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Proliferative response of cells of the dentogingival junction to mechanical stimulation

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SUMMARY The aim of this research was to study the proliferative response of junctional epithelium (JE) and gingival connective tissue (GCT) to mechanical stimulation *in vivo* with regard to the potential occurrence of apical migration of JE and loss of GCT attachment during orthodontic tooth movement. Elastic bands were inserted between the maxillary first and second molars of male rats aged 8 weeks, which were pulse-labelled with ^{3}H-thymidine and subsequently killed in groups, together with labelled control animals (a total of 98 rats) after periods of 1–168 hours. Autoradiographs were prepared from plastic mesiodistal sections, and parameters of cell proliferation for JE and GCT of the papilla between the second and third molars were determined.

Although the distance between the apical limit of JE and the most coronal periodontal ligament (PDL) fibres decreased on the pressure and increased on the tension sides of mechanically stimulated animals, the total cross-sectional area of JE remained unchanged compared with controls. In the basal and suprabasal layers of JE, cell proliferation was reduced on the pressure side and showed no change on the tension side. In the apical JE compartments on both sides, mechanical stressing resulted in lower proliferative activity. Cell proliferation in GCT adjacent to JE in stimulated animals did not differ from the corresponding controls. JE rapidly adapted to mechanical stimulation by means of differential local adjustments of cell proliferation without an occurrence of apical migration or hyperplasia. GCT cells in the vicinity of JE maintained their steady-state proliferative activity.

These results do not support the concept that orthodontic tooth movement might *per se* have detrimental effects on the stability of the dentogingival junction.

Introduction

Stability of the dentogingival junction when subjected to orthodontic forces is essential for maintenance of periodontal health during orthodontic treatment. However, gingival changes, loss of connective tissue attachment and marginal bone loss have been reported in a number of clinical studies as unwanted side-effects of orthodontic tooth movement (Heasman and Millett, 1996). The occurrence of such effects and their extent vary substantially, although this may be the result of differences in the design of these studies, oral hygiene levels, size and type of orthodontic attachments used, and measurement techniques employed (Heasman and Millett, 1996). Although the major aetiological role of plaque and efficacy of oral hygiene practised by orthodontic patients in the development of such unwanted effects has been recognized (Heasman and Millett, 1996), it has been suggested that occurrence of some adverse tissue reactions, such as gingival hyperplasia (Zachrisson and Zachrisson, 1972) and loss of connective tissue attachment (Hamp *et al*., 1982), cannot be explained merely by poor oral hygiene. This indicates that mechanical stimulation of the dentogingival junction *per se* might be one of the causative factors.

In contrast, experimentally induced excessive occlusal loads, while leading to an adaptive widening of the periodontal ligament (PDL), do not

cause apical migration of junctional epithelium (JE) unless inflammation is also present (Polson *et al*., 1974, 1976; Nyman *et al*., 1978). While the multi-directional loading characteristics of occlusal trauma are not identical to those of orthodontic forces, these studies suggest that it is unlikely that orthodontic forces alone will cause migration of JE.

JE has a low level of steady-state proliferative activity as expressed by conventional measures, such as labelling or mitotic indices (Karring, 1973), but greatly increased division can be induced by inflammation, and this may also be associated with apical migration, loss of connective tissue attachment or hyperplasia (Mackenzie, 1988). It has also been shown that disturbances of fibroblast populations in inflamed gingival connective tissue (GCT), including increased single cell deletion and lack of compensatory proliferation, may occur in the immediate vicinity of JE as an early event of inflammation-induced tissue changes (Nemeth *et al*., 1993) and that inflammation can serve as a permissive factor for apically directed epithelial migration (Mackenzie, 1988), thus acting as an important component of the mechanism of attachment loss (Nemeth *et al*., 1993). Although it is known that mechanical stimulation of epithelia may lead to increased proliferative activity both *in vivo* (Mackenzie, 1974; Squier, 1980) and *in vitro* (Brunette, 1984), no information is currently available with regard to proliferative behaviour of JE cells subjected to orthodontic forces. In addition, nothing is known about the kinetics of GCT cells under mechanical stimulation during orthodontic tooth movement. However, the morphological adaptive changes which occur in both gingiva and JE are considerable (Atherton and Kerr, 1968). It can be postulated therefore that even if migration of JE does not occur in the non-inflamed state, a proliferative response to mechanical stimulation in affected periodontal tissues is likely to be an important feature of orthodontic tooth movement, because proliferation within PDL and bone cell lineages is an essential pre-condition of cell differentiation and cell-mediated extracellular matrix remodelling (Rygh, 1992). The aim of this investigation was to gain insights into this aspect of tissue reaction by determining the proliferative activity of JE and GCT cells *in vivo* under experimental conditions simulating orthodontic tooth movement.

Materials and Methods

Animal experiments, tissue processing, and autoradiography

Male hooded Lister rats, aged 8 weeks, were caged separately after weaning, fed a standard laboratory diet and water *ad libitum*, and kept in a room with a 12-hour light/dark cycle beginning at 08.00 hours after an acclimatization period of 1 week. At the start of the experiments the animals weighed 146.3 ± 21.5 g. Mechanical stimulation imitating orthodontic force was induced between 09.00–10.00 hours on rats which were anaesthetized with an intra-muscular injection of Small Animal Immobilon® (C-Vet, Bury St Edmunds, UK), 0.1 µl/g body weight. Orthodontic elastic bands, 0.5 mm thick (size 1.1, Ord.No. 772–103, Dentaurum, Pforzheim, Germany) were inserted between the maxillary first (M1) and second (M2) molars using fine haemostatic forceps and left *in situ* for the entire stimulation period. Immediately after insertion of the bands, the rats were labelled by intra-peritoneal injection of tritiated thymidine (^{3}H-TdR, Specific Activity 25 Ci/mMol, Amersham International, Amersham, UK) in a dose of 1 µCi/g body weight. The isotope solution was prepared immediately before use as a 1:4 solution in Dulbecco's phosphate-buffered saline, pH 7.3.

Mechanically stimulated animals were subsequently killed in groups of six or seven animals together with equal-sized groups of ^{3}H-TdR-labelled unstimulated control animals after 1, 12, 24, 48, 72, 120, or 168 hours. The upper left maxillary molars and adjacent periodontium and alveolar bone were dissected *en bloc*, fixed in 10 per cent v/v neutral buffered formalin, decalcified in 14 per cent w/v EDTA solution, and embedded in plastic (JB-4®, Polysciences, Warrington, PA, USA). Serial 2.5 µm mesiodistal sections were prepared from the tissue blocks and two sections per animal, at least 10 µm apart, were chosen from the col area for preparation of autoradiographs. These sections were dipped in

Ilford K2 emulsion (Ilford, Mobberley, Cheshire, UK), exposed, and developed according to the liquid emulsion procedure described by Rogers (1979) and counterstained with Mayer's haemalum (Romeis, 1968). Additional sections from ^{3}H-TdR-unlabelled animals were likewise chosen, processed at the same time and used to establish the labelling threshold for the number of silver grains over a nucleus of a ^{3}H-TdR-labelled cell at $P < 0.001$ as described by McCulloch and Melcher (1983).

Data collection and analysis

The gingival papilla between M2 and M3 was selected for measurements because the experimental mechanical manipulation caused distal tipping of M2 and, via the inter-proximal crown contact of M3, created both pressure (distal to M2), and tension (mesial to M3) zones in the papilla. Apart from determinations of the total cross-sectional area of JE, all measurements were carried out both on the pressure and tension sides of the papilla of mechanically stimulated animals and the equivalent sites in unstimulated control animals.

Morphometric measurements were accomplished under the ×10 objective of a light microscope using an image analyser (KS 100, rel. 2.0, Kontron Elektronik, Eching, Germany). Parameters assessed were the total cross-sectional area of JE in μm^2, as well as the distance between the apical limit of JE and the most coronal fibres of the adjacent PDL. The distance was measured both on the pressure and tension sides, and was defined as the length in micrometres of the line segment between the most apical limit of JE and the intersection of the root tangent with a perpendicular line dropped onto the latter from the most coronal point of the inter-dental bone crest (Figure 1).

The JE was divided into pressure and tension zones in the image analyser by drawing a line from the most coronal extension of the papillary tip to the mid-point of the crest of the inter-dental bony septum (Figure 2). Cell proliferation of JE was determined under a ×100 microscope objective as the percentage of ^{3}H-TdR-labelled cells (PLC) of the total numbers of cells within the tissue compartments examined. PLCs were determined in the basal cell layer as the fraction of labelled cells of all basal cells on the pressure and tension sides. In the suprabasal cell layer

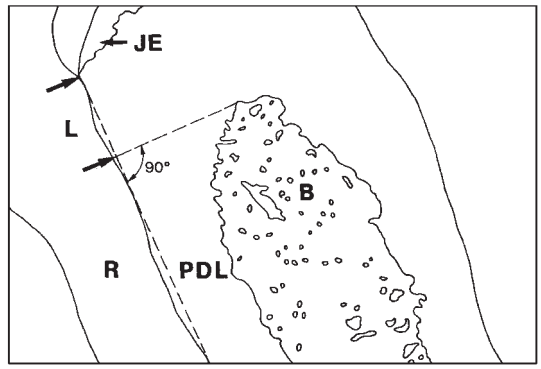

Figure 1 Schematic drawing of the measurement of the distance between the apical limit of JE and the coronal limit of the adjacent PDL. This was defined as the length in micrometres of the line segment between the most apical part of JE and the intersection of the root tangent with the perpendicular line dropped onto the latter from the most coronal point of the inter-dental bone crest. JE, junctional epithelium; R, root; B, inter-dental bone; PDL, periodontal ligament; L, line segment.

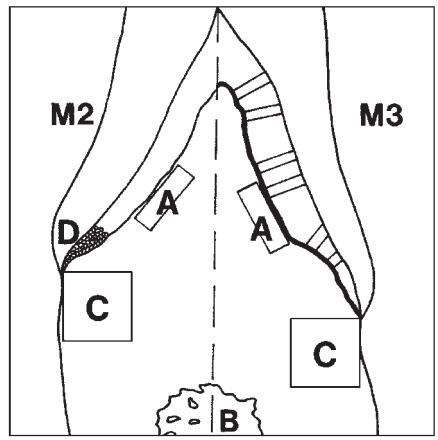

Figure 2 Schematic drawing of the tissue compartments employed for evaluation of cell proliferation on the pressure and tension sides of gingival papilla. The basal cell layer of JE of one side is shown by the solid line and in the suprabasal layer the positions of the six 12.5-μm wide strip-shaped sample areas are indicated. The positions in gingival connective tissue of the 37.5 × 125 μm (A) and 125 × 125 μm (C) sample areas located near the basement membrane or subjacent to the apical limit of JE, respectively, are indicated. M2, M3—second and third molars; B—inter-dental bone; D—position of the 50 most apically located cells comprising the apical compartment of JE.

of both pressure and tension zones PLCs were obtained for six 12.5-μm wide strip-shaped sample areas which were placed perpendicular to the basement membrane (Figure 2). Two of these sample areas were arbitrarily placed in the coronal, two in the middle and two in the apical third of the JE in the pressure and tension zones. Data from all strips were pooled for calculation of PLCs of individual pressure or tension sides. In addition, in the apical compartments PLCs were assessed on the pressure and tension sides for the 50 most apically located JE cells without discriminating between the basal and suprabasal cells (Figure 2). In the GCT, PLCs were determined on the pressure and tension sides within 37.5 × 125 μm and 125 × 125 μm sample areas placed in the immediate vicinity of the basement membrane of JE and subjacent to its apical limit, respectively (Figure 2).

The results obtained for each parameter from stimulated and control groups over the entire time course were tested for statistical significance using a two-way ANOVA (SigmaStat® 2.0, Jandel Scientific Software, San Rafael, CA, USA), followed, depending on the normality of data distribution, by the *t*-test or Mann–Whitney Rank Sum Test with pairwise comparison for each individual stimulation period, if a statistically significant influence of mechanical stimulation was revealed by ANOVA. In all tests, the level of statistical significance was set at $P < 0.05$. Mean values and standard errors of the mean were calculated for data presentation.

Results

As shown in Table 1, the distance between the apical limit of JE and the coronal limit of the adjacent PDL on the pressure side decreased significantly (ANOVA $P = 0.0001$), throughout the time course of 168 hours in mechanically stimulated specimens compared with the values obtained from the corresponding control specimens. This reduction ranged between 50 and 150 μm, and pairwise comparison tests revealed that this difference was significant ($P < 0.05$) after the initial stimulation period of 1 hour and remained so almost at each individual time point. By contrast, on the contralateral side of the interdental papilla which had been subjected to tension, this distance was significantly greater in experimental animals compared with controls (Table 1, ANOVA $P = 0.0001$). The increase amounted to 50–100 μm, and was significant after 12, 72, and 120 hours of stimulation (pairwise comparison tests $P < 0.05$).

Measurements of the total cross-sectional area of JE are shown in Table 2. Comparison of the values obtained from mechanically stimulated tissue with those measured in the corresponding controls revealed no statistically significant differences (ANOVA $P = 0.04502$).

Figure 3 shows the typical appearance of labelled JE cells on the pressure and tension sides at 24 and 72 hours. Calculated PLC values for basal and suprabasal cell layers, and the apical compartment of JE which had been

Table 1 The distance in micrometres between the apical limit of JE and the coronal limit of the PDL on the pressure (distal to M2) and tension (mesial to M3) sides of mechanically stimulated and unstimulated control animals. Results are presented as means ± SE. ANOVA over the time course on pressure side $P = 0.0001$, on tension side $P = 0.0001$, * indicates a statistically significant difference at $P < 0.05$ between stimulated and control groups at individual time periods.

Duration of stimulation (hours)	Pressure side		Tension side	
	Stimulated	Control	Stimulated	Control
1	151.1 ± 10.9*	204.6 ± 3.6	196.3 ± 31.4	128.4 ± 19.1
12	176.3 ± 24.6*	260.1 ± 9.0	257.3 ± 25.5*	150.6 ± 18.2
24	135.2 ± 29.3*	241.0 ± 11.7	205.4 ± 16.1	173.2 ± 13.5
48	83.1 ± 18.7*	229.0 ± 10.8	207.5 ± 30.3	162.4 ± 17.4
72	156.6 ± 48.8*	281.1 ± 18.1	246.0 ± 27.9*	143.4 ± 20.6
120	121.9 ± 31.3	163.7 ± 17.2	228.8 ± 21.9*	148.7 ± 12.7
168	180.0 ± 11.2*	232.3 ± 12.0	186.4 ± 29.4	166.5 ± 13.7

Table 2 Total cross-sectional area (μm²) of JE of the papilla between M2 and M3 of mechanically stimulated and unstimulated control animals. Results are presented as means ± SE, ANOVA over the time course $P = 0.4502$.

Duration of stimulation (hours)	Stimulated tissue area	Control tissue area
1	60151.0 ± 8217.9	60295.7 ± 8217.9
12	50914.9 ± 7608.3	58674.6 ± 7608.3
24	58862.6 ± 7608.3	81029.4 ± 7608.3
48	36948.7 ± 8217.9	49943.2 ± 8217.9
72	59222.7 ± 7608.3	68084.4 ± 7608.3
120	70665.4 ± 7608.3	45976.4 ± 7608.3
168	67098.6 ± 7608.3	61969.1 ± 7608.3

subjected to mechanical pressure are shown in Table 3. It can be seen that mechanical stressing caused a general pattern of reduction of PLCs in all three tissue compartments relative to controls. The overall differences between the PLCs of stimulated and control groups were statistically significant (ANOVA basal cell layer $P = 0.0042$, suprabasal cell layer $P = 0.0001$, apical compartment $P = 0.0015$), while the reduction in the 24- and 48-hour groups of stimulated animals relative to controls was also significant (pairwise comparison tests $P < 0.05$).

Table 4 shows the PLCs obtained for JE on the tension side of the papilla. Both in the basal (ANOVA $P = 0.4237$) and suprabasal (ANOVA $P = 0.4515$) cell layers mechanical stimulation had no significant influence on proliferative activity. In the apical tissue compartment, cell proliferation was significantly reduced in stimulated animals over the entire experimental course (ANOVA $P = 0.0055$), as was proliferation in the 12-hour group, which had been subjected to mechanical stress ($P < 0.05$) compared with controls.

PLCs from GCT adjacent to JE are shown in Table 5. It is apparent that none of GCT compartments evaluated sustained any change in cell proliferation under the experimental conditions employed over the time course, as shown by

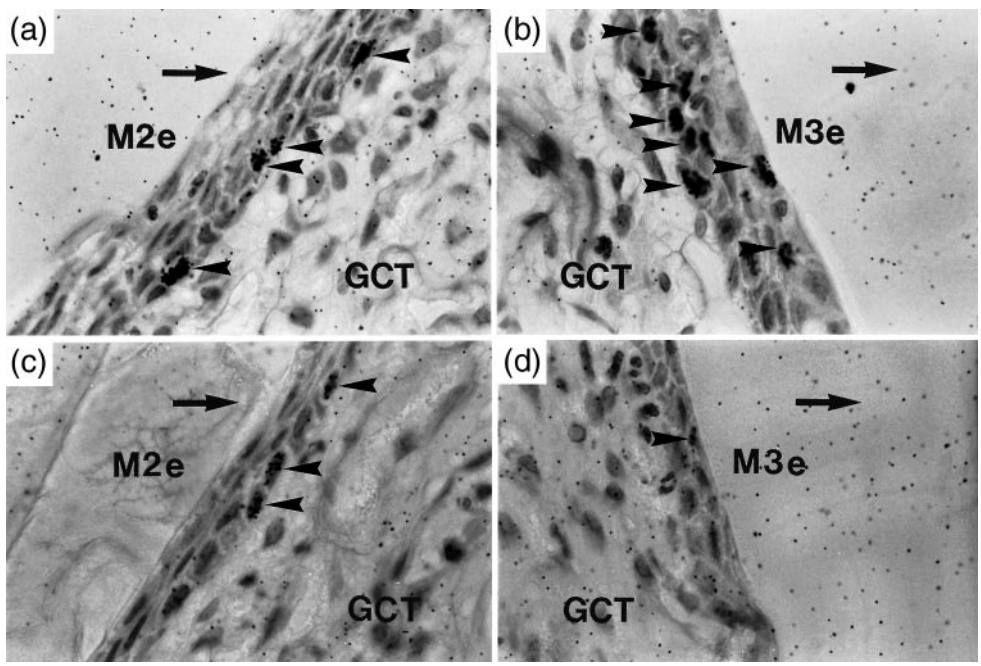

Figure 3 Typical appearances of autoradiographs under ×100 microscope objective. (a) Mechanically stimulated tissue at 24 hours on the pressure side and (b) on the tension side. (c) Mechanically stimulated tissue at 72 hours on the pressure side and (d) on the tension side. M2e and M3e, enamel of the second and third molars, respectively; GCT, gingival connective tissue. Large arrows show the direction of tooth movement, small triangular arrows indicate ^{3}H-TdR-labelled JE cells.

Table 3 Cell proliferation in JE on the pressure side (distal to M2) of the papilla between M2 and M3 of mechanically stimulated and unstimulated control animals. Results are presented as mean percentage of ^{3}H-TdR-labelled cells ± SE obtained from different tissue compartments. *P*-values of the ANOVA over the time course are given for each compartment, * indicates a statistically significant difference at $P < 0.05$ between stimulated and control groups at individual time periods.

Compartments	Basal		Suprabasal		Apical	
	Stimulated	Control	Stimulated	Control	Stimulated	Control
1 h	9.93 ± 1.60	11.75 ± 1.12	2.51 ± 0.42	3.86 ± 0.57	2.83 ± 0.70	3.5 ± 1.18
12 h	14.85 ± 3.00	17.88 ± 2.44	5.33 ± 0.48	5.87 ± 0.43	5.29 ± 2.56	5.43 ± 1.04
24 h	4.47 ± 0.78*	8.02 ± 0.73	4.88 ± 0.55*	13.71 ± 1.02	2.67 ± 0.66*	7.33 ± 0.88
48 h	1.13 ± 0.42*	5.77 ± 1.28	4.83 ± 0.51*	7.43 ± 0.96	2.17 ± 0.88*	6.50 ± 0.99
72 h	2.20 ± 0.74	2.35 ± 0.71	3.73 ± 0.55	4.95 ± 0.82	2.00 ± 0.68	3.83 ± 0.83
120 h	0.12 ± 0.12	1.22 ± 0.53	1.77 ± 0.43	2.17 ± 0.43	1.00 ± 0.31	1.86 ± 0.40
168 h	0.20 ± 0.20	0.38 ± 0.24	0.00 ± 0.00	0.67 ± 0.30	0.00 ± 0.00	0.50 ± 0.34
ANOVA	$P = 0.0042$		$P = 0.0001$		$P = 0.0015$	

Table 4 Cell proliferation in JE on the tension side (mesial to M3) of the papilla between M2 and M3 of mechanically stimulated and unstimulated control animals. Results are presented as mean percentage of ^{3}H-TdR-labelled cells ± SE obtained from different tissue compartments. *P*-values of the ANOVA over the time course are given for each compartment, * indicates a statistically significant difference at $P < 0.05$ between stimulated and control groups at individual time periods.

Compartments	Basal		Suprabasal		Apical	
	Stimulated	Control	Stimulated	Control	Stimulated	Control
1 h	7.52 ± 0.77	8.87 ± 2.16	4.12 ± 0.47	3.77 ± 0.77	0.67 ± 0.21	1.33 ± 0.33
12 h	18.70 ± 2.99	19.80 ± 3.94	8.90 ± 1.51	7.94 ± 0.53	5.43 ± 1.76*	10.86 ± 1.50
24 h	6.38 ± 0.99	6.12 ± 0.63	9.37 ± 0.53	8.42 ± 0.79	2.17 ± 0.60	3.50 ± 1.06
48 h	1.38 ± 0.53	3.67 ± 0.40	5.45 ± 0.55	7.99 ± 0.96	2.00 ± 0.37	3.00 ± 1.34
72 h	1.57 ± 0.59	1.32 ± 0.45	3.20 ± 0.27	5.08 ± 0.86	1.33 ± 0.62	2.00 ± 1.07
120 h	0.18 ± 0.18	0.53 ± 0.34	1.29 ± 0.44	0.94 ± 0.51	1.00 ± 0.31	1.43 ± 0.48
168 h	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.27 ± 0.27	0.00 ± 0.00	0.33 ± 0.21
ANOVA	$P = 0.4237$		$P = 0.4515$		$P = 0.0055$	

statistical comparison of PLCs from mechanically stimulated animals with their corresponding controls, nor were there significant differences between the groups at individual times.

Discussion

The animal model employed in the present work has been widely used and is well characterized with regard to the magnitude of mechanical forces, generalized degree of tissue deformation and resulting tooth movement (Fukui, 1993; Katona *et al*., 1995; Yamane *et al*., 1997). Orthodontic latex elastics of the type used in this investigation, when placed between rat molars create forces around 0.14 N (Fukui, 1993). As shown by measurements of tooth movement occurring under identical (Panagiotis *et al*., 1999) or very similar (Katona *et al*., 1995; Yamane *et al*., 1997) conditions, in this experimental model, initially high force levels tend to decay rapidly, the molars are physically moved within their sockets, and the socket walls are deformed within minutes after initiation of the force. The latex bands stay in place for 5 days, and relapse of the tooth movement takes place between 120- and 168-hour periods of stimulation (Panagiotis *et al*., 1999). Thus, the method has the

Table 5 Cell proliferation in gingival connective tissue of the papilla between M2 and M3 in the vicinity of JE of mechanically stimulated experimental and unstimulated control animals. 'Subjacent'—the compartments subjacent to the apical limit of JE, 'Near BM'—the compartments near the basement membrane. Results are presented as mean percentage of ^{3}H-TdR-labelled cells ± SE obtained from different tissue compartments. *P*-values of the ANOVA over the time course are given.

Compartments	'Subjacent' pressure		'Subjacent' tension		'Near BM' pressure		'Near BM' tension	
	Stimulated	Control	Stimulated	Control	Stimulated	Control	Stimulated	Control
1 h	1.35 ± 0.40	1.17 ± 0.40	2.32 ± 0.27	2.37 ± 0.45	1.36 ± 0.69	2.16 ± 0.74	2.47 ± 0.87	1.51 ± 0.77
12 h	1.51 ± 0.37	1.49 ± 0.50	2.10 ± 0.37	1.90 ± 0.66	1.66 ± 0.71	3.59 ± 0.68	2.97 ± 0.89	1.35 ± 0.56
24 h	2.70 ± 0.98	1.87 ± 0.63	3.37 ± 1.00	1.87 ± 0.43	2.70 ± 1.15	2.40 ± 0.85	4.52 ± 1.38	3.63 ± 0.80
48 h	0.60 ± 0.40	1.60 ± 0.56	2.41 ± 0.58	1.73 ± 0.45	2.06 ± 0.63	2.01 ± 0.88	1.79 ± 0.77	2.65 ± 0.87
72 h	1.62 ± 0.50	1.11 ± 0.59	2.01 ± 0.37	1.11 ± 0.40	4.71 ± 0.98	4.30 ± 0.89	5.40 ± 0.88	4.33 ± 0.58
120 h	1.15 ± 0.26	2.07 ± 0.38	0.94 ± 0.32	1.75 ± 0.40	3.56 ± 0.78	3.86 ± 0.50	1.89 ± 0.97	2.69 ± 0.6
168 h	1.32 ± 0.39	0.91 ± 0.50	0.61 ± 0.45	1.18 ± 0.34	0.79 ± 0.54	2.93 ± 1.25	1.07 ± 0.55	0.84 ± 0.56
ANOVA	$P = 0.9876$		$P = 0.3216$		$P = 0.1586$		$P = 0.3122$	

virtue of simplicity, while possessing many of the effects of an orthodontic appliance.

PLCs calculated for the various tissue compartments of JE and GCT in unstimulated control animals closely correspond to the steady-state levels of proliferative activity reported for rodent JE (Beagrie and Skougaard, 1962) and GCT (Pender *et al*., 1988).

There was a sharp increase in PLCs in the basal layer of unstimulated JE on both sides of the papilla at 12 hours followed by a rapid, progressive and almost complete decline in PLCs at subsequent examination times (Tables 3 and 4). This can be explained on the basis of sequential cell division and attenuation of ^{3}H-TdR-label among daughter cells down to levels approximating background labelling (Scragg and Johnson, 1980). In the suprabasal layer of controls, however, peak PLC values appeared 24 hours after ^{3}H-TdR-administration (Tables 3 and 4) indicating that, in accord with the cell hierarchy model proposed for JE by Kellett *et al*. (1987) and in contrast to stratified squamous epithelia in which keratocyte progenitors lose proliferative capacity after leaving the basal layer (Schroeder, 1986), a concomitant cell division of progenitors, while migrating through the suprabasal layer might occur in JE.

As indicated by the more rapid decline in PLCs (Table 3), on the pressure side, mechanical stimulation brought about a general pattern of significantly reduced proliferation in all compartments over the time course relative to controls. However, it also caused a significant reduction of cell proliferation both in the basal and suprabasal layers themselves over the time course, which was especially prominent 24–48 hours after the onset of mechanical load. A similar response was revealed in the apical compartments on both sides of the papilla (Tables 3 and 4). These results provide novel information regarding the cell kinetic reactions of epithelium when subjected to pressure and suggest that pressure by reducing the volume of JE also reduces the necessity for production of new cells during re-organization of the tissue when it is spatially constrained.

The explanation of the more rapid decline in PLCs revealed on the pressure side of mechanically stimulated JE (Table 3) as a reduction of cell proliferation, is based on the assumption that the mean cycle time of JE cells does not alter in response to the stimulus. However, if reactions of JE cells to mechanical stimulation included a decrease of the cell cycle time, that is a faster progression of cells through the cell cycle, another possibility of data interpretation should be considered. It is conceivable in such an eventuality, that the observed more rapid decline in PLCs might be based on faster ^{3}H-TdR-label dilution resulting from a rapid successive division of labelled cells, and thus imply that a very early transient increase of

proliferation might have occurred in response to mechanical pressure. The mean cell cycle time of oral epithelial cells is approximately 18 hours (Kellett *et al*., 1987) and the S-phase lasts about 7–8 hours (Scragg and Johnson, 1980). Therefore, a very substantial cell cycle time decrease would be necessary in this case to accommodate a potential occurrence of at least two successive mitoses between 1–12 and 12–24-hour periods (Table 3), which on the basis of currently available information seems unlikely. In any event, as nothing is known at present about possible changes of epithelial cell cycle times under mechanical stress, further work is needed to clarify this uncertainty.

On the tension side, mechanical stimulation appeared to have no effect on proliferative activity of cells both in the basal and suprabasal layers compared with corresponding controls (Table 4). Taken together with the absence of significant differences between the measurements of the total cross-sectional areas of JE in stimulated and control specimens at any time, these results indicate that under the conditions tested, JE showed no tendency towards a hyperplastic response to mechanical stimulation. This observation may reflect specific characteristics of JE which in many respects is a unique epithelium (Schroeder, 1986) distinguishing it from other integumental epithelia which normally respond to tension or friction by increased cell division and hyperplasia (Mackenzie, 1974; Squier, 1980; Brunette, 1984). Alternatively, it is possible that mechanical stimulation as employed in this study was not of sufficient duration to elicit a more marked epithelial response. The results of the present investigation cannot provide a definite answer to this question, which certainly warrants further investigation.

The level of proliferative activity revealed in the GCT compartments over the duration of the experiment reflects a considerable cell division among GCT fibroblasts. At least three progenitor cell populations are present in gingival *lamina propria* in distinct tissue locations and with differing cell kinetic properties (McCulloch, 1986; Pender *et al*., 1988; McCulloch and Knowles, 1991). In the inter-dental papilla they reside within a 5–10-μm band adjacent to the basement membrane of JE and to cementum, within the body of the papilla some 20–60 μm from JE, and immediately coronal to the inter-dental bone crest (Pender *et al*., 1988). The size and position of the tissue compartments which were used in the present investigation were chosen in order to include areas known to be rich in progenitor cells. Increased proliferative activity in response to tissue stimulation such as, for instance, wounding is an archetypal attribute of progenitor cells (Lajtha, 1979). It is noteworthy, therefore, that mechanical stimulation had no apparent effect on proliferation of these cells in the GCT compartments under investigation. This finding is in contrast to the behaviour of progenitors in PDL which show pronounced suppression or increase of proliferative activity in response to mechanical pressure (Zentner and Heaney, 1998) or tension (Roberts and Jee, 1974; Smith and Roberts, 1980; Zentner and Heaney, 1998). The lack of proliferative response in GCT compartments under the conditions of the present study may mean that the resident cells are not authentic progenitors representing, instead, perhaps more differentiated progeny with some remaining limited proliferative capacity. Alternatively, it may be speculated that some hypothetical, currently unknown force magnitude threshold necessary to elicit a response in these cell populations might not have been reached in these tissue compartments under the experimental conditions of this study. Specifically it is possible that mechanically induced initial movement of M2 and M3, which under similar experimental conditions involves substantial deformation of PDL and adjacent alveolar bone (Katona *et al*., 1995), by dragging the more movable part of the papilla coronal to the attached trans-septal fibre region, precluded local development of sufficiently high levels of mechanical force within the GCT compartments. In that event, evidence of an increase in PLCs relative to controls might be expected to occur in the papillary zone immediately overlying the crest of the inter-dental septum. Further work is now required to confirm this possibility.

The conspicuous decrease of the distance between the apical limit of JE and the most coronal PDL on the pressure side of the

mechanically stimulated specimens (Table 1) might, at first sight, signify occurrence of apical migration of JE concurrent with connective tissue attachment loss. However, this eventuality must be discounted because the observed reduction occurred very rapidly, that is as early as 1 hour after the initiation of stimulation. Given that epithelial cells require at least several hours for transition from stationary to migratory phenotype (Stenn and Depalma, 1988) it is most unlikely that the apical cells of JE would have migrated some 50 μm in such a short period. Furthermore, no increase of proliferative activity took place in the apical compartments of the JE on either side under mechanical load at any time period tested in this study. Finally, a coincident increase in the distance between JE and PDL occurred on the tension side (Table 1), indicating that the changes of this parameter on either side were merely of a geometric nature associated with distally directed tipping of the crowns of M2 and M3, and distortion of their inter-proximal gingival papilla between the crowns and the inter-dental bone crest. The occurrence of such tipping under the experimental conditions employed was confirmed by detailed measurements carried out by Panagiotis *et al*. (1999).

Moreover, as revealed by the experimental and control PLC values, proliferation of GCT cells in the vicinity of the basement membrane of JE and subjacent to its apical limit in mechanically stimulated animals did not differ from the level of proliferative activity of control animals, that is of the proliferative activity necessary for homeostatic maintenance in these tissue compartments. These findings indicate a certain degree of stability of local resident gingival papillary connective tissue cell populations under mechanical load in contrast to cell death and reduced compensatory proliferative activity which tend to occur in this area during progressive loss of connective tissue attachment and pocket formation in inflammatory gingival lesions (Nemeth *et al*., 1993).

Taken together, the results of this study suggest that as an early reaction to mechanical force, JE may rapidly adapt by means of differential local adjustments of cell proliferation within itself without the occurrence of apical migration or hyperplasia. This does not support the notion that orthodontic mechanical stimulation *per se* might be detrimental to the stability of the dentogingival junction.

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