# Identification of dentine sialoprotein in gingival crevicular fluid during physiological root resorption and orthodontic tooth movement

# Shalene Kereshanan, Pamela Stephenson and Rachel Waddington

School of Dentistry, Cardiff University, Heath Park, Cardiff, Wales, UK

SUMMARY Root resorption is an unwanted effect of orthodontic tooth movement. Analysis of dentine proteins in gingival crevicular fluid (GCF) is a potentially safer method of quantifying root resorption compared with conventional radiographic methods. This study aimed to identify and quantify the dentinespecific matrix protein, dentine sialoprotein (DSP), released into GCF during physiological root resorption and orthodontic tooth movement. GCF was collected using micropipettes from 50 second primary molar sites undergoing physiological root resorption in 9- to 14-year olds [coronal group (Rc) with advanced resorption (n=33) and apical group (Ra) with minimal resorption (n=17)] and 20 subjects aged 8–14 years with erupted mandibular second premolars (control group). In addition, GCF was collected from 20 patients undergoing treatment with fixed appliances at two time points, immediately prior to orthodontic intervention (T0) and 12 weeks following commencement of fixed appliance therapy (T1). GCF samples were analysed for DSP using an immunoassay and levels semi-quantified using image analysis. To determine differences between the means of the various experimental and control groups, data based on the relative optical density volumes, were statistically analysed using a parametric *t*-test.

DSP was raised in sites that were undergoing physiological resorption compared with the non-resorbing controls (P < 0.05). Notably, DSP was detected in some control samples. There was no difference in DSP levels for the Rc or Ra groups. DSP was also raised in GCF samples of teeth at 12 weeks following commencement of fixed appliance therapy (P < 0.001). The results highlight the potential for measuring DSP in GCF as a biomarker to monitor root resorption. Dentine is likely to be the major source for DSP in GCF, although alternative origins of bone and cementum are possible.

## Introduction

Unlike the resorption of roots of primary teeth, which is a physiological phenomenon, the resorption of roots of permanent teeth is a pathological process usually associated with trauma, chronic pulpal pathology, tooth impaction, or mechanically induced pressures within the periodontal ligament during orthodontic tooth movement (Fuss et al., 2003). Apical root resorption is a common clinical problem which may complicate orthodontic tooth movement and presents with variable frequency and extent (Brezniak and Wasserstein, 1993a,b, 2002). It has been accepted that the reported incidence of severe apical root resorption after orthodontic treatment is about 1–2 per cent (Killiany, 1999). Accordingly, apical root resorption is of limited clinical significance for the average patient. However, for the susceptible patient, apical root resorption may limit the outcome of successful orthodontic treatment.

At present, clinical diagnosis of root resorption is largely radiographic. Radiographic examination offers wide accessibility, ease of use, and cost-effectiveness, but is not without its limitations (Sameshima and Asgarifar, 2001). Problems of technique, standardization, limited projection views, and radiation exposure persist. Radiographs do not allow accurate identification of root resorption in the early stages and often fail to reveal surface resorption on the lingual and buccal aspects of the roots (Acar *et al.*, 1999). Computerized tomography and cone beam volumetric imaging have been shown to increase sensitivity in detecting root resorption (Walker *et al.*, 2005). However, the cost and high radiation exposure make it impracticable for routine use in dentistry. Given these current limitations of radiographic methods, there is a need for establishing a safer, reliable alternative method to clinically diagnose root resorption.

This study investigated the potential use of gingival crevicular fluid (GCF) for the analysis of matrix protein components as a biomarker of root resorption. GCF is the inflammatory transudate that flows out via the gingival crevice. The quantity and composition of the fluid varies depending on the health of the periodontium. GCF is known to contain an array of biochemical and cellular factors that reflect the state of the underlying periodontium (Uematsu *et al.*, 1996; Waddington and Embery, 2001). Balducci *et al.* (2006) explored the presence of dentine sialoprotein (DSP), dentine phosphoprotein (DPP), and dentine matrix protein-1 (DMP-1) in the GCF of patients diagnosed with mild and severe resorption after at least 1 year of fixed appliance therapy in comparison with untreated controls as confirmed

by intraoral periapical radiographs. Those authors concluded that the use of DSP and DPP as biomarkers were suitable alternatives for monitoring root resorption during orthodontic tooth movement. Of the various dentinal noncollagenous proteins, DSP and DPP are the most abundant proteins present within dentine. DPP accounting for approximately 50 per cent (Dimuzio and Veis, 1978) and DSP for 5-8 per cent (Butler and Ritchie, 1995) of dentine non-collagenous proteins. DSP and DPP are N- and Cterminal proteolytic cleavage products of dentine sialophosphoprotein (DSPP), respectively, and belong to the small integrin-binding ligand N-linked glycoprotein family of proteins (Fisher et al., 2001). Immunolocalization studies have shown that DSP is localized within odontoblasts, the cells and the extracellular matrix of dental pulp, predentine, and dentine (Butler et al., 1992). DSP is not found in ameloblasts, bone, cartilage, soft tissues, or other components of the oral tissues, suggesting that it is highly dentine specific (Butler and Ritchie, 1995).

The aim of the present study was to further investigate the potential of DSP in GCF as a biomarker of root resorption using alternative clinical models to those previously described by Balducci et al. (2006). Levels of DSP released into GCF were measured during physiological root resorption of primary teeth and from non-resorbing erupted mandibular second premolars. The results were considered alongside levels of DSP in GCF samples immediately prior to fixed appliances therapy and at 12 weeks following the initial phase of levelling and alignment of teeth during orthodontic tooth movement. The ability to detect the presence of dentinal proteins early during pathological root resorption will aid the clinician to instigate prompt treatment if there is a suspicion of root resorption. However, the detection of DSP in these various clinical situations additionally allows for the evaluation of the origins of the biomarkers from dental tissues other than dentine.

#### Materials and methods

#### Collection of GCF

Ethical approval was obtained from the South East Wales Research Ethics Committee and consent from the patients who participated in the study or their parents.

The experimental subjects were divided into two groups. The first group comprised 50 patients between 9 and 15 years of age who had second primary molars undergoing physiological root resorption as confirmed by dental panoramic tomograms (DPTs). Other inclusion criteria were no relevant medical history (e.g. connective tissue or blood disorders), no prior history of orthodontic treatment, caries free, and no associated pulpal pathology. This group was further subdivided into two groups dependent on the degree of root resorption that had taken place. The apical group (Ra) demonstrated resorption from the apex up to half the root length and the coronal group (Rc) more advanced resorption from halfway up the root to the amelocemental junction. The control group (n=20) was 10- to 15-year-old patients attending the orthodontic department who had fully erupted second premolars, no relevant medical history, and no prior history of orthodontic treatment. These teeth were also caries free and had no pulpal pathology.

The second experimental group included 20 patients aged 11–15 years who were attending the orthodontic department for orthodontic treatment with fixed appliances. The inclusion criteria were the absence of any relevant medical disorder, no prior history of orthodontic treatment, no active caries, and no evidence of root resorption as confirmed by DPTs. The samples were collected from the distal gingival crevicular margins of a premolar, canine, and a central incisor in both the upper and lower dental arches. These GCF samples were obtained at two time points: immediately prior to orthodontic intervention (T0) and 12 weeks following the placement of orthodontic pre-adjusted edgewise appliances (T1). The bracket system used was 0.022 × 0.028 inch slot: MBT<sup>™</sup> prescription (3M Unitek, St Paul, Minnesota, USA) with a 0.012 or 0.016 inch round austenitic active nickel-titanium archwire for the purpose of initial levelling and alignment.

Each sample of GCF was collected using  $5 \,\mu$ l micropipettes (Drummond Scientific Co., Broomall, Pennsylvania, USA) by capillary action over a 10 minute period. The samples were stored at  $-80^{\circ}$ C prior to laboratory analysis.

#### Isolation of DSP from dentine

Twenty primary second molars were collected and debrided of any soft tissue and alveolar bone fragments. Isolation of DSP from dentine was carried out based on previously described methods (Waddington et al., 1993) for the chaotrophic dissociation and isolation of non-collagenous proteins and further separation by anion exchange chromatography. Briefly, the teeth were powdered in a ball mill at -70°C and then demineralized in 10 per cent ethylenediaminetetraacetic acid trisodium salt (pH 7.45 and containing protease inhibitors 1 mM iodoacetic acid, 5 mM benzydamine hydrochloride, and 5 mM N-ethylmaleimide) at 4°C for 15 days. The residual organic matrix was exhaustedly dialysed at 4°C against double distilled water containing the above-mentioned protease inhibitors for 4 days followed by 1 day against double distilled water only and then recovered by lyophilization. Non-collagenous proteins were extracted into 4 M GuCl, 0.5 M sodium acetate buffer with protease inhibitors, pH 5.9, at 4°C for 48 hours after which the insoluble collagenous matrix was removed by centrifugation at 3300 rpm at 4°C for 15 minutes and discarded. The supernatant containing the non-collagenous proteins was exhaustively dialysed against double distilled water, after which the dialysate was lyophilized.

Separation of the non-collagenous components was achieved by anion exchange chromatography using a 20 ml, Resource Q anion exchange column, GE Healthcare UK Ltd, Amersham, Buckinghamshire, UK, incorporated into a fast performance liquid chromatography system (Pharmacia LKB, Ramsey, Minnesota, USA). The lyophilized non-collagenous matrix was dissolved in 0.1 M Tris-HCl, pH 7.4, at a concentration of 2 mg/ml and 2 ml samples were applied to a column. Unbound material was eluted with 20 ml of the above elution buffer at a flow rate of 1 ml/minute. Bound material was selectively eluted by the application of an increasing gradient of 0-0.5 M of NaCl over 20 ml elution buffer. Elution profiles were monitored by absorbance at 280 nm and the individual protein fractions were collected, pooled, and dialysed against distilled water and lyophilized. The samples were then reconstituted in double distilled water at 10 mg/ml. Based on the 280 nm elution profile, seven protein peaks were resolved. Immunodetection of DSP for each fraction by western blot analysis (described below) indicated that DSP eluted with a salt concentration of 0.24-0.32 M NaCl (data not shown).

#### Immunodetection of DSP by Western blot analysis

Fractions arising from anion exchange analysis and GCF samples collected from resorbing primary molar sites were examined for immunoreactivity of the antibodies to DSP using western blot analysis. The samples were mixed with equal volume of sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) dissociating sample buffer containing 0.062 M Tris–HCl, pH 6.8, 10 per cent glycerol, 2 per cent SDS, 5 per cent 2- $\beta$ -mercapthoethanol, and 0.002 per cent bromophenol blue. The samples (1 µl) were then separated on 4–15 per cent polyacrylamide gels (GE Healthcare UK Ltd) using the Phastsystem (GE Healthcare UK Ltd) as previously described (Waddington *et al.*, 1993). Each gel also contained a 1 µl sample of BioRad-prestained molecular weight standard protein markers (198–6.9 kDa).

Following electrophoresis, the separated components were electroblotted onto nitrocellulose membranes (Whatman Schleicher and Schuell, Protran®, Maidstone, Kent, UK) using the Phastsystem electroblotting apparatus at 20 V, 1.0 W, 5.0 Vh, at 15°C. Non-specific protein-binding sites on the membranes were then blocked with 5 per cent powdered milk (Marvel<sup>™</sup>, Premier Foods, St Albans, Hertfordshire, UK) in 100 mM Tris, 100 mM NaCl, and 5 mM MgCl2, pH 9.55 [Tris buffered saline (TBS)], for 1 hour at room temperature. Immunoreactivity of the electroblotted proteins was assessed using rabbit polyclonal antibodies LF-151, a primary polyclonal antibody to part of the DSP portion of human DSPP (1:250 dilution in 3 per cent powdered milk in the above TBS), for 1 hour. Following washing with TBS, immunoreactivity was detected by incubating the membranes with an anti-rabbit IgG conjugated to alkaline phosphatase (1:10 000; Sigma–Aldrich Co., Poole, Dorset, UK), a visualization using colorimeric substrate system NBT/BCIP (Promega UK Ltd, Southampton, Hants, UK).

#### Slot blot analysis

Semi-quantification of DSP within the collected GCF samples was carried out using a slot blot analysis. The GCF samples were mixed with 200 µl TBS (100 mM Tris, 100 mM NaCl, and 5 mM MgCl2). The samples were then further diluted in TBS to give 1:2 and 1:4 dilutions. Nitrocellulose membranes (Whatman Schleicher and Schuell, Protran<sup>®</sup>, Maidstone, Kent, UK) were briefly soaked in TBS and then sandwiched within the slot blot manifold containing  $3 \times 12$  wells (Hoefer PR 648, Amersham Biosciences, Amersham, Buckinghamshire, UK). One hundred microlitres of the neat and diluted GCF samples were then loaded into the wells which were then drawn onto exposed nitrocellulose membranes at the base of the wells by application of a vacuum to the apparatus. The membranes were then removed from the apparatus and non-specific protein-binding sites were blocked with 5 per cent powdered milk and immunoreactivity to rabbit polyclonal anti-DSP (LF-154) was detected as described above for the western blot procedure. Following staining, the membranes were dried and staining intensity was quantified using the BioRad image analysis system (Quantity 4.3.1 software Hemel Hempstead, Hertfordshire, UK). Two rows of wells on each of the membranes were loaded with anti-rabbit IgG conjugated to alkaline phosphatase (secondary antibody) diluted 1:40000, 1:80 000, and 1:160 000 as a positive control. The addition of the secondary antibody served to provide a visual aid to the development of the membrane in the colorimetric substrate. Following scanning of the membranes, the pixel count obtained for the 1:40 000 dilution of the secondary antibody was assigned an arbitrary unit value of 1.00, which then allowed quantification of relative DSP levels (represented as staining intensities, OD/mm<sup>2</sup>) obtained for the neat GCF samples. Likewise, the pixel count for the 1:80 000 dilution of the secondary antibody was assigned a value of 1.00 which was used to calculate relative DSP levels in the 1:2 samples and pixel counts for the 1:160000 dilution of the secondary antibody were used to calculate relative DSP levels in GCF samples in the 1:4 diluted samples. To confirm that membranes had not been overdeveloped, thus producing an oversaturated pixel count, for each sample in a series of serial dilutions, the OD/mm<sup>2</sup> should be approximately the same. Those values which demonstrated a difference greater than 10 per cent in OD/mm<sup>2</sup> values between higher (e.g. neat) and lower (e.g. 1:2) dilutions were discarded as they were considered oversaturated in terms of substrate development.

#### Statistical analysis

Analysis of the GCF samples at three serially diluted concentrations provided a method of statistically assessing the data for normality. Thus, frequency and distribution of DSP levels in GCF were assessed at the three different concentrations (neat, 1:2, and 1:4). Quantile–quantile (QQ) plots were constructed to visually check for the fit of a theoretical distribution to the observed data and confirm normality. The data, based on relative optical density, were statistically analysed with a parametric *t*-test using the Statistical Package for Social Sciences (version 12.0 SPSS Inc., Chicago, Illinois, USA) to determine statistical differences between the means of the various experimental and control groups.

#### Results

DSP was isolated from human teeth to provide an analytical comparison for DSP identifiable in GCF samples. Unfortunately, the DSP was not of high purity and following immunoblotting, using the polyclonal antibody to DSP, was identifiable in several of the protein peaks (data not shown). The potential presence of other co-purifying proteins in the recovered protein sample thus prevented its use as a standard amount for quantification of DSP in the slot blot analysis. However, following isolation of the DSPrich fraction from human dentine and further separation by SDS-PAGE, subsequent Western blot analysis allowed for immunoidentification of the DSP within the fraction (Figure 1). DSP migrated with a molecular weight of approximately 89 kDa, similar to that previously reported (Qin et al., 2002) and confirmed the specificity of the antibody used in subsequent analyses of DSP in GCF samples. The separation and immunodetection of DSP within two GCF samples is also shown in Figure 1. DSP was detected in the GCF sample separated in lane 3 with a molecular weight ranging from 54 to 16 kDa.

DSP was semi-quantified using a slot blot immunoassay. DSP levels represent relative staining intensities, related to a known concentration of the conjugated secondary antibody applied to the nitrocellulose membrane in the immunoassay, and not an absolute value reported as a weight (e.g.  $\mu$ g). The semi-quantitative data were subjected to QQ plot (Figure 2) to assess parametric distribution. The near distribution of the data points to the predicted straight line (y=x) suggested an approximately normal distribution of DSP levels in GCF. QQ plots were calculated for all three concentrations and analysed; all demonstrated data points for a near normal distribution.

Figure 3 shows the collective frequency distribution of the relative DSP levels calculated for GCF samples collected from sites where coronal, apical, or no resorption of the tooth root was apparent. The data represent the DSP levels for the analysis of the neat samples only. Values obtained for those teeth where root resorption had been determined



**Figure 1** Western blot analysis for the immunodetection of dentine sialoprotein (DSP) in fractionated dentine and gingival crevicular fluid (GCF). The samples were separated by sodium dodecyl sulphate–polyacrylamide gel electrophoresis prior to immunoanalysis. Lane 1— DSP-rich fraction purified from dentine of human primary teeth; lane 2—GCF collected from around non-resorbing teeth; and lane 3—GCF collected from around resorbing teeth.

indicated a near normal distribution of DSP. Of note, 50 per cent of the control samples (n=20) demonstrated positive, minimal staining for DSP.

Having demonstrated the normality of the data, parametric Student's *t*-tests were used to compare the means of the relative DSP levels in GCF collected from resorbing primary molars and the non-resorbing permanent premolar (control). This was repeated for values obtained at all three dilutions analysed. The mean difference between the resorbing group and the non-resorbing premolars was approximately 0.3 ODu/mm<sup>2</sup> (95 per cent confidence interval of 0.1–0.4 ODu/mm<sup>2</sup>) for all dilutions (Table 1). Uniformity of the mean levels and mean differences for each of the dilutions analysed confirmed the validity of the method used to provide a measure of the relative DSP levels.

Comparison of the mean relative DSP levels in GCF (neat data only shown) collected from the teeth of subjects with Rc, Ra, or no resorption (control) is shown in Figure 4. A statistically significant difference was noted when comparing the mean difference in relative DSP levels between the Rc  $[0.30 \pm 0.10 \text{ ODu/mm}^2; (P < 0.05)]$  or the Ra  $[0.20 \pm 0.10 \text{ ODu/mm}^2; (P < 0.05)]$  groups with the control group. However, there was no significant difference in the relative DSP levels when comparing the Ra and the Rc groups (P > 0.05) with the resorbing primary molar group.



Figure 2 Quantile-quantile plot demonstrating the near normal distributions of the relative dentine sialoprotein levels within gingival crevicular fluid at all three dilutions analysed.



Figure 3 Frequency distribution of the relative dentine sialoprotein levels calculated for the independent gingival crevicular fluid samples collected from teeth associated with apical (Ra), coronal (Rc), or no resorption (control) of the root. Only data obtained for analysis of neat samples shown.

The data obtained for arbitrary DSP levels in GCF collected at T0 and T1 were subjected to statistical analyses similar to that described above. QQ plots suggested that the data obtained followed an approximately normal distribution, despite the small sample size (Figure 5). This was observed for each of the sample dilution analysed. Figure 6 shows the frequency distribution of the relative DSP levels for GCF collected at T0 and T1. Low levels of DSP were observed in most GCF samples at T0. The levels were raised in the GCF collected at T1. Analysis of the data using a paired sample t-test indicated a statistically significant increase in the levels of DSP in GCF samples at T1 compared with T0 levels (Table 2). The mean difference in the levels of DSP between T0 and T1 was  $0.40 \pm 0.10$  ODu/mm<sup>2</sup>. There was a marked increase in DSP being liberated into GCF at T1 at all three concentrations (P < 0.001).

#### Discussion

Given the current diagnostic limitations of radiographs, an alternative method which provides clinicians with the ability to scrutinize the root status of patients at risk of root resorption 
 Table 1
 Mean relative dentine sialoprotein levels in gingival crevicular fluid of resorbing primary second molar sites (apical and coronal group data combined) compared with controls.

Dilution	Mean levels (SD) in ODu/mm <sup>2</sup>		Mean difference in ODu/mm <sup>2</sup> (95% CI)	P value
	Resorbing molars $(n=50)$	Controls $(n=20)$		
Neat 1:2 1:4	0.593 (0.254) 0.506 (0.265) 0.528 (0.260)	0.326 (0.217) 0.238 (0.213) 0.275 (0.213)	0.267 (0.108, 0.375) 0.269 (0.139, 0.397) 0.253 (0.111, 0.394)	0.001 0.001 0.002

through non-invasive, safe, and site-specific means is highly desirable. Crevicular fluid diagnostics is dependent on the identification of specific biomarkers associated with the disease state supported by the appropriate research methodologies that can discriminate between the various biomarkers available. This study identified the presence of DSP in GCF samples. Western blot analysis suggested the presence of a biomarker with molecular weight between 54 and 16 kDa. The intact DSP isolated from human primary teeth was of a higher molecular weight, 89 kDa, thus indicating that the DSP in GCF associated with root resorption likely represents a degradation product. This concurs with parallel studies which have indicated a similar molecular weight of 55 kDa and below for proteoglycan metabolites in GCF (Waddington *et al.*, 1998). This value probably represents the cut-off molecular weight for the passage of biomarkers from



Figure 4 The mean relative dentine sialoprotein levels in gingival crevicular fluid associated with apical (Ra), coronal (Rc), or no resorption (control) of the root. Only data obtained for analysis of neat samples shown.

the deeper seated tissues into the gingival crevice.

This *ex vivo* study was based on the assumption that the composition of the non-collagenous proteins is comparable in both the primary and permanent dentitions. To date, although it is known that there are microscopic differences in the structure of dentine in the primary and permanent dentitions (Ten Cate, 1998), there are no available data in the literature characterizing the structure of non-collagenous proteins in the human primary dentition. Based on a number of studies (Ten Cate and Anderson, 1986; Sahara, 2001; Fukushima *et al.*, 2003; Sasaki, 2003) on the resorption of dentine, researchers have accepted the use of physiological root resorption. It is agreed that although the initiation process of root resorption may differ, the actual biochemical processes that take place are largely similar.

A comparison between the Ra, Rc, and control groups demonstrated that exfoliation of the primary molar involves degradation of the dentine matrix with the subsequent release of the dentinal matrix protein, DSP, into GCF. At both the initial and advanced stages of resorption, crevicular fluid analyses indicated that there was no statistical difference in the expression of DSP in GCF as the primary dentine matrix undergoes physiological resorption. This may reflect the relatively small sample size of the Ra group (n=17) in comparison with the Rc group (n=33). Alternatively, this may reflect no difference in the localization of DSP at the cervical and apical regions of the



**Figure 5** Quantile–quantile plot demonstrating the approximately normal distributions of the mean differences of dentine sialoprotein within gingival crevicular fluid between the start of the intervention and 12 weeks following orthodontic tooth movements. Data shown for each dilution analysed.

**Table 2** Paired sample *t*-test to determine mean differences in relative dentine sialoprotein levels in gingival crevicular fluid samples collected from teeth prior to orthodontic intervention (T0) and 12 weeks following orthodontic movement (T1).

Dilution	Mean levels (SD) in ODu/mm <sup>2</sup>		Mean differences in ODu/mm <sup>2</sup> (95% CI)	P value
	Т0	T1		
Neat (T1-T0)	0.449 (0.259)	0.875 (0.151)	0.425 (0.311, 0.540)	0.001
1:2 (T1–T0)	0.364 (0.250)	0.765 (0.167)	0.401 (0.288, 0.514)	0.001
1:4 (T1–T0)	0.401 (0.240)	0.793 (0.168)	0.392 (0.293, 0.490)	0.001



**Figure 6** Frequency distribution of the relative dentine sialoprotein (DSP) levels calculated for the independent gingival crevicular fluid samples collected from teeth prior to orthodontic intervention (T0) and 12 weeks following orthodontic movement (T1). Only data obtained for analysis of neat samples shown.

roots (Ohma *et al.*, 2000) and may suggest that the rate of resorption may be similar at the apical and cervical regions of the root. The results would indicate that the release of DSP into GCF is determined by the rate of resorption of the dentine matrix as opposed to the site/region of the root undergoing resorption.

In 50 per cent of the control GCF samples, DSP was considered absent as no staining was observed as anticipated on the slot blot analyses. However, the presence of DSP was detected in 10 control GCF samples (Figure 4) although at reduced levels. The presence of DSP in 50 per cent of these control samples was not anticipated as the root forms were not observed to be undergoing any visible structural changes on the DPTs. Similarly, the study carried out by Mah and Prasad (2004) based on the enzyme-linked immunosorbant assay method also found the presence of DPP in GCF collected from their untreated control subjects. These findings may reflect complex cellular and structural changes within the periodontium involved at the mineralization front as the maturation of the root takes place. It has been shown that basal turnover of dentine matrix proteins occurs during the maturation process of the root structures of the young permanent adult dentition and that DSP may be liberated from pulpal cells as the roots of these premolar controls may have patent apices (Chang et al., 1996; MacDougall et al., 1997). Additionally, there are some suggestions that DSP may not be entirely dentine specific (Qin et al., 2002). DSP and DPP are expressed as a single messenger RNA (mRNA) transcript and this transcript encodes for a large precursor protein termed DSPP traditionally considered to be dentine specific. Qin et al. (2002) found that the dspp gene was expressed in osteoblasts cells. Based on Western immunoblots, DSP was detected in extracts of rat long bone at a level of about 1:400 of that in dentine. Using reverse transcriptase polymerase chain reaction techniques with primers specific to the 5' DSP portion and to the 3' DPP

sequence, DSPP mRNA was detected in osteoblast-like cells and mouse calvarial osteoblasts. However, this gene was expressed at a much lower level in osteoblasts than in odontoblasts. The data indicated that different regulatory mechanisms governing DSPP expression are involved in teeth and bone. The presence of DSP in bone, although at a very low level, may reflect the slight staining of the slot blotassociated control GCF samples during the maturation stages of the premolar root within the periodontium. Finally, although not supported by the literature, a plausible explanation is that cementum contains DSP within its matrix, such that DSP is released into GCF as a natural consequence of the resorption/repair process of cementum during physiological root resorption and also during orthodontic tooth movement. Collectively, these may explain the slight expression of DSP in the control subjects.

Balducci et al. (2006) explored the presence of DSP, DPP, and DMP-1 in the GCF of patients diagnosed with mild and severe resorption after at least 1 year of fixed appliance therapy in comparison with untreated controls as confirmed by intraoral perapical radiographs. They concluded that the use of DSP and DPP as biomarkers were suitable alternatives for monitoring root resorption during orthodontic tooth movement. The findings of the present study add value to these results as the increase in the levels of DSP could be detected as early as 12 weeks following orthodontic force application. This further confirms that during the initial stages of fixed appliance therapy, DSP is liberated into GCF as the dentine matrices of the permanent roots undergo intermittent phases of surface resorption and repair. The dentine surfaces were being resorbed during the early stages of fixed appliance therapy with light orthodontic forces applied using superelastic round 0.012 or 0.016 inch nickel-titanium archwires (P < 0.05). Parallel to these findings, it has been shown, using confocal laser scanning microscopy, that following initial superelastic archwire placement at 12 weeks (Weiland, 2003), dentine resorption takes place.

### Conclusions

Collectively, the results of this study demonstrate the potential of DSP as an analytical biomarker in GCF for root resorption. The findings highlight the potential for DSP in GCF to originate from non-dentinal tissues such as bone and cementum. Thus, in the development of any bioassay, the contribution of DSP from such periodontal tissues will need to be assessed. However, the development of a bioassay based on this analytical biomarker would enable the clinician to instigate a prompt treatment response when there is a clinical suspicion of root resorption.

#### Address for correspondence

Dr Pamela Stephenson Dental Health and Biological Sciences School of Dentistry Cardiff University Heath Park Cardiff CF14 4XY Wales UK E-mail: pamelastephenson@lineone.net

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#### References

- Acar A, Canyurek U, Kocaaga M, Erverdi N 1999 Continuous vs. discontinuous force application and root resorption. Angle Orthodontist 69: 159–163
- Balducci L, Ramachandran A, Hao J, Narayanan K, Evans C, George A 2006 Biological markers for evaluation of root resorption. Archives of Oral Biology 52: 203–208
- Brezniak N, Wasserstein A 1993a Root resorption after orthodontic treatment: part 1. Literature review. American Journal of Orthodontics and Dentofacial Orthopedics 103: 62–66
- Brezniak N, Wasserstein A 1993b Root resorption after orthodontic treatment: part 2. Literature review. American Journal of Orthodontics and Dentofacial Orthopedics 103: 138–146
- Brezniak N, Wasserstein A 2002 Orthodontically induced inflammatory root resorption. Part II: the clinical aspects. Angle Orthodontist 72: 180–184

- Butler W T Ritchie H 1995 The nature and functional significance of dentin extracellular matrix proteins. International Journal of Developmental Biology 39: 169–179
- Butler W T. *et al.* 1992 Isolation, characterization and immunolocalization of a 53-kDal dentin sialoprotein (DSP). Matrix 12: 343–351
- Chang S R, Chiego Jr D, Clarkson B H 1996 Characterization and identification of a human dentin phosphophoryn. Calcified Tissue International 59: 149–153
- Dimuzio M T, Veis A 1978 Phosphophoryns-major noncollagenous proteins of rat incisor dentin. Calcified Tissue Research 25: 169–178
- Fisher L W, Torchia D A, Fohr B, Young M F, Fedarko N S 2001 Flexible structures of SIBLING proteins, bone sialoprotein, and osteopontin. Biochemical and Biophysical Research Communications 280: 460–465
- Fukushima H, Kajiya H, Takada K, Okamoto F, Okabe K 2003 Expression and role of RANKL in periodontal ligament cells during physiological root-resorption in human deciduous teeth. European Journal of Oral Sciences 111: 346–352
- Fuss Z, Tsesis I, Lin S 2003 Root resorption—diagnosis, classification and treatment choices based on stimulation factors. Dental Traumatology 19: 175–182
- Killiany D M 1999 Root resorption caused by orthodontic treatment: an evidence-based review of literature. Seminars in Orthodontics 5: 128–133
- MacDougall M, Simmons D, Luan X, Nydegger J, Feng J, Gu T T 1997
  Dentin phosphoprotein and dentin sialoprotein are cleavage products expressed from a single transcript coded by a gene on human chromosome
  4. Dentin phosphoprotein DNA sequence determination. Journal of Biological Chemistry 272: 835–842
- Mah J, Prasad N 2004 Dentine phosphoproteins in gingival crevicular fluid during root resorption. European Journal of Orthodontics 26: 25–30
- Ohma N, Takagi Y, Takano Y 2000 Distribution of non-collagenous dentin matrix proteins and proteoglycans, and their relation to calcium accumulation in bisphosphonate-affected rat incisors. European Journal of Oral Sciences 108: 222–232
- Qin C *et al.* 2002 The expression of dentin sialophosphoprotein gene in bone. Journal of Dental Research 81: 392–394
- Sahara N 2001 Cellular events at the onset of physiological root resorption in rabbit deciduous teeth. Anatomical Record 264: 387–396
- Sameshima G T, Asgarifar K O 2001 Assessment of root resorption and root shape: periapical vs panoramic films. Angle Orthodontist 71: 185–189
- Sasaki T 2003 Differentiation and functions of osteoclasts and odontoclasts in mineralized tissue resorption. Microscopic Research and Technic 61: 483–495
- Ten Cate A R 1998 Oral histology. Development, structure and function 5th edn. C V Mosby, St Louis
- Ten Cate A R, Anderson R D 1986 An ultrastructural study of tooth resorption in the kitten. Journal of Dental Research 65: 1087–1093
- Uematsu S, Mogi M, Deguchi T 1996 Interleukin (IL)-1 beta, IL-6, tumor necrosis factor-alpha, epidermal growth factor, and beta 2-microglobulin levels are elevated in gingival crevicular fluid during human orthodontic tooth movement. Journal of Dental Research 75: 562–567
- Waddington R J, Embery G 2001 Proteoglycans and orthodontic tooth movement. Journal of Orthodontics 28: 281–290
- Waddington R J, Embery G, Hall R C 1993 The influence of fluoride on proteoglycan structure using rat odontoblast *in vitro* system. Calcified Tissue International 52: 392–398
- Waddington J, Embery G, Smith A J 1998 Immunochemical detection of the proteoglycans decorin and biglycan in human gingival crevicular fluid from sites of advanced periodontitis. Archives of Oral Biology 43: 287–295
- Walker L, Enciso R, Mah J 2005 Three-dimensional localization of maxillary canines with cone-beam computed tomography. American Journal of Orthodontics and Dentofacial Orthopedics 128: 418–423
- Weiland F 2003 Constant versus dissipating forces in orthodontics: the effect on initial tooth movement and root resorption. European Journal of Orthodontics 25: 335–342

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