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# Characterization of root surface periodontal ligament following avulsion, severe intrusion or extraction: preliminary observations

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Correspondence to: Prof David J Kenny, The Hospital for Sick Children, 555 University Avenue, Toronto, ON, Canada M5G 1X8 Tel.: +1 416 813 6010 Fax: +1 416 813 6375 e-mail: davidjkenny@sympatico.ca Accepted 10 May, 2007 Abstract – This study employed novel topographic and histological techniques to assess remaining periodontal ligament (PDL) in a convenience sample of avulsed and intruded human permanent incisors and extracted premolars. Seventeen human teeth (eight avulsed, five severely intruded and four uninjured extracted) were evaluated for the distribution and physical characteristics of adherent root surface PDL. The topographic distribution of PDL was assessed by staining roots with malachite green and determining the proportion of remaining PDL within selected regions on four aspects of each tooth. In order to characterize mechanical damage to PDL, serial transverse sections of roots were stained with hematoxylin and eosin and examined at magnifications of  $40 \times$  to  $800 \times$ . The sections were photographed and imaging software was used to calculate the percentage of remaining PDL in the circumference of each root section. Topographic analysis demonstrated that 54% of the PDL remained on roots of avulsed and severely intruded incisors and 36% of the PDL remained on the extracted single-rooted premolars. Examination of serial transverse root sections revealed that 58% of the PDL remained on roots of avulsed or severely intruded incisors and 54% on extracted premolars. Avulsed and severely intruded incisors demonstrated similar amounts of retained PDL. In both injuries, almost half of the root surface was denuded of PDL.

Avulsion, severe intrusion injuries and extraction produce catastrophic mechanical damage to the periodontal ligament (PDL) (1-3). It has been speculated that rupture of the principal fibers of the PDL occurs midway between the root surface and alveolus following extraction of primate teeth (4). Because of the paucity of clinical material, details of mechanical damage to the root surface PDL have not been described for avulsions or severe intrusions of human teeth. Description of the pattern of damage to actual intruded and avulsed permanent incisors would extend knowledge of the biological extent of these injuries and assist in developing therapies for the prevention of inflammatory or replacement resorption that inevitably lead to tooth loss (5, 6). Although extraction has been used as a model for avulsion in replantation experiments in animals (7, 8), there is no study that compares PDL damage between avulsed and extracted teeth in humans.

The objectives of this investigation are to quantify and characterize the remaining root surface PDL among avulsed, severely intruded and extracted human teeth, to compare remaining PDL between avulsed and extracted teeth and to compare topographic and histological techniques as methods for evaluation of retained PDL on traumatized teeth.

# Methods

## Sample

The experimental material was gathered from a convenience sample of 10 children (Table 1) treated in the dental department of the Hospital for Sick Children, Toronto, Canada. All teeth were obtained after informed consent from the subjects or their guardians. The Research Ethics Board of the hospital approved the protocol (Ref. #1000005485). Eight maxillary incisors were acquired from children whose parents declined replantation of their avulsed incisors, five maxillary incisors were obtained from children whose parents declined reduction and splinting of their severely intruded incisors (coronal submergence of more than 6 mm) (9) and four mature functional single-rooted premolars were obtained from two children who required extractions for orthodontic purposes. All teeth employed in the study were immediately transferred to 10% neutral buffered formalin solution. Extractions were performed exclusively with forceps and minimal luxation in order to minimize mechanical damage as a result of surgical manipulation of the root surfaces. The four premolars used had conical, mature roots and single apices. All avulsed teeth arrived at the

Table 1. Age and gender for samples

	Age (years) $\pm$ SD	Gender	п
Avulsion	9.6 ± 1.4	2 F	4
		3 M	4
Severe intrusion	11.3 ± 4.3	1 F	2
		3 M	3
Extraction	15.5 ± 0.5	1 F	2
		1 M	2
Note: one male child p incisor to the sample. F female: M male	provided one avulsed incisor	and one severely i	ntruded

clinic in storage media and had been out of the mouth for more than 30 min.

### Calculation of PDL and root surface area

In order to delineate the remaining root surface PDL, each root was stained with malachite green (ICN Biomedicals Inc, Aurora, Ontario, Canada) diluted with distilled water to highlight PDL connective tissue (collagen) (Fig. 1a,b). The root surface was photographed in four aspects (mesial, distal, labial and palatal) using a



*Fig. 1.* (a) Lateral view of avulsed maxillary central incisor stained with malachite green to highlight remaining periodontal ligament (PDL); (b) palatal view of the same incisor.

digital camera (Nikon Coolpix 995, Nikon Corp., Japan) through a light microscope. Regions of interest (ROI), a series of the largest attainable rectangles superimposed on the two-dimensional image of the root surface image, were aligned on the root surfaces (Fig. 2a). The area of the remaining PDL within each ROI was calculated using ImagePro<sup>®</sup> 5.0 software (Media Cybernetics, Houston, TX, USA). For the software to recognize and calculate the area of remaining PDL, the operator identified stained PDL and denuded surfaces within each ROI (Fig. 2b).

Total root surface area was calculated from the cemento-enamel junction (CEJ) to the apex. The total surface area of each tooth was approximated to be the sum of the surface areas of two contiguous truncated cones using a modification of the conical frustum equation (Fig. 3) (10). The conical frustum equation describes the surface area of a truncated cone. The modifications made for this study excluded the area of the cone base as it is not part of the root surface. Calculation of the surface area of two half-height cones for the frustum equation was nearly as accurate as taking continuous truncated cones at 1 mm increments along the root length. A pilot study demonstrated the more easily measured half-height cones produced a surface area that was within 3% of the surface area calculated by using fifteen 1-mm cones. Consequently, it was decided to use two half-height blunted cones in the frustum equation.

The combined ROI area of each root was divided by the calculated total root surface of the same root to estimate the proportion of sampled root surface per root. The values for all root surfaces were combined, divided by the combined ROI areas and a mean value determined for percentage of total root surface sampled.



*Fig. 2.* (a) Outline of region of interest (ROI) using ImagePro, from cemento–enamel junction to near apex; (b) digital identification of remaining periodontal ligament (PDL) in red using ImagePro.



Area =  $\pi(R1 + R2) \sqrt{((R1-R2)^2 + h1^2 + \pi(R2 + R3) \sqrt{((R2-R3)^2 + h2^2)}}$ Radius (R) = (d1 + d2)/4

*Fig. 3.* Calculation of total root surface area using two blunt cones and the modified conical frustum equation:

Area = 
$$\Pi(R1 + R2)\sqrt{[(R1 - R2)^2 + h1^2]}$$
  
+  $\Pi(R2 + R3)\sqrt{[(R2 - R3)2 + h2^2]};$   
Radius(R) =  $(d1 + d2)/4.$ 

Similarly, the proportion of remaining PDL within each ROI was calculated for all the ROI for each tooth. Then, the areas of retained PDL for all ROI per tooth were combined and an average value was calculated for each root. Mean percentages of the remaining PDL for each group (avulsion, severe intrusion and extraction) were calculated.

### Histological evaluation of root surfaces

Upon completion of topographic analysis, the roots were decalcified in 5% formic acid in an incubator at 36.9°C.



*Fig. 4.* Calculation of remaining periodontal ligament (PDL) using ImagePro<sup>®</sup> software. Yellow outline indicates length of remaining PDL.

Decalcification was confirmed radiographically and was considered complete when the roots had the radiodensity of soft tissue. The roots were embedded in hard paraffin wax and 5-µm thick sections were made perpendicular to the long axis of the root using a microtome. Sections were taken every 100 µm. This systematic sampling ensured that cross-sections of 100 µm apart were taken from the apical, middle and cervical (A, M, C) thirds of each root. The sections were stained with hematoxylin and eosin (SPI-Chem Supplies, West Chester, PA, USA) and all the measurable representatives from each sample site were evaluated microscopically at  $40 \times$  and  $800 \times$ magnification. The percentage of the remaining PDL was calculated using ImagePro® software to analyze the PDL on digitized light microscopic images of each section. Using the software, the circumferential length of the remaining PDL was identified by the operator (Fig. 4) and divided by the total root circumference to give the percentage of remaining PDL on each section.

### Results

### **Topographic analysis**

Sixty-four per cent of root surface area was evaluated for the total sample of teeth. Individual root surface areas sampled ranged from 44% to 87%. Within the ROI, the average remaining PDL was 54% for both avulsed and severely intruded teeth and 36% for extracted teeth (Table 2).

Microscopic observation of the root surface revealed that the remaining PDL was often torn or folded in avulsed and extracted teeth (Fig. 5). Alternatively, the PDL was often crushed and torn in severely intruded incisors. It was observed that the amount of remaining PDL was greater on the palatal than labial root surfaces for both avulsed and severely intruded teeth. This observation for avulsed and severely intruded incisors was not present on extracted premolars.

### Histological analysis

Histological analysis demonstrated that 54–59% of root surface PDL remained on all teeth in the total sample

Table 2. Topographical analysis of avulsed (A), severely intruded (I) and extracted (E) root surfaces

	A1	A2	A3	A4	A5	A6	A7	A8	11	12	13	14	15	E1	E2	E3	E4
PDL in ROI (mm <sup>2</sup> )																	
Labial	8.9	6.8	11.6	3.6	2.8	22.1	38.7	27.9	2.0	21.7	8.6	3.4	19.5	3.4	7.8	6.4	5.3
Palatal	11.1	14.5	25.3	7.9	6.5	20.1	31.1	17.8	19.1	21.6	14.5	2.2	33.3	2.5	8.3	4.0	12.4
Mesial	15.9	20.7	35.0	17.7	22.8	33.5	37.9	24.5	26.9	23.1	11.5	3.3	37.6	6.8	12.5	7.3	27.8
Distal	1.5	15.2	22.6	11.7	12.8	15.1	38.5	29.7	25.6	19.1	5.3	6.7	35.1	12.2	6.0	22.0	21.6
ROI area (mm <sup>2</sup> )																	
Labial	31.0	22.5	24.5	18.4	25.7	30.9	41.0	37.0	30.5	32.7	22.6	18.8	36.3	21.6	15.7	28.9	18.5
Palatal	27.0	28.5	28.5	21.3	35.7	29.9	39.0	33.3	33.4	22.6	25.1	16.0	34.8	31.5	16.6	22.2	26.2
Mesial	33.5	31.2	49.6	29.8	46.3	41.7	40.9	45.5	44.0	31.6	29.5	23.1	44.4	47.3	31.5	32.6	45.2
Distal	28.8	27.9	38.1	27.8	45.0	36.0	46.0	54.8	36.4	25.2	26.9	24.9	36.0	39.8	30.1	44.5	41.0
Proportion PDL in ROI (%)	31.1	51.9	67.2	42.0	29.4	65.6	87.6	58.6	51.5	77.0	38.3	19.0	82.8	17.8	36.9	29.9	57.5
Proportion PDL in ROI (%) per group				54.2 (A)							53.7 (I)				35.5 (E)		
Total root area per modified frustum (mm <sup>2</sup> )	228.7	230.2	248.6	184.8	226.8	194.6	253.6	258.8	170.6	154.1	161.7	228.7	201.6	188.9	186.9	214.2	205.9
Proportion root surface evaluated (%)	52.6	47.8	56.6	52.6	67.0	70.9	65.8	65.9	87.4	67.6	64.4	44.3	75.2	74.3	50.2	68.6	77.1
PDL, periodontal ligament; ROI, region of interest.																	



*Fig. 5.* Avulsed incisor with torn and folded root surface periodontal ligament (PDL).

regardless of whether the tooth had suffered an injury or was extracted (Table 3). Histological sections pooled by injury demonstrated 58% remaining PDL on avulsed incisors, 58% on severely intruded incisors and 54% on extracted premolars. Severely intruded incisors and extracted premolars demonstrated differing patterns of root damage (Fig. 6). Intruded incisors showed less PDL loss at the cervical third than extracted premolars. Extracted premolars showed less PDL loss at the apical third of the root.

Light microscopic evaluation of sections revealed the presence of tears along the PDL fibers between the alveolar bone and root surface (Fig. 7) and areas of denuded root surfaces including pockets of exposed dentin (Fig. 8). There was also evidence of PDL crushing on some root surfaces.

### Discussion

This is the first report of the extent and nature of mechanical damage to PDL in avulsed, severely intruded and extracted human teeth using topographic and histological analysis. Although histomorphometric studies have described root resorption following replantation in animal investigations (11, 12) no study has reported on mechanical damage to the root surface PDL of injured human teeth. Direct traction with forceps was used to extract premolars in this study in order to

Table 3. Histological analysis of root sections for avulsed (A), severely intruded (I) and extracted (E) teeth

	A1	A2	A3	A4	A5	A6	A7	A8	11	12	13	14	15	E1	E2	E3	E4	
Apical																		
No. of histomorphometric sections	7	8	5	8	8	8	3	10	6	5	7	5	2	3	9	5	5	
Proportion of remaining PDL (%)	47.4	32.0	55.6	59.4	32.4	55.6	73.8	84.9	32.1	57.0	52.3	88.9	46.7	71.6	74.2	43.6	45.7	
Middle																		
No. of histomorphometric sections	7	14	5	9	5	8	9	15	4	4	4	8	6	2	4	3	8	
Proportion of remaining PDL (%)	54.4	47.5	46.7	55.0	15.2	66.8	82.5	72.3	34.2	34.7	33.5	86.4	66.1	49.0	71.5	28.2	44.2	
Cervical																		
No. of histomorphometric sections	5	4	8	6	6	16	5	5	7	8	4	4	3	4	5	6	6	
Proportion of remaining PDL (%)	60.5	44.6	37.5	47.9	25.5	56.2	86.7	83.8	72.1	71.2	45.8	85.9	54.0	55.9	64.9	25.5	51.4	
Proportion of remaining PDL				57.6 (A)							58.3 (I)				53.5 (E)			
per group (%)																		
PDL, periodontal ligament.																		

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*Fig. 6.* Plot showing 25th–75th percentiles (boxes) and median (line within each box) percentage of periodontal ligament (PDL) remaining on study teeth.



Fig. 8. Arrow indicates area of exposed surface dentin (800 ×).



Fig. 7. Arrow indicates PDL tears on root surface  $(800 \times)$ .

minimize damage to the cervical PDL during extraction. Previous extraction models for replanted teeth have used elevators that may have produced mechanical damage to root surface PDL (11).

The two mandibular first and two maxillary second premolars used in this study were extracted for orthodontic reasons. While their root configuration is not identical to incisors, each of the premolars had conical mature roots with single apices. For ethical reasons, extraction of functional maxillary human incisors is impractical and therefore it is unlikely that a study employing these teeth as controls will be performed in the future. Under this restriction, single-rooted premolars were considered practical substitutes for incisors.

All avulsed teeth had an extra-oral time of more than 30 min and all intruded teeth were displaced more than 6 mm apically. Upon the decision not to replant for avulsed incisors or at time of extraction of severely intruded incisors, all teeth were immediately stored in buffered formalin solution which allowed preservation of their remaining PDL for topographic and histological assessment. As this study aimed to examine the mechanical damage to root surface PDL, damage produced at the cellular level by extra-oral time and storage variables did not affect this investigation.

### Topographic assessment

Surface staining and digital image analysis led to assessment of a greater root surface area than the histomorphometric analyses used in studies of root surface resorption in monkeys. An average of 64% of the root surfaces were sampled in this investigation compared with less than 5% in previous histomorphometric sampling studies of root resorption (11, 12). A limitation of topographic image analysis was that the ROI did not include areas with substantial root curvature. Curved root surfaces as captured in a two-dimensional digital image produced a distorted view of the root. As a result of this limitation, it was not possible to extrapolate the topographic analysis of the complete root surface area. With accurate image analysis limited to approximately planar root surfaces, ROI were restricted to an average of 64% of total root surfaces. The remaining 36% of the total root surface of the sample consisted of areas where distortion of images prevented assessment of root surface features.

The characteristics of mechanical damage to PDL have been speculated upon for avulsed and severely intruded teeth. The greater loss of PDL on labial root surfaces of avulsed and severely intruded incisors observed in this sample may have been because of the nature of the impact. In avulsion injuries, the traumatic force commonly is applied to the labial aspect of the tooth, displacing the crown palatally and the root tip labially while the incisor is translated out of the socket. Consequently, the palatal PDL is likely to be torn and the apical aspects of the labial PDL crushed. In an intrusive injury, the root is forced into the socket and the palatal and labial PDL are both crushed and torn. The average total root surface area calculated using the modified frustum equation was  $208.2 \text{ mm}^2 \pm 31.7 \text{ mm}^2$  (mean  $\pm$  SD). This result compares well with the findings of other investigators that calculated the average root surface area for maxillary central incisors as  $200.7 \text{ mm}^2 \pm 25.9 \text{ mm}^2$  through the use of an acetate membrane technique (13).

### Histological assessment

Histological analysis of the 5 µm transverse sections provided detailed assessment of the remaining circumferential PDL. Previous histomorphometric studies have employed vervet monkey teeth to evaluate both remaining PDL and postreplantation sequelae (11, 12). The total number of sections assessed in each of these studies varied from 30 to 51 by Andersson et al. (11) and 5 to 7 by Andreasen (12). An average of 19 sections per tooth were evaluated in the current study but how this number compares with Andersson et al. (11) and Andreasen (12) is unknown owing to anatomical tooth differences between the species. The current study is the first in the literature to have evaluated actual avulsed and intruded human incisors. As the teeth studied were available in their near-original postinjury state only the characteristics of the remaining PDL were studied.

Even with the limited root surface area evaluated, this method provided specific information on the nature of PDL damage that occurred during injuries and extractions. However, histological evaluation of transverse root sections of avulsed and severely intruded incisors suggests that the location of PDL tearing during trauma is different from extraction and is characterized by multiple denuded areas where cementum and dentin were exposed (Fig. 7). In severely intruded teeth, the apical and middle thirds of the roots demonstrated more PDL loss than the cervical thirds. Hence, most of the root surface damage was in the apical two-thirds. This is likely due to the wedging and shearing effect on the root surface as it is pushed into the narrow cross-section of socket bone. Conversely, avulsed and extracted teeth showed markedly more remaining apical PDL than intruded teeth because the nature of the forces on these teeth were opposite to that of intrusions.

The topographic and histological assessments of root surfaces were different in nature, with the former being macroscopic and the latter microscopic. Nonetheless, both produced similar findings with respect to the quantity of remaining PDL. Topographic analysis exhibited greater variation than histological analysis possibly because of the greater area assessed (64% vs 5%) of root surfaces. Both methods may be considered useful for characterization of remaining PDL.

Further investigations with larger samples will be necessary to assess the quantitative and qualitative findings of these preliminary observations. This investigation will serve as a benchmark for future studies that evaluate avulsion and intrusion in humans as well as assess the reliability and validity of extraction as a model for avulsion in replantation studies.

### Conclusions

- 1. Topographic and histological techniques were consistent for quantifying remaining PDL on extracted, avulsed and severely intruded incisors.
- 2. Avulsion and severe intrusions produce similar catastrophic damage to root surfaces and the amount of remaining root PDL.
- 3. Further investigation with larger samples may provide assessments of the validity and reliability of extraction as a model for avulsion or severe intrusion.

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