peak force is clearly increased.

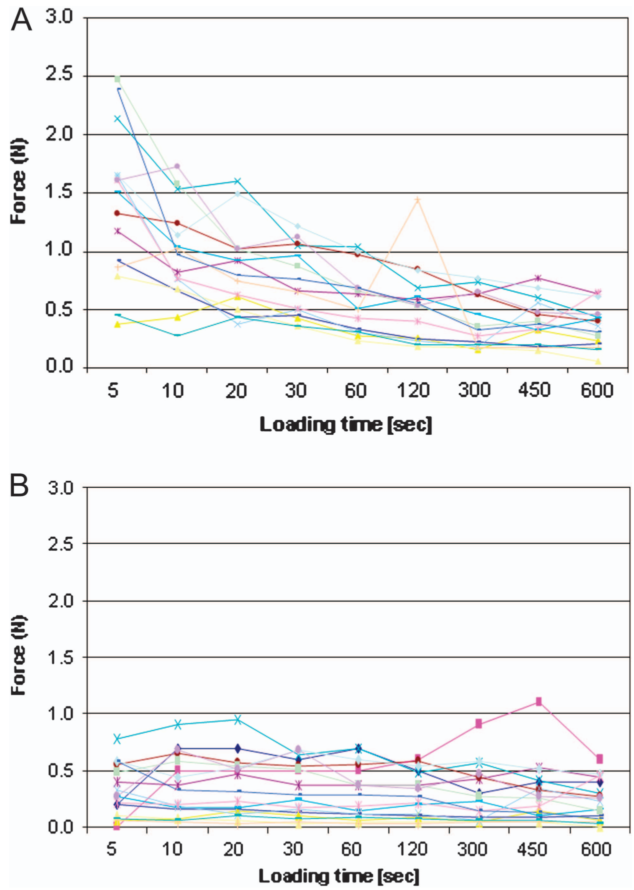


**Figure 4** (A) Maximum forces of all specimens at 0.1 mm displacement with all different time spans examined. Each curve stands for an individual specimen. An extreme variation of maximum forces is obvious; however, a common boundary value seems to exist around 0.40 N. (B) Force values at the end of the relaxation phase for the same displacement (0.1 mm) for all specimens and loading times. Except for several outliers, forces for all specimens decay to a limit between 0.16 and 0.46 N.

The force relaxation shown in Figure 4B concretes that forces decay towards a constant level for each individual specimen, independent of the loading time, i.e. loading times of 5 and 600 seconds result in, for example, a final force value of 0.05–0.50 N, provided the relaxation period is sufficiently long.

Means and standard deviations calculated from the results of all specimens (Figure 4A) for each deflection/time combination on maximum load values are depicted in Figure 5A, taking the 0.1 mm deflection as an example. Due to the extreme force variations between specimens, errors of the means are up to 50 per cent or even more. With increasing loading time, it was observed that the maximum forces decreased clearly. Mean values are 1.40 and 0.36 N for 5 and 600 seconds for 0.1 mm displacement, respectively. Forces after relaxation converged to boundary values of 0.20 N (Figure 5B). For the 0.2 mm displacement, the maximum force values increased and reached 4.38 and 2.06 N for 5 and 600 seconds, respectively (Figure 6A). Forces after relaxation converged to boundary values of 1.14 N (Figure 6B).

In order to estimate the impact of the root volume and surface area on the presented results, the root surface and volume were calculated using an idealized paraboloid geometry formula (Bronstein *et al.*, 2007). The Spearman rank correlation test did not show any significant correlation of forces with root volumes or root surfaces.

## Discussion

The tests on loading of the PDL for multiple controlled velocities demonstrate a clear dependence of the material properties of the PDL with the loading rate since maximum force varies according to the loading rate. The displacement of interstitial fluid takes place in the initial phase of tooth mobility (Mühlemann, 1954; Mühlemann and Zander, 1954; Andersen *et al.*, 1991; Nishihira *et al.*, 1996; Van Driel *et al.*, 2000; Yoshida *et al.*, 2001).

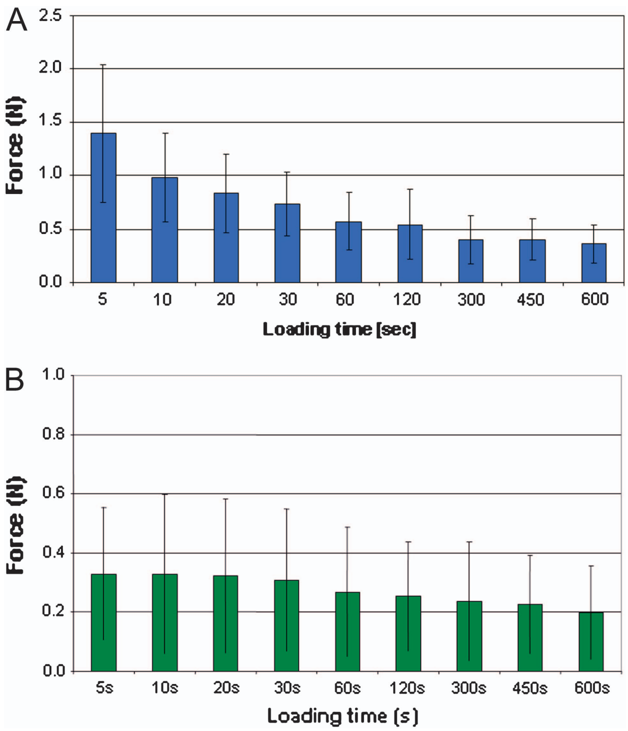


**Figure 5** (A) Diagram representing the mean values and standard deviations (SDs) obtained for the maximum forces at 0.1 mm displacement taken from Figure 4A. (B) Diagram representing the mean values and SDs obtained for the forces at 0.1 mm displacement after the relaxation period of 600 seconds taken from Figure 4B.

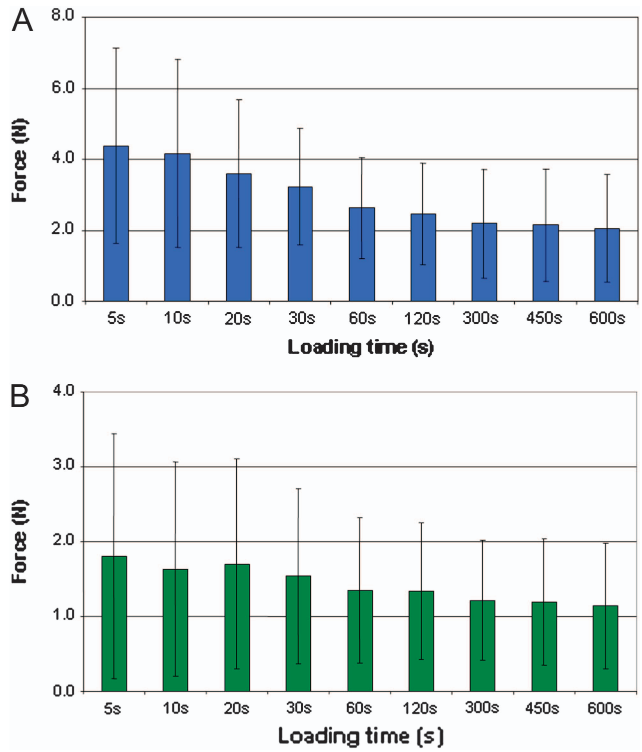


**Figure 6** (A) Diagram representing the mean values and standard deviations (SDs) obtained for the maximum forces at 0.2 mm displacement. (B) Diagram representing the mean values and SDs obtained for the forces at 0.2 mm displacement after the relaxation period of 600 seconds.

Research results postulate that the components of the fluid phase show reduced mobility with increased loading rates. Thus, the specimen then exhibits a higher stiffness (Wills *et al.*, 1972, 1976; Picton, 1990). The impact of the fluid-phase components on the material biomechanical behaviour of the PDL was studied experimentally *in vivo* by Mühlemann (1954), demonstrating the time-dependent behaviour of the PDL. Mühlemann and Zander (1954) observed, while applying load on teeth in a horizontal direction, that tooth displacement is not linearly related to the magnitude of the force applied. It was shown that tooth mobility is divided into ITM and secondary tooth mobility. The ITM pattern of movement consists of a high displacement of the tooth while applying small forces up to 1 N. It can be assumed that this corresponded to movement of the root within the PDL space. With higher force application, tooth movement progresses slowly. Although this was an *in vivo* study, the exhibited behaviour of the PDL is qualitatively similar to our study. Van Driel *et al.* (2000) also conducted *in vivo* studies on the time-dependent mechanical behaviour of the PDL concluding that its fluid component has a significant role in the transmission and damping of forces acting on teeth.

When the force is released, the tooth will return to its original position. This is attributed to the restoring forces of the surrounding bone and soft tissue, as well as the refilling of blood vessels and interstitial fluid (Parfitt, 1961; Körber, 1971; Wills *et al.*, 1972).

Measurement techniques of ITM include magnetic sensors (Yoshida *et al.*, 2001, 2000), strain gauge techniques (Pedersen *et al.*, 1991), laser measurements (Castellini *et al.*, 1998; Hinterkausen *et al.*, 1998; Kawarizadeh *et al.*, 2003), or non-contact optical measurement technique (Göllner *et al.*, 2010).

Applying small forces, the initial type of tooth mobility is most likely a tipping around an axis of rotation inside the PDL space (Natali *et al.*, 2004). With the application of forces of higher magnitude, the PDL is compressed and a continuing force increase leads to a displacement of both the alveolar bone and the tooth (Poppe *et al.*, 2002; Kawarizadeh *et al.*, 2003; Sanctuary *et al.*, 2005). In our study, we apply low initial forces only.

Deformation patterns in the entire periodontium are strongly dependent on the geometrical profiles and material properties of the individual elements that constitute the periodontal tissue including the PDL configuration. The PDL shows a non-linear viscoelastic behaviour which regulates tooth movement as well as deformation fields of the whole periodontium (Picton, 1990; Frost, 1992; Hinterkausen *et al.*, 1998; Toms *et al.*, 2002a,b; Komatsu *et al.*, 2004).

The viscoelastic behaviour of the PDL is known since longer time to be closely related to its fluid phase and wavy configuration of the collagen fibres (Shackleford, 1971; Wills *et al.*, 1972). The internal fibre structure and orientation and therefore biomechanical properties of the PDL vary among different teeth, different mandibles, different animals, or human beings. In the present study, specimens of minipig mandibular two-rooted premolars were investigated. The studied mandible specimens were carefully mechanically sliced and prepared before testing and therefore variation of initial force magnitudes can be attributed to the individual biological characteristics among specimens and different states of dental development of the animals.

Direct measurement of the properties of the PDL is an invasive technique and very complex due to the alterations in blood flow and subsequent differentiation of the tissue response to load. There are however several *in vivo* studies conducted to investigate the PDL behaviour (Mühlemann and Zander, 1954; Göz *et al.*, 1992; Tanne *et al.*, 1995; Van Driel *et al.*, 2000; Yoshida *et al.*, 2001; Kawarizadeh *et al.*, 2003; Cronau *et al.*, 2006).

It has been suggested by several authors that the root surface and volume have a marked influence on tooth mobility (Tanne and Sakuda, 1983; Tanne *et al.*, 1991; Geramy, 2000). Therefore, experimental data should be combined with numerical results for reliability valuation of the proposed model. Several researchers have used only numerical data to propose a constitutive model of the PDL (Middleton *et al.*, 1990, 1996; Toms and Eberhardt, 2003; Natali *et al.*, 2004; Field *et al.*, 2009). Other studies provide only experimental data (Pilon *et al.*, 1996; Pini *et al.*, 2002). Andersen *et al.* (1991) were the first to conduct combined experimental and numerical studies of material parameters and stress profiles within the PDL. Other combined experimental and numerical studies towards the investigation of the biomechanical behaviour of the PDL were conducted by Van Driel *et al.* (2000), Natali *et al.* (2007), Qian *et al.* (2009), Sanctuary *et al.* (2005), and Vollmer *et al.* (1997). Numerical model analysis helps to demonstrate and interpret readily the biomechanical response of tooth and surrounding structure. Our experimental data of this *in vitro* study will be taken into account as reference data in the numerical study, which is needed for further evaluation. A good agreement among the experimental and numerical data is an index of the reliability of the proposed model.

## Conclusions

1. The time-dependent viscoelasticity of the PDL under load is confirmed.
2. During the relaxation period, the load decreased noticeably demonstrating clearly the viscoelasticity of the tissues of the specimens.
3. With increasing loading time, it was observed that the maximum force values decreased.
4. The initial force value deviations observed can be attributed to the biological tissue characteristics of the respective specimen and probably to the different morphology of the alveolar bone around the root of the teeth and the different states of dental development of the animals.

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