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Biomechanical behaviour of the periodontal ligament of the beagle dog during the first 5 hours of orthodontic force application

S. H. Jónsdóttir, E. B. W. Giesen and J. C. Maltha
Department of Orthodontics and Oral Biology, Radboud University Nijmegen Medical Centre, The Netherlands

SUMMARY The aim of this study was to describe the mechanical behaviour of the periodontal ligament (PDL) in response to loading with different forces for a period of 5 hours. Seven young adult male beagle dogs (age 1.0–1.5 years) were used. After extractions and placement of implants, custom-made appliances on both sides of the mandible were used to measure the displacement of the second premolars. Tooth displacement was measured during 5 hours of force application. Each dog underwent two measurement sessions. One premolar was moved with a force of 100 cN in the first session and with 50 cN in the second. The contralateral premolar was moved with forces of 100 and 300 cN, respectively.

Time–displacement curves showed a rapid instantaneous response lasting only a few seconds followed by a slowly decreasing creep displacement. The instantaneous response demonstrated a large individual variability, caused by both a dog and a force effect. Differences in tooth and PDL anatomy and in the orientation of the periodontal fibres are probably important in this respect. The individual variability faded after the first seconds of tooth displacement, when the viscoelastic properties of the periodontal fibres became more pronounced. The force effect was non-linear for the first minute. Higher forces did not lead to proportionally larger displacements. The non-linearity decreased in the second response. The PDL is a complex material that might be considered as a non-linear fibre-reinforced poroviscoelastic material.

Introduction

The periodontal ligament (PDL) plays an essential role in orthodontic tooth movement. Forces exerted on a tooth lead to a cascade of events in the PDL: tissue and cell strain are induced, which in turn leads to the expression of a variety of regulation factors, followed by cell differentiation, and the remodelling of soft tissues and alveolar bone. All these events ultimately lead to tooth movement.

The mechanical characteristics of the PDL determine, to a large extent, the strain induced by a certain force. This strain is considered as the initial trigger for orthodontic tooth movement. Most classical studies consider the PDL as an isotropic and linear-elastic material (Tanne and Sakuda, 1983; Williams and Edmundson, 1984; Andersen *et al.*, 1991; Wilson *et al.*, 1994). More recently, increasing evidence has been reported on anisotropic material characteristics of the PDL, in which collagen fibres play an important role (Provatidis, 2000; Qian *et al.*, 2001). However, others still consider it to be linear or bi-linear elastic and isotropic (Poppe *et al.*, 2002; Kawarizadeh *et al.*, 2003, 2004; Clement *et al.*, 2004).

Van Driel *et al.* (2000) determined experimental bodily tooth displacement over a 5-hour period in beagle dogs. An instantaneous response changed to a creep response after a few seconds. These data were used to validate their three-dimensional finite element (FE) model of a dog's premolar and PDL. Best fits were achieved if the PDL was considered to be biphasic with a fluid (the interstitial fluid) and a solid (the extracellular matrix) phase and if simultaneously different porosities were assumed for two subsets of PDL elements. They concluded that the PDL behaved as a poroelastic material showing a two-phase response in time on loading. Pietrzak *et al.* (2002) fitted a FE model to experimental data, which were reported by Parfitt (1960) on intrusive tooth displacement. They achieved the best fit if the PDL was considered to be non-linear elastic. An *ex vivo* push–pull test of the PDL also indicated non-linear characteristics (Pini *et al.*, 2002). Limbert *et al.* (2003) simulated transitional forces and showed the anisotropy to be particularly important at the tension side of the tooth where the non-fibrous component of the matrix had a much lower contribution in the generation of strain than the fibres. They stressed the importance of accurate modelling of the complex structure of the PDL, including, among others, non-linear behaviour, anisotropy, different behaviour in tension and compression, and fibre characteristics.

As intercellular matrix, and especially collagen fibres, appear to be essential for the mechanical behaviour of the PDL (Van Driel *et al.*, 2000; Limbert *et al.*, 2003), recent findings in cartilage biomechanics seem to be promising.

Cartilage can well be described as a poroviscoelastic fibre-reinforced material (Wilson *et al.*, 2004). It was hypothesized that this approach is also promising for FE modelling of the PDL. However, a thorough mechanical characterization of the PDL is essential for testing the hypothesis, and up to now only limited data are available that describe the *in vivo* behaviour of the PDL after controlled transitional force application (Limbert *et al.*, 2003; Kawarizadeh *et al.*, 2004). Therefore, the purpose of this study was to provide experimental data that can be used in that approach. The time-dependent characteristics of *in vivo* tooth displacement using different forces will yield important information on the possible non-linear mechanical behaviour of the PDL. To obtain this information, a dog model for tooth displacement was used (Van Driel *et al.*, 2000). In this model, the response of the PDL during 5 hours of application of different predefined forces was studied.

Materials and methods

Tooth displacement was measured in a 5-hour experiment in beagle dogs. The design of the experiment required several preparation steps. First, teeth were extracted and after a healing period an implant was placed. Crowns were placed on the study teeth to enable controlled force application. The implant was used as an anchor for the measurement device. Different force regimes were applied to the teeth and the measurement device registered the displacement.

Preparation of the animals

Young adult male beagle dogs (n = 7; age 1.0–1.5 years) with a complete permanent dentition were used for the experiment. Ethical permission for the study was obtained according to the guidelines of the Radboud University Nijmegen, The Netherlands.

All extractions, surgical interventions, X-ray procedures, appliance placements, and measurements were performed under general anaesthesia. To that end, the dogs were pre-medicated with 0.2 ml/kg of a 1:1 mixture of Fentanyl (50 μg/ml, Hameln Pharmaceuticals, Hameln, Germany) and Haloperidol (5 mg/ml, Janssen-Cilag, Tilburg, The Netherlands), and 0.25 ml of atropine sulphate (0.5 mg/ml), followed by 0.5 ml/kg Nembutal (sodium pentobarbital 60 mg/ml, Ceva Sante Animale, Maassluis, The Netherlands). After intubation, a closed system of Isofluran (Rhodia Organique Fine, Avonmouth, Bristol, UK), N_2O, and O_2 was used to maintain the anaesthesia.

In the lower jaw, the third and fourth premolars on both sides were extracted. In the upper jaw, the second and third premolars and the first molars were also extracted, in order to avoid interference with the measurement device.

After a healing period of 3 months, an incision was made at the alveolar ridge at both sides of the mandible between the second premolar and the first molar. Implant holes were prepared with burs with a diameter 2.5 and 2.85 mm, respectively, and a depth 10 mm (Nobel Biocare, Almere, The Netherlands), and then custom-made sand-blasted titanium implants (height 10 mm, diameter 3.1 mm) with locking screws were press fitted into the holes. The soft tissue was closed over the implants with sutures (Ethicon Coated Vicryl 4-0, Johnson & Johnson Medical, Amersfoort, The Netherlands). The dogs were medicated with Albipen L A (ampicillin 100 mg/ml, Intervet, Boxmeer, The Netherlands) after implantation.

Three months after implant placement, healing was radiographically evaluated. At both sides, the second premolar and the first molar in the lower jaw were prepared for crowns, and impressions were made (Express™, 3M ESPE, Seefeld, Germany). The impressions were poured in stone (Silky-Rock, ADA Type IV, Whipmix, Louisville, Kentucky, USA) and custom-made chrome-cobalt crowns for the second premolars and first molars were prepared on the casts. A tube and a hook were soldered on the buccal side of each crown.

In a next session, the crowns on both sides were cemented in place with PanaviaEx dental adhesive (Kuraray Medical, Okayama, Japan) and a suprastructure was fitted on the implant. A holder for a stainless steel sliding bar was placed into this suprastructure. Silicone bearings were fitted into the tubes on the crowns, and a sliding bar, 2 mm in diameter, was placed through the bearings and the holder in the implant. The silicone bearings were then fixed with Fissurit F (Voco, Cuxhaven, Germany) into the tubes. Finally, the holder for the sliding bar was fixed with Ketac-Cem (3M Espe) into the suprastructure. This resulted in a situation in which the second premolar was free to move bodily, but no external force was applied to the system.

Tooth displacement measurements

The dogs were pre-medicated and anaesthetized as before. On both sides of the mandible, the sliding bar and the suprastructure on the implant were removed and a special suprastructure for the measurements was placed (Figure 1). On this new suprastructure, a displacement transducer (MSH 707, Sony Magnescale, Tokyo, Japan) was fixed with Clearfil™ AP-X (Kuraray Medical). This transducer contained a centric hole with a diameter of 2.0 mm. A magnetic ruler (SR-721 SP, Sony Magnescale) with a diameter of 2.0 mm was inserted through this centric hole and the tubes on the premolars, and was fixed at the second premolar. The magnetic ruler was divided alternately into north and south magnetic poles, 200 μm apart, which changed the electromagnetic fields within the transducer by displacement of the ruler. The transducer was connected to a digital counter (LH 20C, Sony Magnescale) to compute a 0.5-μm accurate displacement of the magnetic ruler passing through the transducer. The counter was connected to a personal computer to store the data.

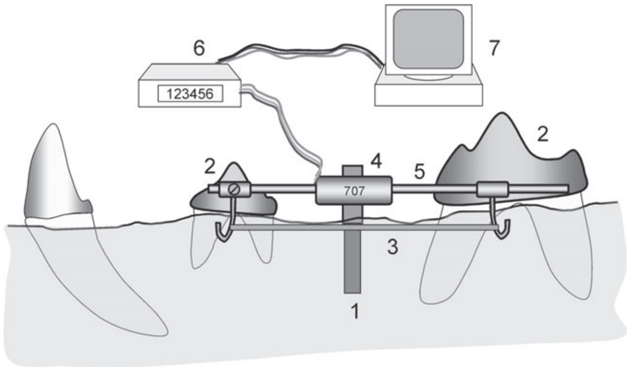

Figure 1 Schematic drawing of experimental appliance and measuring system. (1) Implant, (2) crown, (3) elastic, (4) displacement transducer, (5) magnetic ruler, (6) digital counter, and (7) personal computer.

Tooth displacement was induced on both sides by pre-stretched elastics (Z-pak elastics, Ormco, Glendora, California, USA). Pre-stretched elastics are able to exert a predetermined force over the experimental period (Pilon *et al.*, 1996). The displacement was sampled at 1 Hz for 5 hours per session. Each dog had two measurement sessions with at least 2 weeks between to allow the dog to recover from the measurement and anaesthesia, and to permit full re-establishment of the ligament. In each dog, one premolar was moved with a force of 100 cN in the first session and with 50 cN in the second, while the contralateral premolar was moved with a force of 100 and 300 cN, respectively.

Data analysis

From the data, individual time–displacement curves were constructed. For a comparison of tooth displacement between and within animals with different forces, the data were split over time into an initial phase of 1 minute and five phases of 1 hour each. A two-way analysis of variance (ANOVA) was performed after square root transformation, with the dogs and the forces as independent variables. To further analyse the dog effect on the rate of tooth displacement within the group with 100 cN force, one-way ANOVA was performed after square root transformation, with the dogs as the independent variable. To study non-linear behaviour, the displacement was fitted against different force levels by power and linear relations.

Results

All time–displacement curves showed the same general outline (Figure 2a). There was an initial phase that lasted for only a few seconds in which an instantaneous rapid tooth displacement was found. During the remainder of the 5-hour period, a gradual creep movement occurred. The rate of tooth displacement decreased with time and reached a plateau at the end of the experimental period.

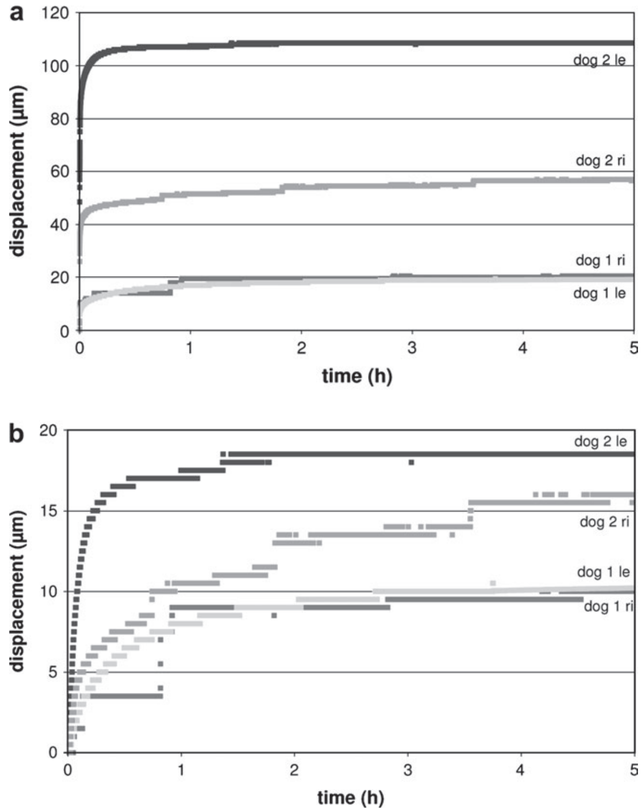

Figure 2 Tooth displacement curves of two dogs with a force of 100 cN on (a) both sides and (b) after resetting the measurements after the first minute of force application (le, left side; ri, right side).

A force of 100 cN resulted in considerable individual differences in the total amount of tooth displacement between different dogs and between both sides within one dog (Figure 2a). These differences, however, were mainly caused by the variation in displacement in the initial phase. In the 5-hour period following the first minute, the amount of tooth displacement was similar, as is illustrated in Figure 2b.

The rate during the first minute for all measurements demonstrated a very wide range from 90 to 5400 μm/h (median 1065 μm/h). In the subsequent 1-hour period, all measurements showed a dramatic decrease to a median rate of 10.2 μm/h (range 0.5–20.4 μm/h). A further decrease was found until, in the period from 4 to 5 hours, the teeth showed a median rate of movement of 1.1 μm/h (range 0–5.5 μm/h). For the 100-cN group, the data from the first hour are presented in Figure 3.

One-way ANOVA on square root transformed data was performed for the 100 cN group. Significant differences ($P < 0.05$) between dogs were found for the first minute, and for the subsequent periods of force application up to 3 hours. Thereafter, no significant differences were found between the dogs.

When all force groups were considered, the force effect appeared to be more pronounced than the dog effect. The

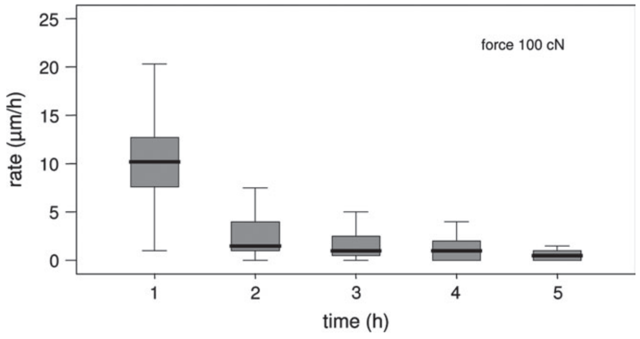

Figure 3 Box-and-Whisker plot of tooth displacement in µm/h for the periods after the first minute (force = 100 cN). Significant differences between dogs were found until 3 hours of force application.

rate of movement was significantly larger for 300 cN than for 50 cN for all time periods, including the first minute. In the period between the first minute and 3 hours, 300 cN also induced a higher rate than 100 cN. Finally, the rate induced by 100 cN was significantly larger than that induced by 50 cN in the second and the third hour (Figure 4).

For the first minute, a non-linear relationship was found between the force level and the displacement (Figure 5). The explained variance for the power relationship was higher than the explained variance for the linear relationship, $r^2 = 0.938$ and $r^2 = 0.580$, respectively.

Discussion

Under normal physiological conditions, a tooth is at rest within its socket. The vast majority of the periodontal fibres will be slack. Depending on the physiological–mechanical conditions and the orientation of the periodontal fibres, their condition will change if a tooth is loaded. Since a relationship between force level and tooth displacement is expected, the

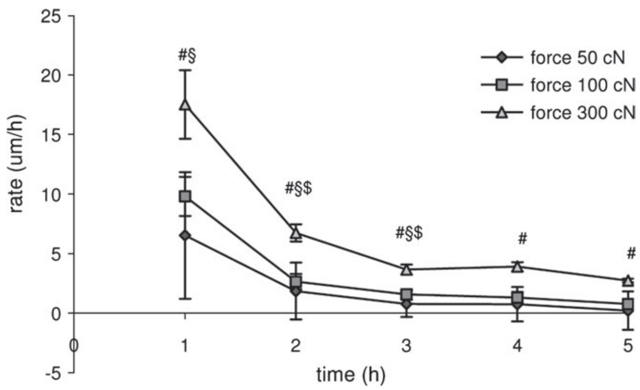

Figure 4 Means ± standard error of the mean of the rates of tooth displacement for the different forces in µm/h for the periods after the first minute. #, significant difference between 300 cN and 50 cN, §, significant difference between 300 cN and 100 cN, and $, significant difference between 100 and 50 cN.

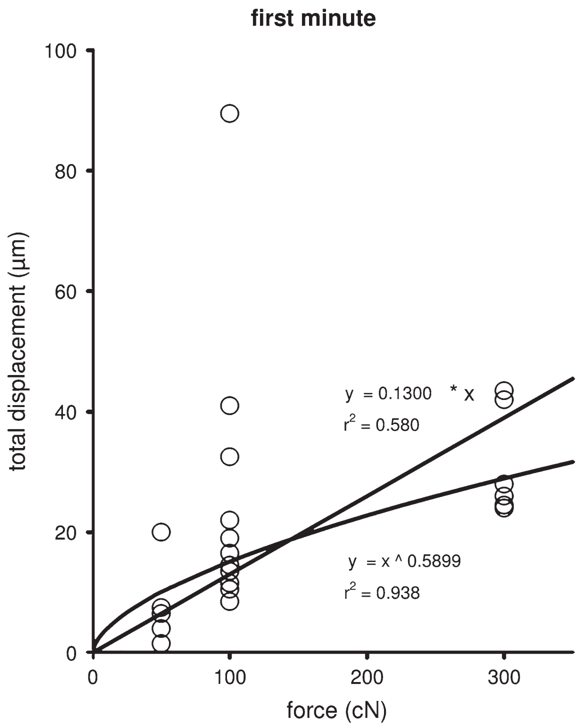

Figure 5 Force–displacement relationship for the first minute of the experiment. The data are fitted by both a power and a linear relationship. The equations and explained variance of the fits are given.

use of defined constant forces is required to study this relationship.

In the present study, orthodontic elastics were used for force application. It has been shown that 23–28 per cent of force reduction takes place in moist surroundings during the first 24 hours of stretching (Hwang and Cha, 2003), and that most will occur within the first hour (Kanchana and Godfrey, 2000; Kersey *et al.*, 2003). However, this problem can be overcome by pre-stretching the elastics after which force degradation is limited to a few per cent for the following 24 hours (Pilon *et al.*, 1996). Therefore, the use of pre-stretched elastics provided a suitable force application system for the 5-hour experiment.

The tooth displacement measurements in the present study indicated two distinct responses. An instantaneous response showing a very fast movement for only a few seconds and a secondary response that showed a dramatic decrease in the rate of movement in the subsequent hours. Van Driel *et al.* (2000) suggested that the very rapid initial change in the position of the tooth might be facilitated by the rapid relocation of fluid, which is possibly due to the high porosity of the PDL. They were able to fit the subsequent restriction of the movement in their model by assuming two subsets of periodontal elements with different porosities. However, an instantaneous increase in the number of fibres under tension, as supposed by a fibre-reinforced poroelastic model (Wilson *et al.*, 2004) yields the same effect.

The initial response in the present study showed large differences, which could be attributed to both a dog and a force effect. The dog effect might be caused by differences in root and alveolar bone morphology and in periodontal fibre orientation and density, either between dogs or between teeth within the same dog. The behaviour of the PDL in this phase is difficult to predict unless the orientation of the fibres and the relative number of fibres under tension are correctly described on an individual level. The force effect during the initial response appeared to be non-linear, as larger forces did not proportionally lead to larger displacements. At the higher force levels, the number of periodontal fibres under tension will probably reach its maximum within a few seconds, and thereby will decrease the effect of increasing the force.

If the first minute is excluded, the time–displacement curves become similar. The dog effect decreased, and after 3 hours no significant dog effect was found. The force effect was also restricted, as only the difference between 300 cN and 50 cN was significant in all phases after the first minute. Since all fibres involved are under tension in this secondary phase, they are the major factor that restricts tooth displacement. Therefore, their presumed viscoelastic characteristics become increasingly important. This is supported by the finding that the time–displacement curves showed viscoelastic creep characteristics. The findings from the present study therefore suggest that a fibre-reinforced poroviscoelastic material model, as developed by Wilson *et al.* (2004), that includes viscoelastic collagen fibres, is not only applicable for cartilage but might be feasible to describe the viscoelastic behaviour of the PDL in the secondary phase.

Other reports are also available that attempt to describe the mechanical behaviour of the PDL. The classical study of Tanne and Sakuda (1983) described the material properties of the PDL by means of FE analysis after *in vivo* measurements of tooth displacement in young individuals. However, it is not clear for what period of time the displacement was measured. Thus, it is not possible to determine if their measurements were restricted to the initial, highly variable phase, or also included the creep phase. Therefore, their data, which are often used by other authors (e.g. Tanne *et al.*, 1987; Andersen *et al.*, 1991; Wilson *et al.*, 1994), have to be interpreted with caution. Recently, Yoshida *et al.* (2001) tried to determine the biomechanical behaviour of the PDL *in vivo* in two adults by measuring tooth displacement after force application. However, the force application lasted for only 3 seconds, and thus their results only include the instantaneous response and disregard the subsequent creep phase, which appears to be essential for a correct description of the biomechanical behaviour.

Other authors have simulated a two-step response by assuming the elasticity of the PDL to be bi-linear (Poppe *et al.*, 2002; Kawarizadeh *et al.*, 2003), which means that below a certain strain the PDL has a low linear stiffness, whereas above that strain the PDL has a much higher linear stiffness. However, in this approach the time effect, which is important, is disregarded. Recent studies (Pietrzak *et al.*, 2002; Limbert *et al.*, 2003; Kawarizadeh *et al.*, 2004) support the opinion that a more accurate experimental study on the behaviour of the PDL *in vivo* is necessary. In the present study, the behaviour of the PDL was analysed over the first 5 hours after orthodontic force application, and it was concluded that a fibre-reinforced poroviscoelastic material model is probably suitable to describe the biomechanical behaviour of the PDL. In addition, the data show that a longer measuring period is essential for accurately describing the behaviour of the PDL.

Average orthodontic treatment, however, takes months or even years. During that time, continuous tissue remodelling occurs. The morphology of the PDL changes continuously and bone resorption and deposition takes place around the tooth. The biomechanical properties of the PDL are therefore also changing throughout treatment. Hence, its stress and strain levels are changing with time, as is the cellular behaviour. This means that more experimental data are needed to provide information about the behaviour of the PDL in the later phases of orthodontic treatment.

Address for correspondence

J. C. Maltha
Department of Orthodontics and Oral Biology
Radboud University Nijmegen Medical Centre
P.O. Box 9101
6500 HB
Nijmegen
The Netherlands
E-mail: j.maltha@dent.umcn.nl

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