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Experimental and numerical determination of initial tooth mobility and material properties of the periodontal ligament in rat molar specimens

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SUMMARY The mechanical parameters of the periodontal ligament (PDL) in rat specimens were investigated in a combined experimental and numerical approach. Tooth mobility of the rat mandibular first molar was measured *in vitro* using a high precision experimental set-up. Finite element models (FEM) were developed, based on histological sections of the measured specimens, to simulate tooth mobility numerically under the same force systems as used in the experiment. Force/deflection curves from the measurements showed a significant non-linear behaviour of elastic stiffness of the PDL. A bilinear material parameter set was assumed to simulate tooth deflections. The numerical force/deflection curves were fitted to the experimental curves by repeatedly calculating theoretical tooth deflections and varying the parameters describing the non-linearity. Mean values of E_1 = 0.15 MPa, E_2 = 0.60 MPa and an ultimate strain of ε_{12} = 6.3 per cent were derived for the elastic behaviour of the rat PDL. Comparing fresh specimens and those frozen in a 0.9 per cent saline solution, differences between the measurements were significant. Using the agent, Periston, for freezing significantly reduced the deviation. The results indicated that strains in the PDL with a maximum of 14 per cent at the furcation were 10^4 times higher than strains in the bone, while the variability of stress values in both PDL and bone was not significant.

Introduction

In orthodontic practice, teeth are moved within the alveolar bone. This movement is based on the ability of the surrounding bone and the periodontal ligament (PDL) to react to a mechanical stimulus with remodelling processes. Application of an orthodontic force system to a tooth causes displacement of the root within its alveolus. Maintaining the force for a short time, the tooth returns to its pre-loaded position. This process is called initial tooth mobility (Mühlemann and Zander, 1954), which causes stresses and strains in the involved structures. It is believed that stresses and strains induced in the PDL by an orthodontic force system are the appropriate mechanical signals initiating orthodontic bone remodelling processes (Storey, 1973; Davidovitch and Shanfeld, 1975; Davidovitch *et al.*, 1980; Roberts and Chase, 1981; Reitan and Vanarsdall, 1994; Melsen, 2001).

The study of mechanical stress/strain distributions requires knowledge of the material properties of all involved structures. The material properties of bone and tooth can be found in the literature (see, for example, Abé *et al.*, 1996). Although the interdependence between bone density, orientation and material properties is not yet completely clarified, the parameters show minor variations. However, a great variety of values for Young's modulus of the PDL can be found, with values ranging from 0.07 MPa (Andersen *et al.*, 1991) to 1379 MPa (Thresher and Saito, 1973). These differences can be explained in part by the use of different methods, the underlying various assumptions for the mechanical behaviour of the PDL and the complexity of the involved tissues.

The finite element (FE) method is a useful tool for solving mechanical structural problems. Using the finite element method, structures of a modelled object are discretized into a large number of elements which are connected by nodes. The first step in performing a FE analysis is the generation of the element mesh, representing the structures. This plays a crucial role, determining the quality and accuracy of the numerical analysis. To date there is no report on the experimental determination of initial tooth mobility in rat molars under controlled force systems, and only one numerical study reports on the use of a two-dimensional finite element model (FEM) of a rat molar in association with bone remodelling (Katona *et al.*, 1995). On the other hand, in many studies evaluating the biological mechanisms of orthodontic tooth movement, rats are used for the experiments.

It was, therefore, the aim of this study to develop a three-dimensional (3D) FEM of the rat mandibular first molar in order to:

1. calculate initial tooth mobility under orthodontic force systems;
2. determine the elastic properties of the PDL using a combined experimental and numerical approach; and

3. display the stress and strain pattern in the surrounding tissues associated with the tooth displacement.

Furthermore, the method of intermediate storage of the specimens was investigated to determine whether frozen specimens are suitable for later combined numerical and experimental investigations.

Materials and methods

Twelve Wistar rats, 8–15 weeks of age and 200–300 g in weight, were used to measure the mobility of the first molar using the mobility measurement system (MOMS), described in detail previously (Hinterkausen *et al.*, 1998; Vollmer *et al.*, 2000). The rats were used for animal experiments in basic medical research in the Department of Experimental Surgery of the University of Bonn, ensuring that all experiments comply with ethical guidelines. After experimentation, the rats were anaesthetized with ether, decapitated and the mandibles were removed and dissected into right and left segments. The soft tissues around the jaw bone, except the gingiva propria, were removed. The basal part of the right mandible was mounted immediately into a socket made of a self-curing resin (Technovit® 4004, Kulzer, Germany). To avoid drying of the tissues during polymerization and later during measuring, free surfaces were coated with moistened cellulose using 0.9 per cent saline solution. The left mandibles were randomly assigned to two groups. The first group was frozen in saline solution and the second group was frozen in Periston (NaCl 0.9 per cent + 6 per cent polyvinylpyrrolidon) in an airtight container at –20°C for up to 3 days. The frozen specimens were slowly thawed at room temperature and then measured.

The combined experimental and numerical approach is shown schematically in Figure 1. The MOMS consists of a laser–optical component to measure tooth deflections non-invasively, and a mechanical component to apply and register a force system on the tooth. In view of the very small size of a rat molar (height approximately 4.0 mm), measurement of tooth mobility was difficult. Therefore, a resin-based adapter (Palapress®, Kulzer) was constructed to transfer the force to the tooth (Figure 2). After the occlusal surfaces of the mandibular first molar were prepared by grinding a hole with a dental diamond burr, etching with phosphoric acid (Esticid®-Gel, 35 per cent, Kulzer) for 60 seconds and treating with bonding material (Syntac®, single component, Vivadent, Liechtenstein), the adapters were fixed to the tooth crown with a light-curing restorative composite material (Tetric®, Vivadent). Three right-angled laser diodes were mounted on the adapter and fixed with a fast-hardening adhesive (Loctite® 496, Loctite, Germany).

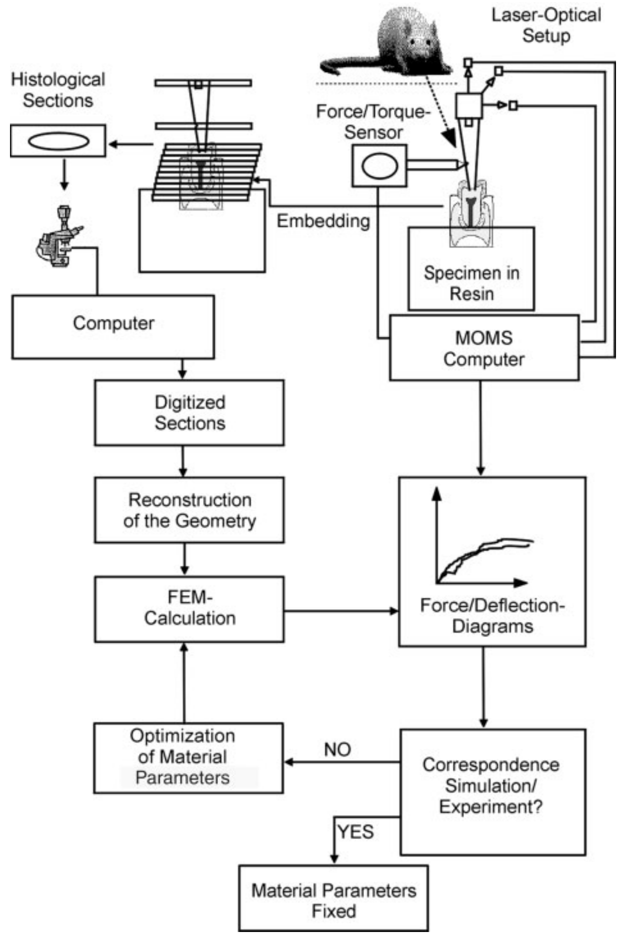

Figure 1 The combined experimental and numerical approach used to determine the material parameters of the periodontal ligament in rat specimens.

Forces of up to 0.15 N in a linguo-buccal direction were applied stepwise to the adapter and thereby to the tooth crown. The force system was applied and measured using a 3D force/torque transducer, mounted on to a set of translatory stages in rectangular arrangement. By moving the transducer stepwise into the corresponding direction, loads were applied to the tooth by a fine tip. The laser beams were focused on to the surfaces of three position-sensitive detectors, and deflections were registered, amplified and transferred to a control computer via an A/D converter board (ADLINK Technology, Taipei, Taiwan). The data collected were subsequently shown as force/deflection curves. An idle time of 40 seconds and an increment of 1 μm between two load steps was chosen to reduce the influence of hydrodynamic flow processes in the PDL. This resulted in approximately 150 steps per measurement, which lasted approximately 2 hours. The accuracy of the laser–optical component has been confirmed to be 10 μm and 0.02 degrees for the registration of tooth mobilities (Hinterkausen *et al.*, 1998). The 3D force/torque transducer

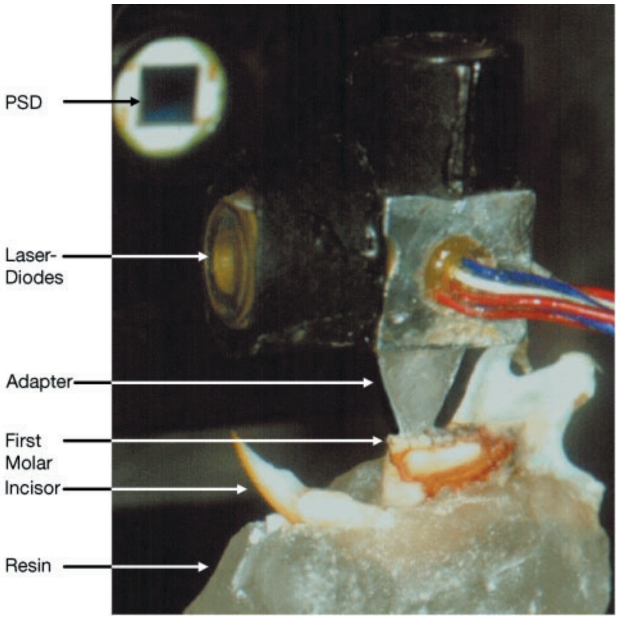

Figure 2 The laser–optical set-up with the segment of a rat's mandible used for experimentation.

used (ATI, Industrial Automation, Garner, NC, USA) has a resolution of 0.0125 N for forces and 0.0625 Nmm for torques.

After measuring the force/deflection curves, the specimens were embedded in resin (Technovit® 4004) for histological processing. Parallel horizontal sections were prepared using a precision inner diameter saw (Microtom 1600, Leitz, Wetzlar, Germany) every 300 µm with a material loss of 200 µm in each section. Ten sections (eight from the tooth and surrounding tissues and two from the resin adapter) were digitized using a scanner camera (Pentacon, Dresden, Germany) mounted on a microscope (Wild-Heerbrugg, Heerbrugg, Switzerland). The scanned sections were processed using the software CAGOG (computer-aided generator for orthodontic geometries), a program specially developed for the reconstruction of tooth geometries (Haase, 1996). Using CAGOG, structures could be identified semi-automatically. Thus, several sets of contours were determined in different planes. The contours were fitted to the outlines of the anatomical structures (Figure 3). Previous studies have indicated that a differentiation between enamel and dentine on the one side and cortical and cancellous bone on the other does not have a decisive influence on the calculated tooth mobility (Bourauel *et al.*, 1999; Vollmer *et al.*, 1999). The reason for this behaviour can be seen in the solid structure and the relatively high Young's modulus of tooth and bone compared with the PDL (see Table 1). To reduce calculation times, the structures were thus subdivided into tooth, PDL and surrounding alveolar bone.

Table 1 Mechanical parameters of tooth, periodontium and bone used in the study [the values for bone and tooth were taken from Abé *et al.* (1996) and Hall *et al.* (1973)].

Material	Young's modulus (MPa)	Poisson's ratio µ
Tooth (mean)	20 000	0.30
Enamel	79 000	0.30
Dentine	18 000	0.30
Bone (mean)	2000	0.30
Cortical bone	20 000	0.30
Cancellous bone	100–3000	0.30
Periodontal ligament	Bilinear elastic	0.30
Adapter (resin)	5000	0.30

CAGOG has been designed to reconstruct single- or two-rooted teeth. The rat lower first molar has four roots. Therefore, the two small lateral roots were modelled as processes to the distal root (cf. Figure 3). The quadrangular meshes were processed using the finite element package COSMOS/M (version 2.6, SRAC, Los Angeles, CA, USA) to reconstruct the 3D geometry of the specimens. The models were generated with eight-noded isoparametric volume elements. Figure 4 shows a resulting FEM consisting of approximately 5700 elements and about 6400 nodes. The conventions of the co-ordinate system are indicated. A FE analysis was performed on these realistic models using the measured force systems from the corresponding experiments, and the basic initial material properties listed in Table 1. The structures of tooth and bone were modelled as being homogenous and isotropic. The corresponding mean values for a combined elastic behaviour of dentine/enamel and cortical/cancellous bone can also be taken from Table 1.

Results

Material parameters

In the experiment, a single force applied in the X-direction resulted in a translation in the X-direction (Tx) and a rotation around the Y-axis (Ry). The quantity of other deflections (translation in the Y- or Z-direction and rotation around the X- and Z-axis) was negligible. The reliability of the method of measuring tooth mobilities was determined by estimating the error of a single measurement. The error was assessed to be a maximum of 40 per cent of the main deflection values (Tx and Ry being in the range of 0.15 mm and 0.7 degrees, respectively).

Figure 5 shows the measured force/deflection, which may be described as bilinear behaviour with two different Young's moduli of elasticity of the PDL: in the initial phase there is a low modulus (E_1) resulting in high mobility and in the second phase a high modulus (E_2) reduces the mobility. Both linear regimes are

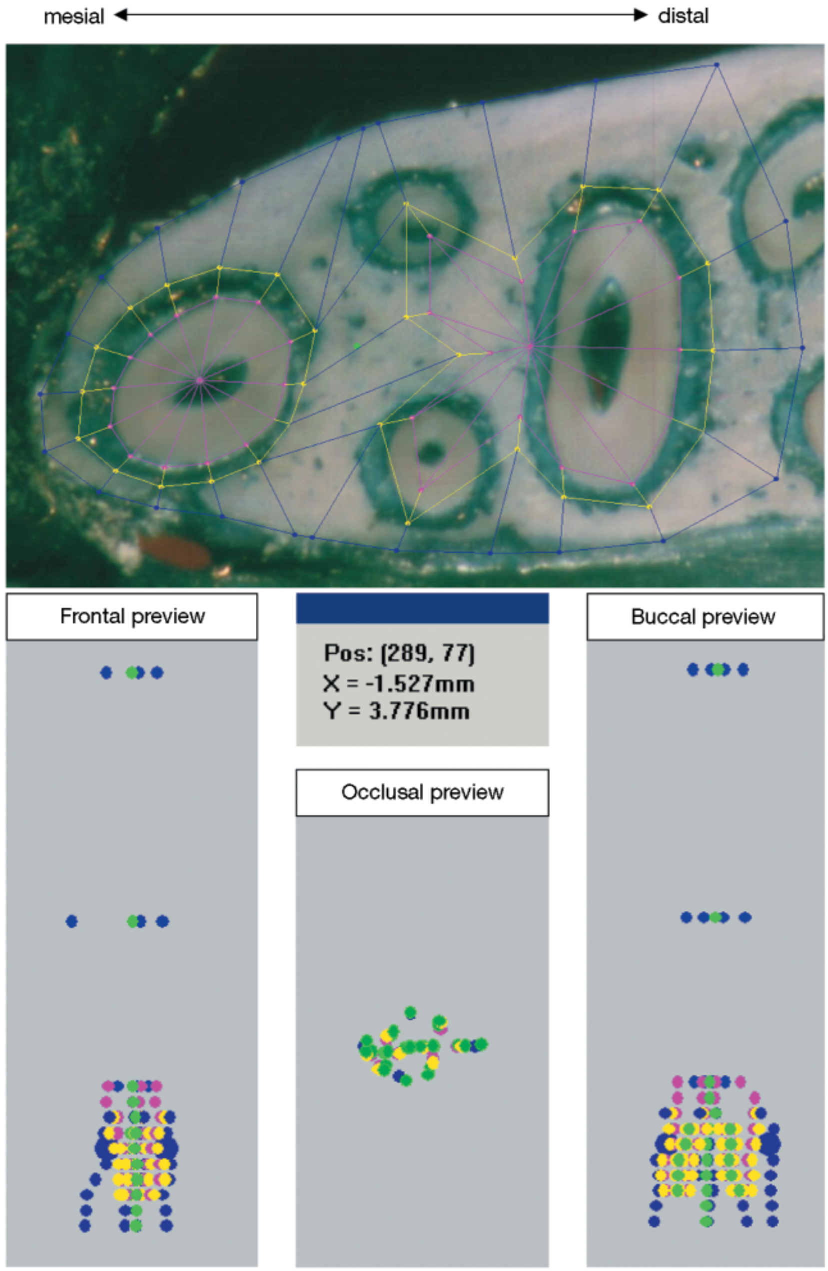

Figure 3 Polygons fitted to the outlines of the anatomical structures in transverse sections taken above the furcation of a rat's mandibular first molar.

separated by an ultimate strain ε_{12}. Therefore, a bilinear parameter set for the material behaviour of the PDL was assumed to simulate the measured curves.

In a first approach, the bilinear elasticity parameters of the PDL determined for pig specimens (Siebers, 1999; Vollmer *et al.*, 2000) were chosen for the numerical analysis of the rat models. The determined Young's moduli and the ultimate strain were varied to fit the calculated results to the measured curves (Figure 6). Achieving a good congruence of both measured and calculated curves, the chosen parameter set was taken as the final result for the specimen under investigation. A mean was calculated for the fitted material parameters of the PDL of all 12 specimens (Table 2). Figure 7 displays this behaviour in a stress/strain diagram.

Influence of freezing

In contrast to measurements of pig specimens (Haase, 1996), clear differences were observed between fresh rat specimens and those frozen in a physiological saline solution. Assuming that the crystallization process during freezing may damage the periodontium, Periston was tested as an alternative; the crystallization process is reduced by admixing polyvinylpyrrolidon to a saline solution (Böck, 1989). Investigations using Periston for freezing showed lower deviations from the results of fresh specimens in comparison with the measurements using pure saline solution (Table 3).

Stress/strain distributions

The stresses and strains in the tissues associated with initial tooth displacement were investigated using the validated FEM and the determined parameter set for the elastic behaviour of the PDL. Figure 8 shows the stress distribution in the PDL and the bone in response to a mesializing force (uncontrolled tipping). Major differences in the values of calculated stresses were not observed between the bone and the PDL. The magnitude of the von Mises stress in the PDL was greatest at the furcation, reaching a peak value of 0.065 MPa at a force level of 0.1 N. The highest stresses in the bone were about 0.144 MPa at the same location.

In the PDL, in the region of the furcation, strains were also significantly higher than in other regions. In this region, strains of up to 14 per cent (0.14) were calculated, whereas the calculated strains in the bone in the same region were as low as 0.001 per cent (10^{-5}, Figure 9). Additionally, loads due to the mesializing force resulted in strains that were concentrated in the PDL around the apex of the mesial root and at the alveolar crest, reaching a maximum of 8 per cent (0.08). The strain patterns in the bone in these regions, as well as in the tooth, approached zero.

Discussion

A review of the literature underlines uncertainties concerning the material properties of the PDL (Table 4). Not only different concepts about material non-linearity or linearity, anisotropy and inhomogeneity, but also viscoelastic behaviour or hydrodynamic flow processes occurring in the PDL have been discussed controversially. In most studies, a linear behaviour was assumed and a single value for Young's modulus was determined. Most

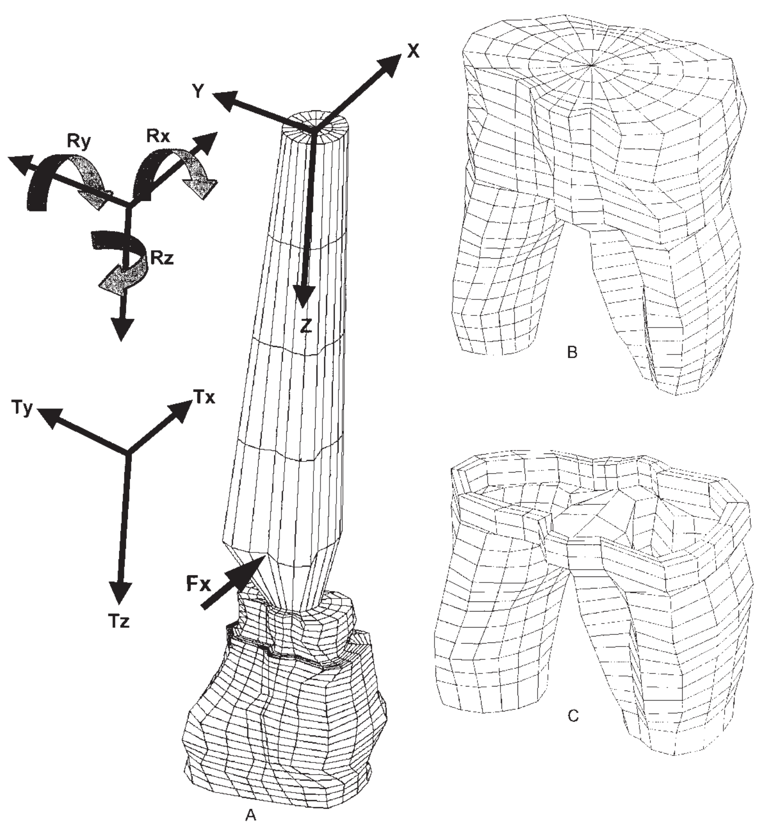

Figure 4 (a) The finite element mesh of a rat's mandibular first molar and supporting tissues. For the point of force application, the resin-based adapter, fixed to the crown, was also modelled. The co-ordinate conventions for translations and rotations are shown on the left; arrowheads mark positive directions. Fx, applied force. (b) A model of the molar without the periodontal ligament and bone. (c) A model of the periodontal ligament.

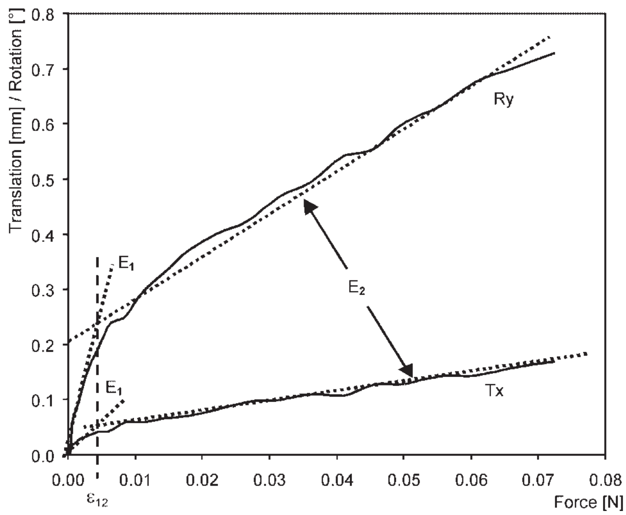

Figure 5 The measured deflection/force curves indicate approximately bilinear behaviour of the periodontal ligament.

Table 2 Material properties of the periodontal ligament of all specimens determined by fitting theoretical curves to experimental curves.

Specimen	E_1 (MPa)	E_2 (MPa)	ε_{12} %
a	0.07	0.40	8
b	0.22	0.45	5
c	0.10	0.35	4
d	0.09	0.45	8
e	0.20	0.50	12
f	0.15	0.80	8
g	0.08	0.72	4
h	0.15	0.80	5
i	0.15	0.50	6
j	0.25	0.80	6
k	0.25	0.80	6
l	0.09	0.65	4
Mean (standard deviation)	0.15 (0.07)	0.60 (0.18)	6.3 (1.7)

E, elastic (Young's) modulus; ε_{12}, ultimate strain.

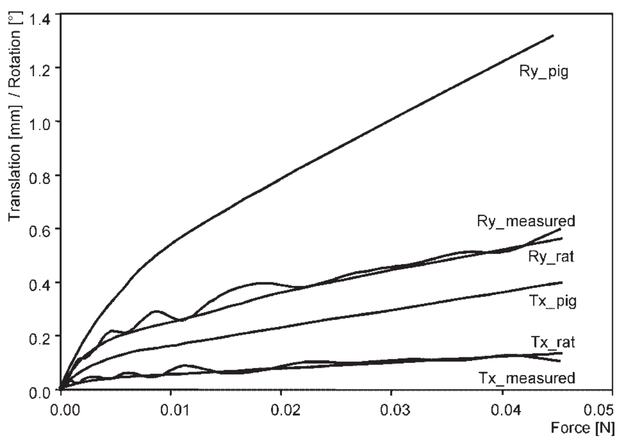

Figure 6 Fit of the calculated translation (Tx) and rotation (Ry) to the measured curves based on bilinear mechanical behaviour of the periodontal ligament. The first calculation was performed with a parameter set for pig specimens (denoted pig), the second one with an optimized parameter set for rat specimens (denoted rat).

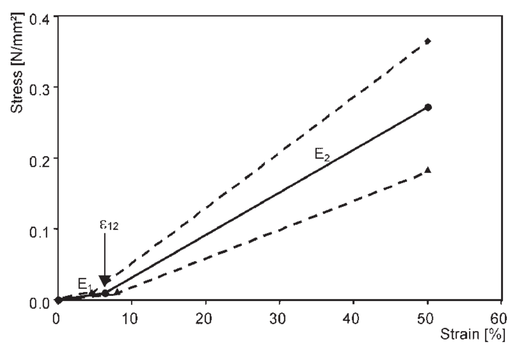

Figure 7 A schematic stress/strain diagram depicting idealized behaviour of the periodontal ligament. The dotted lines illustrate the error range, based on the standard deviations.

of these studies have described the PDL as incompressible and have used a value of 0.45–0.49 for Poisson's ratio. In this study, a purely material elastic behaviour in combination with a compressibility of the PDL was assumed and a Poisson's ratio of 0.3 was used, which resulted in independence of the calculated tooth mobilities from the value of Poisson's ratio (Vollmer *et al.*, 2000). The assumption of compressibility of the PDL is equivalent with the decay of all flow processes in the PDL, which in the case of orthodontics is ensured by long-term force application. In the experiment, this was simulated by very low force rates of about 40×10^{-6} N/second.

In the most frequently cited study (Tanne *et al.*, 1987), average anatomic data were used to create a human premolar. The results of their finite element analysis were compared with the *in vivo* measured tooth mobility in previous investigations. Using a relatively high value of force for an incisor (1 N), they found a mean value of 0.68 MPa, which is close to the second value (E_2 = 0.60 MPa) determined in this study. In a more recent investigation, Tanne *et al.* (1995) described a non-linear behaviour of tooth mobility according to varying force levels between 0 and 5 N. However, they did not report the measurements of the material properties. Using a magnet-magnetic sensing system, Yoshida *et al.* (2001) determined several Young's moduli from 0.25 to 0.96 MPa in a load range from 0 to 2 N.

Similar combined experimental and numerical work on the rat PDL has not been reported until now. However, in a comparable study, Andersen *et al.* (1991)

Table 3 Summary of the determined material properties of three groups of specimens. Values are given as mean (standard deviation).

Material properties	Group A (n = 12)	Group B (n = 6)	Group C (n = 6)
E_1 (MPa)	0.15 (0.07)	0.24 (0.22)	0.10 (0.05)
E_2 (MPa)	0.60 (0.18)	0.45 (0.37)	0.55 (0.17)
ε_{12} %	6.3 (1.7)	18.8 (8.3)	8.5 (3.6)

Group A, fresh specimens; group B, specimens frozen in physiological saline solution; group C, specimens frozen in Periston. E, elastic modulus; ε_{12}, ultimate strain.

found a stiffness of the PDL of 0.07 MPa for human specimens. As the applied forces were relatively low, the results might correspond with the initial phase of the force/deflection curve found in the present investigation. However, the value reported is lower than the first value (E_1 = 0.15 MPa). Using the same method as in the present study, the elastic behaviour of pig and human specimens was investigated (Siebers, 1999; Poppe *et al.*, 2002). By comparing the results presented in Table 4, only minor variations were noted between pig and human material parameters of the PDL. However, the results from the rat investigations differed significantly.

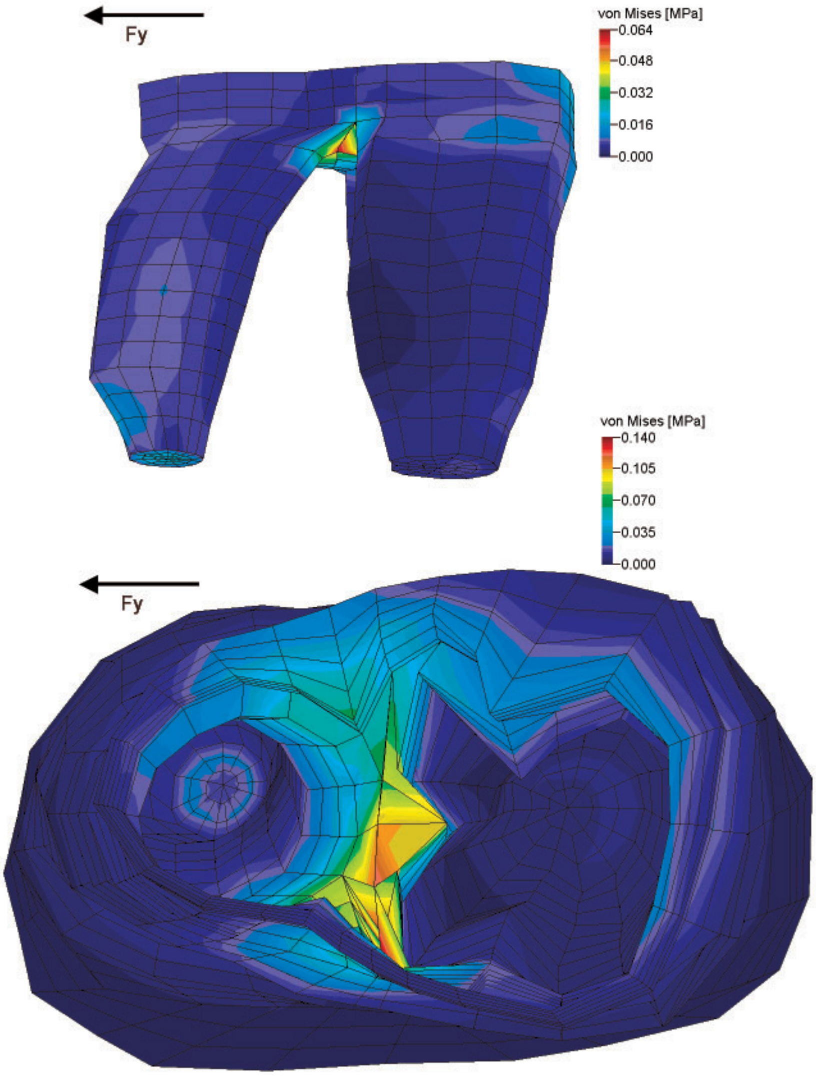

Figure 8 Von Mises stress distribution in the periodontal ligament and the bone. A high stress concentration is observed at the furcation. Further regions with relatively high stress were around the cervical region of the distal aspect and the apex of the mesial root in the periodontal ligament and the bone.

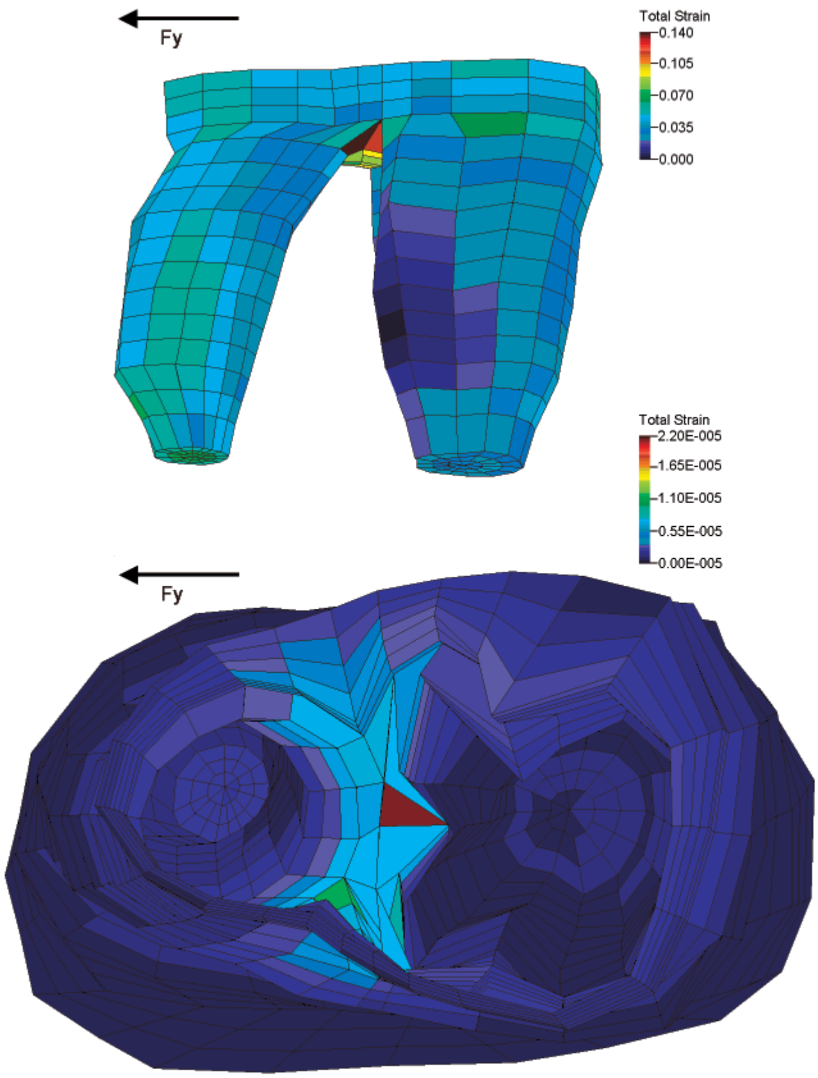

Figure 9 Distribution of the total strain (dimensionless, relative number: root of sum of squares of shear and normal strains) in the periodontal ligament and the bone. A clear difference in the maximum values was observed between strain in the periodontal ligament and the bone. In the periodontal ligament, uniform strains were noted around the cervix, on the mesial aspect and at the apex of the mesial root, while there was no strain in these regions in the bone.

In the rat, both Young's moduli determined were approximately three times higher, indicating that the PDL in rats seems to be stiffer than that in humans or pigs.

The influence of freezing in Ringer's solutions and changing the temperature of the autopsy material were tested by Pedersen *et al.* (1991). They did not find a significant effect on their measured results. Haase (1996) also detected no differences between measurements on frozen and fresh pig specimens. In contrast, in the present study, significant deviations between fresh and frozen rat specimens were found. Using Periston to reduce crystallization during freezing resulted in reduced variability as well as lower deviations compared with fresh rat specimens. However, it could not be clarified whether other factors, i.e. the freezing temperature or the duration of freezing and storage, might have decisive effects on the results. A future investigation, taking all of these factors into consideration, will have to show the true limitation of using frozen rat specimens.

To explain the biological responses of surrounding tissues to orthodontic forces, there is a need to investigate whether stress or strain may be the initiating factor in orthodontic tooth movement. In this study, a high concentration of strain in the PDL was observed, particularly at the furcation. However, the magnitude of stress in the bone and in the PDL seemed to be similar.

Table 4 The material properties of the periodontal ligament used in previous studies.

Reference	Young's modulus (MPa)	Poisson's ratio	Species	Tooth	Method
Yamada (1970)	1.4	–	Human	All teeth	Experimental
Thresher and Saito (1973)	1379	0.45	Human	Upper incisor	2D-FEM
Atkinson and Ralph (1977)	3.8	–	Human	Lower premolar	Experimental
Yettram *et al.* (1977)	0.18	0.49	Human	Upper incisor	2D-FEM
Takahashi *et al.* (1980)	9.8	0.45	Human	Lower teeth	2D-FEM
Atmaran and Mohammed (1981)	175–350***	0.45	Human	Molar	2D-FEM
Williams and Edmundson (1984)	0.5–100**	0–0.45**	Human	Single rooted	2D-FEM
Mandel *et al.* (1986)	3	–	Human	Lower premolar	Experimental
Siegele *et al.* (1986)	0.26, 8.5*	0.28	Human	Upper incisor	2D-FEM
Tanne *et al.* (1987)	0.69	0.49	Human	Lower premolar	3D-FEM
Farah *et al.* (1988)	6.9	0.45	Human	Lower molar	2D-FEM
Andersen *et al.* (1991)	0.07	0.49	Human	Lower premolar	Experimental/3D-FEM
Jones *et al.* (2001)	1	0.45	Human	Upper incisor	Experimental/3D-FEM
Middleton *et al.* (1996)	0.75–1.5***	0.45	Human	Canine	2D-FEM
Rees and Jacobsen (1997)	50	0.49	Human	Lower premolar	Experimental/2D-FEM
Siebers (1999)	0.05, 0.22*	0.30	Pig	Canine	Experimental/3D-FEM
Qian *et al.* (2001)	2, 10–90***	0.45 or 0.35	Dog	Canine	3D-FEM
Yoshida *et al.* (2001)	0.25–0.96**	0.45	Human	Upper incisor	Experimental/3D-FEM
Poppe *et al.* (2002)	0.05, 0.28*	0.30	Human	Incisors, canines	Experimental/3D-FEM

*Calculations with bilinear behaviour of the elastic modulus; **calculations with various values of the elastic modulus; ***calculations with two types of element of the periodontal ligament.
2D-FEM, two-dimensional finite element method; 3D-FEM; three-dimensional finite element method.

In agreement with the results of this study, high strain levels in the PDL and low strain in the alveolar bone have been found using FEM (e.g. Middleton *et al.*, 1996; Bourauel *et al.*, 2000; Jones *et al.*, 2001). Those authors suggested that only strains in the PDL should play the major role in initiating orthodontic tooth movement. However, further refinement of the geometric reconstruction is necessary to improve the accuracy of numerical studies. In addition, *in vivo* investigations are desirable to verify the numerical results and combine them with the biological mediators of orthodontic bone modelling and remodelling.

Conclusions

Three-dimensional finite element models of rat mandibular first molars with surrounding tissues were generated to reconstruct the geometry of experimentally investigated rat specimens.

The numerical and experimental methods used were suitable for the determination of tooth mobility in the rat model.

A bilinear approximation of material properties of the PDL is a suitable description of measured force/deflection diagrams.

As the rat model is widely used in experimental orthodontics, the numerical results can be used to describe mechanical processes, especially stress–strain distributions in the PDL, accurately.

The material parameters of the rat PDL, however, seem to differ from those of the human and porcine PDL, which should be taken into account when interpreting the results and their relevance to clinical orthodontics.

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