

The biomechanical behaviour of the hyalinized periodontal ligament in dogs during experimental orthodontic tooth movement

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SUMMARY During orthodontic tooth movement, the mechanical behaviour of the extracellular matrix of the periodontal ligament (PDL) determines the cellular processes involved in turnover of the PDL and alveolar bone. This mechanical behaviour is the basis for finite element (FE) models and FE analyses. Five young adult male beagle dogs were used to test the null hypothesis that the mechanical behaviour of the PDL is identical in normal and hyalinized PDL. Therefore, tooth transposition was measured after standardized force application by super-elastic nickel titanium (NiTi) coil springs, exerting a constant force of 100 cN for 5 hours in both conditions.

A rapid transposition during the first few seconds was found. However, it was significantly less for hyalinized than for non-hyalinized PDL. Subsequently, a short-lived creep movement was found for hyalinized PDL, while creep persisted at the non-hyalinized sides (analysis of variance and Tukey's multiple comparisons *post hoc* tests). The results showed substantial biomechanical differences between hyalinized and non-hyalinized PDL at different time points (Mann–Whitney). This indicates that FE models in the study of long-term orthodontic tooth movement, which are based solely on the characteristics of normal PDL should be reconsidered.

Introduction

Orthodontic tooth movement depends largely on the biomechanical properties of the periodontal ligament (PDL). Force application on a tooth results in strain within the PDL and alveolar bone. This, along with subsequent fluid flow, leads to cell strain and induces the expression of a variety of regulatory factors. In response, cell differentiation, remodelling of soft tissues, and local resorption and deposition of alveolar bone occurs. Together, these events ultimately lead to tooth movement (Ten Cate and Nanci, 2003; Krishnan and Davidovitch, 2006, 2009; Henneman *et al.*, 2008). This mechanism, however, is complicated by changes in the structure of the PDL during orthodontic tooth movement. It was hypothesized that these changes will alter the biomechanical behaviour of the PDL.

After a first phase that represents the initial movement of the tooth within its socket, tooth movement generally shows a temporary standstill due to hyalinization of the PDL. This is caused by obliteration of the blood vessels and subsequent local necrosis. Immediate turnover of extracellular matrix and direct bone resorption are disabled until the hyalinized tissue has been removed by macrophages and replaced by new loose connective tissue. Only then are osteoclasts recruited to the area and direct bone resorption can start (Pilon *et al.*, 1996; Nakamura *et al.*, 2000; Ten Cate and Nanci, 2003).

If no hyalinization of the PDL has taken place, a rapid remodelling of the extracellular matrix is found in the compression areas (Pilon *et al.*, 1996). The principal collagen type I fibres are lost within a few days and replaced by a loose connective tissue that contains mainly randomly orientated thin collagen fibres.

As cellular reactions during orthodontic tooth movement depend on the strain within the PDL (Krishnan and Davidovitch, 2006), knowledge of the changes in the mechanical behaviour of the PDL caused by structural changes is essential for understanding this process (Wakabayashi *et al.*, 2008). However, to date, only limited data are available on the *in vivo* behaviour of the normal PDL (Limbert *et al.*, 2003; Kavarizadeh *et al.*, 2004; Jónsdóttir *et al.*, 2006). Finite element (FE) models used to analyse the effects of orthodontic forces on the PDL and alveolar bone are based on these normal physiological conditions (Tanne and Sakuda, 1983; Yoshida *et al.*, 2001; Vicilli *et al.*, 2008) or on *ex vivo* measurements (Pini *et al.*, 2002; Poppe *et al.*, 2002; Dorow *et al.*, 2003). No data on the mechanical behaviour of the PDL during the different phases in the course of orthodontic tooth movement are available.

The aim of the present study was therefore to test the null hypothesis that the mechanical behaviour of the hyalinized

and non-hyalinized PDL, and thus tooth transposition within the socket under standardized loading conditions is equal. The term tooth transposition is used to distinguish tooth movement within the socket without bone remodelling from the movement that involves bone resorption and deposition.

Material and methods

Preparation of the animals

Five young adult male beagle dogs (aged 1.0–1.5 years) with a complete permanent dentition were used for the experiment. Ethical permission was obtained according to the guidelines of the Radboud University Nijmegen, The Netherlands. The animals were housed in groups of two or three animals per cage under normal laboratory conditions and were fed standard dog chow and drinking water *ad libitum*.

All interventions were performed under general anaesthesia. The dogs were premedicated 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 incubation, a closed system of isofluran (Rhodia Organique Fine, Avonmouth, Bristol, UK), N₂O, and O₂ was used to maintain the anaesthesia.

The mandibular third and fourth premolars on both sides were extracted, as well as the maxillary second and third premolars and the first molars, in order to avoid interference with the orthodontic appliance. After 3 months, custom-made titanium implants (height 10 mm, diameter 3.1 mm, and sandblasted) with locking screws were placed bilaterally in the mandible between the second premolar and first molar. The dogs were medicated with Albipen L A (ampicillin 100 mg/ml; Intervet, Boxmeer, The Netherlands) after implantation.

Orthodontic appliance

Crowns for the second premolars and first molars were made 3 months after implant placement. A tube and a hook were soldered on the buccal side of each crown. The crowns were cemented in place with PanaviaEx dental adhesive (Kuraray Medical Inc., Okayama, Japan) and a suprastructure with a holder for a stainless steel sliding bar was fitted on the implant. Low friction silicone bearings with an internal diameter of 2.01 mm were fitted in the tubes on the crowns, and a 2 mm sliding bar was placed through the bearings and through the holder on the implant. The silicone bearings were then fixed into the tubes with Fissurit F (Voco, Cuxhaven, Germany). Finally, the holder for the sliding bar was fixed into the suprastructure with Ketac-Cem (3M Espe

AG, Seefeld, Germany). This enabled bodily movement of the second premolar and first molar with virtually no resistance and an axial play of 0.01 mm. At day zero of the experiment, a super-elastic NiTi coil spring, exerting a constant force of 100 cN (GAC International, New York, USA), was fixed to the hooks on the crowns to induce reciprocal movement of the second premolar and first molar. At day 6, the coil springs were removed in order to allow the ligament to relax.

Tooth transposition measurements

On day 7, tooth transposition was carried out on the second premolar. It was assumed, in most cases, that hyalinized tissue was present at that time (Pilon *et al.*, 1996; Van Leeuwen *et al.*, 1999). This was verified by histology or time-displacement curves in the subsequent period.

The dogs were anaesthetized as previously. The sliding bars and the suprastructures were removed from the implants and a special suprastructure for the measurements was fixed on the implant (Figure 1). On this new suprastructure, a displacement transducer with a centric hole of 2.0 mm in diameter (MSH 707; Sony Magnescale Inc., Saitama, Japan) was fixed with Clearfil™ AP-X (Kuraray Medical Inc.). A magnetic ruler (SR-721 SP; Sony Magnescale Inc.) with a diameter of 2.0 mm was inserted through this centric hole and through the tubes on the crowns and fixed at the second premolar by a locking screw. The magnetic ruler could then move through the transducer with the movement of 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 Inc.) to calculate the movement of the magnetic ruler passing through the stable transducer and thus the distal displacement of the second premolar with an accuracy of 0.5 µm.

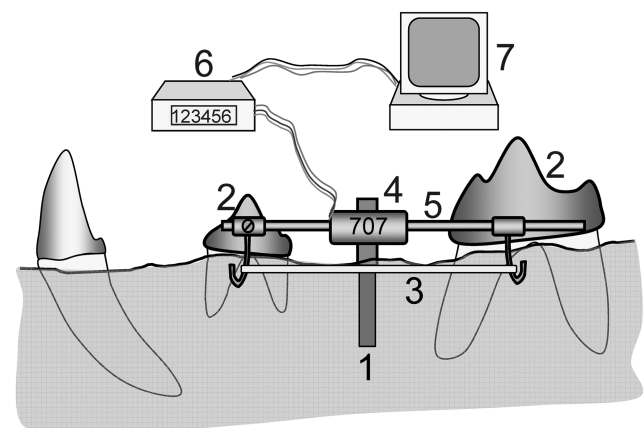


Figure 1 The experimental appliance with the measuring system. 1. implant, 2. crowns, 3. elastic, 4. displacement transducer, 5. magnetic ruler, 6. digital counter, and 7. personal computer.

Displacement of the second premolar was induced on both sides by pre-stretched elastics (Z-pak elastics,Ormco® Orange, California, USA) that were selected to exert a force of 100 cN. The transposition was sampled at 1 Hz for 5 hours.

Hyalinized or non-hyalinized PDL

Two dogs were sacrificed for histological evaluation during general anaesthesia by a lethal dose of narcovet (sodium pentobarbital 60 mg/ml; Apharmo, Arnhem, The Netherlands). The second mandibular premolars and their surrounding bone were dissected. After fixation in 4 per cent buffered formaldehyde solution in 0.1 mol/l phosphate buffered saline and decalcification in 20 per cent formic acid and 5 per cent sodium citrate, the tissue blocks were dehydrated and embedded in paraplast (Monoject Scientific, Athy, Ireland). Serial mesiodistal sections of 7 µm were cut and stained with haematoxylin and eosin. If hyalinized tissue was present in the PDL, the tooth was considered to be in the hyalinization phase.

The three remaining dogs were prepared for further orthodontic tooth movement, and time-displacement curves were constructed, based on intra-oral measurements. The teeth were considered to be in the hyalinization phase if they showed no movement for at least 2 weeks.

Data analysis

Time-transposition curves were constructed from the 5 hour measurements for the second premolars at both sides of each dog. Two different categories of curves were found; one showing limited tooth transposition over the 5 hour measurements of 23.5 ± 4.4 µm and the other a transposition of 66.7 ± 13.7 µm. The first category represented the sides with hyalinized PDL ($n = 7$) and the latter the non-hyalinized sides ($n = 3$).

For statistical analyses, the tooth transposition data for the hyalinized and the non-hyalinized group were split into five periods of 1 hour each. Separate analyses were performed, including or excluding the measurements of the first minute. The data were normally distributed (Kolmogorov–Smirnov), and based on a previous study (Jónsdóttir *et al.*, 2006), data from different sides were considered to be independent. Therefore, time dependency of the amount and rate of transposition were analysed by analysis of variance and Tukey's multiple comparisons *post hoc* tests. The differences between the hyalinized and non-hyalinized sides at different time points were analysed with Mann–Whitney tests as Levene's tests showed unequal variances between the hyalinized and non-hyalinized group.

Results

Not all experimental sides had reached the hyalinization phase as shown by long-term time-displacement curves or

post hoc histological evaluation (Figure 2). The 5 hour measurement data of the hyalinized sides ($n = 7$) and the non-hyalinized sides ($n = 3$) are summarized in Figure 3A.

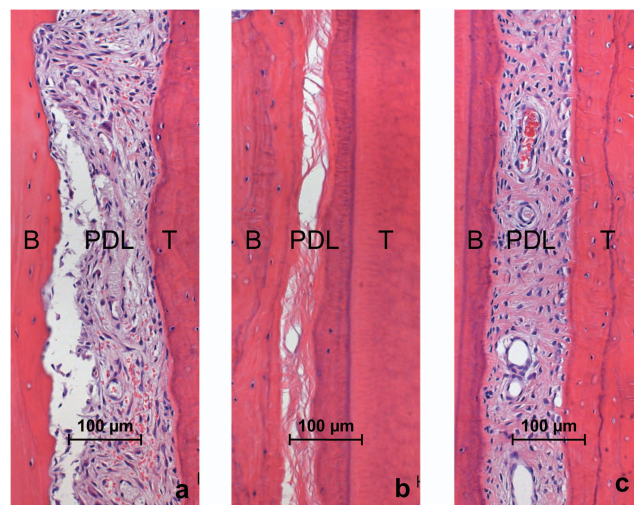


Figure 2 Histological sections showing the periodontal ligament (PDL) of (a) non-hyalinized PDL at the pressure side, (b) hyalinized PDL at the pressure side, and (c) PDL at the tension side. B, alveolar bone; T, tooth.

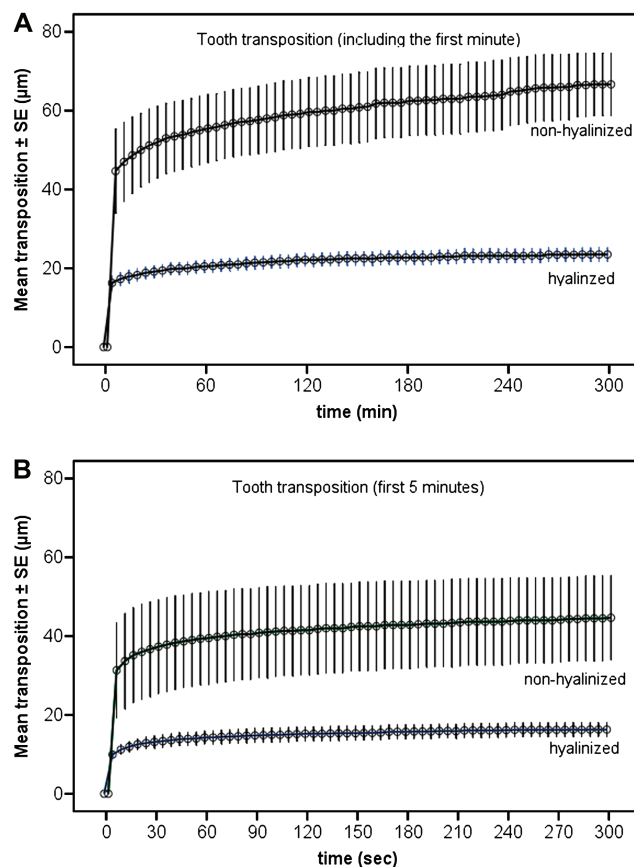


Figure 3 Tooth transposition curves (means \pm standard error) of the hyalinized and the non-hyalinized groups for (A) the 5 hour and (B) first minute of the measurements.

In both the hyalinized and the non-hyalinized groups, an initial rapid transposition was found, followed by a decreasing creep response during the remainder of the experimental period. The mean tooth transposition in the first 5 seconds for the hyalinized group was $9.9 \pm 2.3 \mu\text{m}$ and for the non-hyalinized group $31.3 \pm 20.1 \mu\text{m}$ ($P < 0.02$; Figure 3B).

The total transposition in the first hour was $20.5 \pm 4.0 \mu\text{m}$ in the hyalinized group and $55.5 \pm 14.9 \mu\text{m}$ in the non-hyalinized group ($P < 0.02$; Figure 3A). If the first minute was excluded, the mean remaining transposition in the first hour for the hyalinized group was $6.1 \pm 4.0 \mu\text{m}$ and for the non-hyalinized group $16.0 \pm 5.7 \mu\text{m}$ ($P = 0.03$). The transposition rate for both groups decreased significantly ($P \leq 0.002$) during the subsequent 1 hour periods (Figure 4). However, the transposition rate for the four subsequent 1 hour periods after the first hour showed no significant mutual differences within each of the groups. Comparison of the hyalinized and non-hyalinized groups showed significantly more creep in the non-hyalinized group for all 1 hour periods, except for the last hour (Figure 4).

Discussion

In the present study, a standardized orthodontic force of 100 cN was applied to the second premolars of dogs for 6 days. Thereafter, the PDL was allowed to relax for 24 hours. Then, a standardized force of 100 cN was again applied, and tooth transposition was measured over a 5 hour period to study the biomechanical behaviour of the PDL. The 24 hour relaxation period allowed for tooth transposition measurements under unstressed conditions, while the PDL

morphology differs from normal. For the compression areas, this meant that in most cases, hyalinized tissue was present while the other cases showed non-hyalinized PDL. In the latter group, the PDL was devoid of principal fibres; it contained mainly loose connective tissue with randomly orientated thin collagen fibres as described in other studies (Ren *et al.*, 2008; Lv *et al.*, 2009). The tension areas in the hyalinized as well as in the non-hyalinized group still contained principal fibres and blood vessels (Maltha, Jónsdóttir, unpublished observations). This is in agreement with other studies (Pilon *et al.*, 1996; Van Leeuwen *et al.*, 1999).

Under physiological conditions, teeth show a very rapid initial transposition upon force application that lasts for a few seconds to minutes (Jónsdóttir *et al.*, 2006; Slomka *et al.*, 2008). This can be explained by a rapid fluid reallocation within the PDL (Van Driel *et al.*, 2000). The initial phase is followed by a gradually decreasing creep movement that lasts for approximately 5 hours. This is probably related to the viscoelastic material properties of the PDL at the tension areas (Van Driel *et al.*, 2000; Jónsdóttir *et al.*, 2006). In a previous investigation, it was shown that normal PDL can be considered as a poroviscoelastic fibre-reinforced material (Jónsdóttir *et al.*, 2006). In the present study, the initial transposition in the presence of non-hyalinized PDL was approximately $39.5 \pm 20 \mu\text{m}$ in the first minute, which is higher than in the normal PDL ($23 \pm 22 \mu\text{m}$). Although this difference was not statistically significant, and the number of experimental sides was low, it suggests that the structural changes in the PDL at the compression areas, where the normal PDL is replaced by a loose connective tissue, allow for more rapid fluid reallocation. In the hyalinized group, however, the initial transposition in the first minute was significantly less than normal ($14 \pm 4 \mu\text{m}$), suggesting that fluid reallocation is inhibited by the hyalinized dense necrotic material (Kuroi and Owman-Moll, 1998; Melsen, 2001; Tomizuka *et al.*, 2007).

The total creep after the first minute in the non-hyalinized group was approximately $27 \pm 7 \mu\text{m}$, which is comparable with that found in a previous study with a normal PDL ($33 \pm 16 \mu\text{m}$; Jónsdóttir *et al.*, 2006). This suggests that the mechanical characteristics of the PDL in the tension areas remain the same during the first 6 days of orthodontic tooth movement.

The creep after the first minute in the hyalinized group was completely different. Although the histology of the PDL in the tension areas in this group was comparable to that in the non-hyalinized group, the creep was significantly reduced. Apparently, the high density of the hyalinized PDL and the absence of blood vessels in the compression areas inhibit creep movement. The final result is that during the 5 hour period, the transposition is significantly less in the hyalinized than in the non-hyalinized group. Assuming the normal width of the PDL to be about 0.2 mm, the evoked strain can be roughly estimated as 25–30 per cent for normal

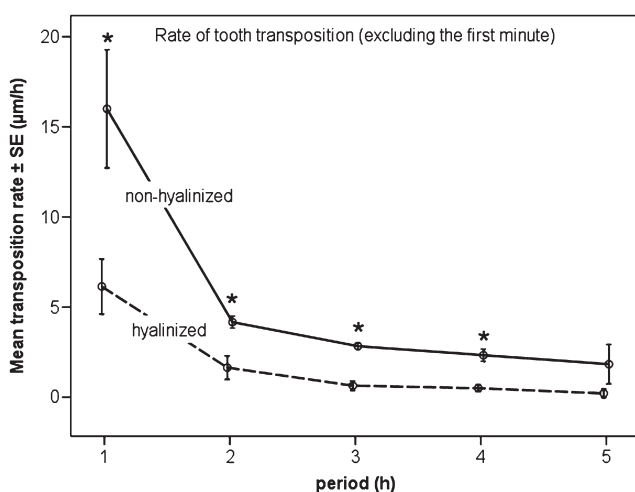


Figure 4 Transposition rates (means \pm standard error) for the hyalinized and non-hyalinized groups (excluding the first minute) over five subsequent 1 hour periods. The decrease in transposition rate from the first to the second period was significant for both groups ($P < 0.01$). Asterisk indicates significant difference between groups at the indicated time points ($P < 0.05$).

and non-hyalinized PDL and as 7 per cent for the hyalinized PDL. Consequently, the strain in the PDL and the alveolar bone is probably less if hyalinization is present. This might affect the signalling pathways that are responsible for the differentiation and/or recruitment of osteoclasts, osteoblast progenitors, and inflammatory cells (Krishnan and Davidovitch, 2006; Henneman *et al.*, 2008).

Conclusions

The present study reports for the first time on the biomechanical behaviour of the PDL during orthodontic tooth movement in the presence or absence of hyalinization. It can be concluded that the null hypothesis that mechanical behaviour of the hyalinized PDL and non-hyalinized PDL is equal, is not supported by the present findings. This should be considered in future studies on the effects of force-induced tissue strain on cell and tissue reactions to orthodontic forces. Furthermore, these data can be used in FE analyses as these are only valid if realistic mechanical properties of the PDL are accurately incorporated (Limbert *et al.*, 2003; Cattaneo *et al.*, 2005; Bosshardt *et al.*, 2008; Wakabayashi *et al.*, 2008).

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