



PDL Oxytalan Fibres, Microvasculature
and
Clinical Orthodontics

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A series of publications submitted for the degree of
Doctor of Dental Science

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DEDICATION

This thesis is dedicated to the spirit and vision of my forbears, and to Helen, Anthony and Michael, whose many sacrifices made this work possible.

Also to Alan.

“The lyf so short, the crafte so long to lerne”

Geoffrey Chaucer

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- Paper 2 Sims MR 1976. Reconstitution of the human oxytalan system during orthodontic tooth movement. *Am J Orthod* 70:38-58
- Paper 3 Sims MR 1980. Angular changes in collagen cemental attachment during tooth movement. *J Periodontal Res* 15:638-645
- Paper 4 Sims MR 1980. Tooth ankylosis in man and the oxytalan fibre meshwork. *Aust Orthod J* 6:147-153
- Paper 5 Sims MR 1981. The periodontal ligament - new concepts. *Annals, Roy Australas Coll Dent Surgeons* 7:71-80
- Paper 6 Sims MR 1982. Tooth ankylosis in man and the oxytalan fibre meshwork. Yearbook of Dentistry. Eds. Hale ML, Hazen SP, Moyers RE, Redig DF, Robinson HBG and Silverman SI. 1. Dental and Occlusal Development. pp 10-11. Yearbook Medical Publishers Inc. Chicago.
- Paper 7 Sims MR 1983. The microvascular venous pool and its ultrastructural associations in mouse molar periodontal ligament – Periodontal microvasculature & nerves. *Aust Orthod J* 8:21-27
- Paper 8 Sims MR 1983. Electron-microscopic affiliations of oxytalan fibres, nerves and the microvascular bed in the mouse periodontal ligament. *Archs oral Biol* 28:1017-1024
- Paper 9 Sims MR 1984. Ultrastructural analysis of the microfibrillar component of mouse and human periodontal oxytalan fibres. *Conn Tiss Res* 13:59-67
- Paper 10 Sims MR 1984. Ultrastructural evidence of nerve and oxytalan fibre associations in the endoneurium of human periodontal ligament. *Archs oral Biol* 29:565-567

SECTION II. PDL MICROVASCULATURE AND FUNCTION

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- Paper 11 Sims MR and Weekes WT 1985. Resorption related to orthodontics and some morphological features of the periodontal microvascular bed. *Internat Endo J* 18:140-145
- Paper 12 Sims MR 1987. A model of the anisotropic distribution of microvascular volume in the periodontal ligament of the mouse mandibular molar. *Aust Orthod J* 10:21-24

- Paper 13 Sims MR 1987. Ultrastructure of the venous ampulla in the interradicular microvascular bed of the mandibular molars of *mus musculus*. *J Morphol* 191:217-224
- Paper 14 Sims MR, Sampson WJ, Fuss JM 1987. Glomeruli in the molar gingival microvascular bed of germ free rats. *J Perio Res* 23:248-251
- Paper 15 Sims MR 1995. The Morphology of the vasculature of the periodontal ligament. In: *The Periodontal Ligament in Health and Disease*. Eds. Berkovitz B.K.B., Moxham B.J. and Newman H.N. Eds. 2nd Edition. Chap. 5, pp.107-120. Mosby-Wolfe. London
- Paper 16 Sims MR, Leppard PI, Sampson WJ, Dreyer CW 1996. Microvascular luminal volume changes in aged mouse periodontal ligament. *J Dent Res* 76:1503-1511

SECTION III. MICROVASCULAR BED IMMUNOLABELLING

OVERVIEW:

- Paper 17 Sims MR 1999. Blood vessel response to pan-endothelium (RECA-1) antibody in normal and tooth loaded periodontal ligament. *Europ J Orthod* 21:469-479
- Paper 18 Sims MR 2000. Early changes in the endothelium of alveolar bone microvasculature with continuous tooth loading : ET-1 and α -SMA immunolabelling. *Ann Roy Australas Coll Dent Surg* 15:292-298
- Paper 19 Sims MR 2001. Endothelin-1 expression in the microvasculature of normal and 3 hour continuously loaded rat molar periodontal ligament. *Europ J Orthod* 23:647-662
- Paper 20 Sims MR, Ashworth JR, Sampson WJ 2003. Upregulation of immunoreactivity of endothelin-1 and α -SMA in PDL microvasculature following acute tooth loading: an immunohistochemical study in the marmoset. *Orthod Craniofacial Res* 6:74-82

SECTION IV. CLINICAL PUBLICATIONS

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- Paper 21 Sims MR 1964. The Begg philosophy and fundamental principles. *Am J Orthod* 50:15-24
- Paper 22 Sims MR 1966. The Begg technique and a radiographic method of recording arch wire co-ordination. *Aust Orthod Bull* 5:3-11 ‡
- Paper 23 Sims MR 1968. Reciprocal orthodontic phenomena. *Aust Orthod J* 1:76-80
- Paper 24 Sims MR 1969. Bijzonderheden tijdens de slotfase van de orthodontische behandeling: De consequenties van een andere zienswijze. (Detailed finishing in orthodontic treatment: the consequences and long term effects.) *Nederlands Tijdschrift voor Tandheelkunde* 76:449-458 ¶
- Paper 25 Sims MR 1970. Relevance and reality in orthodontics. *Aust Dent J* 15:365-370

¶ English translation attached

‡ Japanese translation attached

- Paper 26 Sims MR 1971. Anchorage variation with the light-wire technique. *Am J Orthod* 59:456-469 ‡
- Paper 27 Sims MR 1972. De stabiliteit van de occlusie na overmatige correctie van een klasse III - afwijking. (Occlusal stability following overtreatment of a Class III malocclusion.) *Nederlands Tijdschrift voor Tandheelkunde* 79:94-97 ¶
- Paper 28 Sims MR 1972. Torque application to maxillary incisors. *Aust Orthod J* 3:5-12
- Paper 29 Sims MR 1972. Psychological disturbances associated with a mutilated malocclusion. *J Clin Orthod* 6:341-345
- Paper 30 Sims MR 1972. Loop systems – A contemporary reassessment. *Am J Orthod* 61:270-278 ‡
- Paper 31 Sims MR 1973. Diagnosetechnik. (Diagnostic Engineering.) *Orthodontie und Kieferorthopadie* 4:386-404 ¶
- Paper 32 Sims MR 1973. Diagnostic engineering. *Revue d'Orthopédia Dento Faciale* 7:471-485 ¶
- Paper 33 Sims MR 1973. Técnica del diagnóstico. *Revista Española de Ortodoncia* 3:331-347 ¶
- Paper 34 Sims MR 1975. Anchorage – a continuing crisis. *Trans Third Internat Orthod Congress*. Crosby Lockwood Staples, Hertfordshire. pp.410-419
- Paper 35 Sims MR, Cran JA 1977. A dentigerous cyst associated with an Angle Class II malocclusion. *Aust Dent J* 22:196-198
- Paper 36 Sims MR 1977. Conceptual orthodontics. *Am J Orthod* 71:431-439 ‡
- Paper 37 Sims MR 1992 Les 7 myths de la technique Begg. *Nouvelles de la Société Française de Begg* No.6, pp.5-11 ¶
- Paper 38 Sims MR 1992. La technique de Begg et le milieu facial. (Begg technique and the facial milieu.) *L'orthodontie Française* 62:773-792 ¶
- Paper 39 Sims MR 1995. Are we still looking for the perfect attachment? 95th American Association of Orthodontists Annual Session & International Orthodontic Congress. San Francisco. Keynote Address – Published as Conference Audio-tape
- Paper 40 Sims MR 1999. Brackets, epitopes and flash memory cards – a futuristic view of orthodontics. *Aust Orthod J* 15:260-268

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Abstracts 1.1–1.38

APPENDIX 3. CD-ROM : Movies of ET-1 and α -SMA immunolabelling

Movies 1-15

SIGNED STATEMENT

The principal publications presented in this volume have not been submitted for a degree in this or any other university.

Where jointly authored works are listed, the candidate has made the major contribution to the publication of that particular paper. This role involved the project planning, evaluation of tissues, interpretation of results, manuscript preparation and editing.

The first author for Paper No 11 was the invited speaker who presented his own research and also that of supervised postgraduates, including vascular perfusion illustrations by WT Weekes. Author's contribution 80%.

The author devised the project for Paper No 14, including planning, evaluation of tissues, interpretation of results, manuscript preparation and editing. J Fuss undertook the laboratory procedures and SEM. Author's contribution 80%.

MR Sims, WJ Sampson and CW Dreyer were the joint recipients of the grant to conduct the investigation reported in Paper No 16. The project planning, evaluation of tissues, interpretation of results, stereology, manuscript preparation and editing were carried out by the first author. PI Leppard undertook the additional statistical analyses. Author's contribution 80%.

In Paper No 20, the animal experimentation, section preparation and some initial immunolabelling were conducted by the second author under the writer's direction. WJ Sampson was the co-supervisor for JR Ashworth. The completion and renewal of immunolabelling, confocal microscopy, analysis and interpretation of results, together with manuscript preparation and editing, were the responsibility of the first author. Contribution 80%.

Paper No 35 illustrates the treatment of an orthodontic problem by the author for which JA Cran conducted the oral surgery, biopsy and report. The first author was responsible for manuscript preparation and editing. Contribution 80%.

The papers listed in Appendix 1 were derived from MDS postgraduate research programmes, designed and personally supervised by the present author.

Appendix 2 comprises published abstracts of research by the author and supervised postgraduate students.

I consent to this copy of my submission, when deposited in the University Library, being available for loan and photocopying.

Milton R. Sims

15/7/2003

July 15th, 2003

ACKNOWLEDGEMENTS

Over the years these investigations have involved invaluable support from many individuals. A particular debt of gratitude is due to the late Dr Lafayette Barrett of Atlanta, Georgia, who long ago generously contributed funds to the Adelaide University Orthodontic Laboratory at a time when the research cupboard was bare and the orthodontic programme was about to be abandoned. Subsequently, Messrs Bob Jamieson and Ross Hall of Coca-Cola Bottlers, Adelaide gave major support to the present investigations and allied research being undertaken by the postgraduate MDS candidates.

A significant donation to the Adelaide University Electron Microscope Facility in 1980 by J B Were & Son, through former Adelaide Director Mr Derek Lowings, provided the impetus for Adelaide University to fund a new transmission electron microscope which enhanced University TEM research, including projects in this thesis.

The Australian Society of Orthodontists Foundation for Research and Education has been a major source of research funding. Invaluable support has also been given by the National Health & Medical Research Council, the Australian Dental Research Foundation Inc and private sources.

Initial studies of the oxytalan fibre system were undertaken at the suggestion and encouragement of the late Dr J A Cran, many of whose concepts of dental tissue structure and function were in advance of his era.

Great indebtedness is due to the late Dr John R Casley-Smith who was a constant source of support and encouragement in the electron microscopy studies, as was Ken Crocker who assisted with technical advice. Professor I L Gibbins, Head of Anatomy and Histology, Flinders University, graciously provided access to immunohistochemical facilities. Without the generous encouragement and ongoing expertise of Associate Professor Brendon J Gannon, the vascular perfusion and immunohistochemistry studies, along with several of the related postgraduate research projects reported at conferences, would not have come to fruition. Special acknowledgement is also made to Mr Philip Leppard and Dr Ray Correll for their invaluable statistical service and Dr Peter Kolesik for expert confocal microscopy.

The laboratory studies involved a large amount of skilled technical application and I would particularly mention the contributions of Donald Smale, Anne Menzel, Lorraine McMahon, Vicki Tuck and Margaret Leppard over the years. Pat Berry and Joni Lewis played instrumental roles in typing many of the manuscripts.

The "Big Bang" behind the clinical aspect of this enterprise was the influence of the late Dr P Raymond Begg AO, whose orthodontic doctrines swayed my clinical world between deep despair and an excitement that extended to the edge of the orthodontic universe. Indirectly, he propelled me towards a curiosity and wish to explore periodontal ligament morphology and function. Particular acknowledgement is accorded my teaching colleagues Professor Wayne J Sampson and Dr Craig W Dreyer for their co-operation and friendship during this research.

One's family life is always disadvantaged by the intensity of academic demands. Without the forbearance and sacrifices of Helen, Anthony and Michael these studies could not have been pursued.

PREFACE

For a clinician, the orthodontic relocation of teeth poses an ongoing challenge to achieve a better understanding of the processes involved in the rapid reconstitution of the periodontal tissues. Every long-term research venture has its inspirations. In this endeavour, it was Dr Kaare Reitan who lectured to us as postgraduate students at St. Louis University. Also my Boston University research classmate, Dr Harold J Hayes, tragically killed soon after graduating, who shared with me the excitement of our joint investigations of tooth movement in dogs.

In this thesis, studies of the PDL oxytalan fibre meshwork and microvascular blood vessel system comprise the first three biological sections. Evidence is provided for the reconstitution of the human oxytalan fibre system and statistical data support the hypothesis that collagen and oxytalan fibres perform different dynamic functions. Tooth ankylosis confirms the maintenance of the oxytalan fibre cementum-vascular meshwork, while ultrastructural studies reveal the oxytalan fibre relationships to the peripheral neural system and the blood vessel wall. Statistical data show species differences in oxytalan microfibrillar structures. These additional findings provide further support for the author's original PhD hypotheses which remain extant.

The intimate associations existing between the oxytalan fibre system and PDL microvasculature have led the author to study this peripheral vascular bed. Blood vessels play a pivotal role in the maintenance and function of tissue. Nevertheless, knowledge of PDL vessel geometry and wall ultrastructure has long been limited. The physiologically active postcapillary-sized venule segment is shown to contain ~60% of the blood volume. Most of these venule profiles present as endothelial tubes with pericyte cells. Stereologically, aged PDL microvasculature is proved to undergo major changes in anatomy, distribution and physiological function.

Normally, the PDL vasculature is subjected to intermittent masticatory loads or, at the other extreme, orthodontically superimposed continuous loads in excess of 18 months duration. The latter loads can result in the ~350 μ m wide ligament being reconstituted through the bone at ~60 μ m/day over distances of 7-8mm. Thus, the vessel endothelium, pericytes and arteriolar smooth muscle must undergo a rapid and continuous processes of remodelling, including both shutdown and proliferation.

Early responses of vascular endothelial cells to continuous tooth loading for periods from 1 to 3 hours have been evaluated using immunolabelling in rodents and non-human primates. Significant region by treatment interactions occur for endothelin-1 labelling in both vascular endothelium and bone surface cells. Furthermore, it is demonstrated that upregulation of both ET-1 and α -SMA occurs in the PDL vessels of non-human primates after 1½ hours. Clearly, immunolabelling offers a valuable technique to examine early PDL vascular cell responses to tooth loading.

Section IV comprises a series of clinical publications. These papers were requested principally from the author's clinical orthodontic courses and lectures. The material includes descriptions of the theory and application of the Begg orthodontic technique. Other subjects cover diagnosis, anchorage control and conservation, bite opening, incisor torque, finishing and concepts of facial aesthetics. The final paper in this group suggests some possible avenues of future orthodontic development.

These personal investigations resulted in the design, supervision and publication of a series of postgraduate research studies directed primarily to the microvasculature.

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SECTION I. HUMAN AND RODENT OXYTALAN FIBRE MESHWORKS

OVERVIEW: Knowledge of PDL oxytalan fibres from the author's previous publications is greatly extended with new histological, TEM and data studies. These papers cover orthodontic tooth movement, fibre system permanence, vertical tooth loading, functional aspects, ankylosis, anatomical associations, microfibrillar ultrastructure and species specificity.

Paper 1 provides the first published information that during continuous orthodontic tooth movement in man the PDL oxytalan fibre system undergoes remodelling while maintaining the cementum-vascular association.

Paper 2 describes an histological study of clinical tooth movement from 3 to 28 days in ten adolescents. Maintenance of the oxytalan fibre system and its anatomical associations were confirmed within the PDL. It was hypothesized that the oxytalan system existed and remodelled in a pre-stressed state and, in man, also assisted in the regulation of blood flow by registering tensional changes in the vessel walls.

In **Paper 3** the effects of intrusive and extrusive molar loads transmitted to normal and lathyritic mouse molar PDL fibre systems were analysed. It was shown that acute loads resulted in contrasting and statistically significant changes to PDL collagen and oxytalan fibre angles of attachment to the cementum.

Long term ankylosis of human permanent first molars, was histologically evaluated in **Paper 4**. Findings revealed that the PDL cementum-vascular oxytalan fibre system was maintained, albeit with a more random distribution. This meshwork permanence refuted functional hypotheses put forward by other investigators.

Paper 5 comprises an ultrastructural study of mouse oxytalan fibre relationships to PDL fibroblasts. Analysis of variance showed significant differences in the major axis of the fibre profiles. Differences with human oxytalan fibre profiles were also reported.

Paper 6 is a Dental Yearbook review article of Paper 4 in the annual survey of international journals. These selected articles were considered to warrant the attention of clinicians.

The TEM study in **Paper 7** confirmed the oxytalan fibre-microvascular association in all categories of PDL blood vessels. The predominant postcapillary-sized venule in the mouse had an ultrastructure that differed from the traditional vessel classification of Rhodin (1968). The neural relationship to oxytalan fibres was further defined.

Paper 8 is a TEM study which investigated the anatomical associations of oxytalan fibres, nerves and blood vessels. The identification of morphological models in the PDL further supported the author's hypothesis of an oxytalan fibre-proprioceptor role.

Publication of **Paper 9** provided the first ultrastructural analysis comparing microfibril populations in two samples of mouse and one group of human PDL oxytalan fibres. Data plots for total microfibrillar area against total fibril area revealed that the three groups were significantly different at the 1% level and demonstrated species specificity.

Paper 10 provided new TEM information on the morphological association of oxytalan fibres with myelinated and unmyelinated nerves in the endoneurium.

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SECTION II. PDL MICROVASCULATURE AND FUNCTION

OVERVIEW: A group of morphological investigations using light microscopy, TEM and SEM provide evidence to make functional inferences of the PDL vascular bed. Furthermore, stereological analyses of the normal and aged mouse blood vessel system revealed significant aged changes in architecture and physiological efficiency.

Paper 11 is a conference review presentation covering different types of laboratory techniques and microscopy studies to examine vascular architecture in rodents and cementum resorption after human tooth movement. Vascular loops and their possible role in root and bone resorption are outlined.

Paper 12 provided TEM and SEM descriptions of a previously unreported venous reservoir straddling the mouse molar interradicular bone. The ultrastructure of the wall and its regional variations, wall thickness ratios, as well as the input and output vascular components, were detailed. Also recorded were the pericyte and oxytalan fibre associations. A name was assigned to this vascular structure.

A general logistic model was employed in **Paper 13** to analyse the PDL vascular volumes between the cervical and apical regions of the mouse molar root. Anisotropic blood vessel distribution was associated with significant differences in quadrant and depth effects. As a consequence, variable gradients of functional operation were postulated to occur.

The findings in paper **Paper 14** were based on microcorrosion casting in germ free rats. SEM imaging of vascular glomeruli on all aspects of the gingival crevice provided evidence to negate the prevailing opinion that such glomeruli form primarily as a response to inflammation.

Paper 15 presented a review of PDL vascular morphology and emphasizes the venous character of the system. This chapter refutes the traditionally accepted, but inaccurate, concepts of morphology proposed by Kindlova and Matena (1962) and also the determinations of volume distribution published by Götz (1976, 1980). A body of new data on this microvascular bed is provided from the investigations of the author and his co-workers.

Paper 16 is the first published PDL stereological study of mouse molar PDL which demonstrates the aged changes that occur in vascular morphology and architecture of the different PDL microvascular segments. The data demonstrate the diminution in the physiological gradients of capillary efficiency and changes in the diffusion barrier, both of which undergo major reductions.

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SECTION III. MICROVASCULAR BED IMMUNOLABELLING

OVERVIEW: Investigations directed to extending knowledge of the presence of the cytokine endothelin-1 (ET-1), and vascular smooth muscle actin (α -SMA), in the normal and short-term, continuously loaded PDL microvascular bed.

In **Paper 17**, confocal laser scanning microscope imaging of RECA-1 antibody demonstrated that it reacted with blood vessel endothelium in normal PDL and alveolar bone. Tooth loading for 10 minutes resulted in statistically significant upregulation of the vascular endothelium immunoreactivity with characteristic regional variations. This modulation in RECA-1 expression is a small vessel effect, and suggests that endothelial cell activity is an important early consequence of tooth loading.

Paper 18 is a combined morphological and statistical investigation. Immunolabelling patterns of ET-1 and α -SMA antibodies were examined in the microvascular bed of normal and loaded PDL. Activity of ET-1 was established as the default state in normal vascular endothelium. Analyses of ratio and raw data provided the first statistical proof that tooth loading produced significant regional ET-1 upregulation changes in vascular endothelium, bone socket surface cells, cementum surface cells and PDL background. Concepts of ET-1 influence on vascular function and interaction with bone and cementum cells were discussed in some detail.

The study in **Paper 19** displayed patterns of ET-1 and α -SMA microvascular immunolabelling within normal and tooth loaded alveolar bone of the rat. Marked upregulation of ET-1 immunofluorescence occurred in most parts of this vascular bed. The use of α -SMA demonstrated the first reported immunohistochemical identification of pericytes in the alveolar bone microvasculature. A notable feature was pericyte clustering at vessel branching regions. Some possible roles of blood vessel ET-1 and pericytes in vascular control and bone remodelling are considered. A previously unreported dental example of arteriole penetration of a postcapillary-sized venule is illustrated.

Paper 20 describes a study which tested the hypothesis that acute continuous tooth loading would increase the immunoreactivity of ET-1 and α -SMA in the PDL microvasculature of a non-human primate. Three categories of postcapillary-sized venule (PCV) endothelial cell immunolabelling with ET-1 and α -SMA were identified. When standardized confocal microscope settings were applied to control and experimental pairs, loading showed a significant upregulation of both ET-1 and α -SMA in the blood vessel endothelium. The increased antigenicity of vascular endothelial cells to both ET-1 and α -SMA, and of arteriolar smooth muscle to α -SMA, was a response to PDL shear and compression loads. Thus the hypothesis was confirmed. Because interspecies heterogeneity of ET-1 dependent vascular responses are known to occur, the use of a primate model provided a comparison with the author's previous rat PDL publication and, therefore, a more confident basis for extrapolating the animal results to tooth loading in man.

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SECTION IV. CLINICAL PAPERS

OVERVIEW: A group of clinical papers related to presentations at postgraduate courses and orthodontic meetings and subsequently requested by journal editors for publication.

Paper 21 reviews the application of the Begg theory of attritional occlusion to a rationale for clinical orthodontic extraction and reviews some treatment concepts and principles applied in using the light wire appliance.

Paper 22 directs attention to Begg round archwire control, intraoral anchorage management and the clinical potential of round wire torquing devices.

The importance of reciprocal orthodontic appliance factors and some of their effects associated with in the use of full banding techniques is reviewed in **Paper 23**.

In **Paper 24** the argument is presented that contrary to the concept of precise positioning of individual teeth at the time of appliance removal, malocclusions should be "overtreated" ie. a Class II to a Class III tendency, overbites to an open bite, and the occlusion allowed to settle under normal physiological and functional influences.

The subject of orthodontic retention is addressed in **Paper 25**. Consideration is given to the role of the periodontal ligament, tooth overmovement and dental equilibration.

Considerations of optimal anchorage management and torque control with the Begg technique are demonstrated with the presentation of the treatment records of five different malocclusions in **Paper 26**.

Paper 27 describes the treatment of a female skeletal Class III malocclusion to a Class II dental relationship and after a non-retention period of 3 years. (Journal requested paper from a postgraduate course at Groningen University).

Clinical maxillary incisor torque can be a major requirement for the correction of malocclusions with large overjets. **Paper 28** describes a method is to manage this problem.

Paper 29 indicates the important role that orthodontic treatment can play in alleviating the intense psychological disturbances afflicting some individuals with malocclusion.

Paper 30 reviews the disadvantages of the prolonged use of loop systems with round orthodontic archwires when applied in the Begg or other orthodontic systems.

Paper 31 is from a series of postgraduate course lectures given at Groningen University, The Netherlands, and requested by journal editors for European publication. This paper describes the diagnostic application of shapes, sizes and relative positions of jaw, tooth and bone anatomy is related to the three stages of Begg appliance management.

Paper 32 "Diagnostic engineering" is the French publication of Paper 32.

Paper 33 "Técnica del diagnóstico" is the Spanish publication of Paper 32.

The assigned lecture topic in **Paper 34** provided the occasion to demonstrate the outstanding potential of Begg intra-oral anchorage and signify the contraindication of excessive incisor retroclination during active treatment.

Malocclusions with pathological involvement necessitate a team approach as indicated in **Paper 35**.

In **Paper 36** the application of anchorage bends with round wires for simultaneous control of molar and incisor correction is outlined and demonstrated with a treatment example.

The review of the Begg technique in **Paper 37** addressed some common misconceptions associated with the clinical application of this appliance.

An assigned congress lecture topic in **Paper 38** discussed the age old orthodontic issues of facial aesthetics and challenged the misrepresented view that tooth extraction is detrimental to optimal facial profiles. Fallacies of soft tissue orthodontic diagnosis and treatment planning are illustrated using computerized soft tissue reimaging.

Paper 39 was an allocated keynote presentation, given at the 95th Annual Session of the American Association of Orthodontists and 4th International Orthodontic Congress, San Francisco, 1995. Published as a Conference audio tape.

A hypothetical scenario in **Paper 40** outlines some future changes in orthodontic protocols with an alternative view of postgraduate training needs. Rapid progress in biology and technology will necessitate professional vigilance and prompt response to new challenges.

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APPENDIX 1. Co-authored publications arising from personally designed and directed postgraduate MDS research projects

- Paper 1.1 Sampson WJ, Sims MR 1977. Oxytalan fibre organization in marsupial mandibular periodontal tissues. *J Morphol* 154:115-131
- Paper 1.2 Twelftree CC, Cocks GJ, Sims MR 1977. Tensile properties of orthodontic wire. *Am J Orthod* 72:682-687
- Paper 1.3 Barber AF, Sims MR 1981. Rapid maxillary expansion and external root resorption in man: a scanning electron microscope study. *Am J Orthod* 79:630-652
- Paper 1.4 Langford SR, Sims MR 1981. Upper molar root resorption because of distal movement. *Am J Orthod* 79:669-679
- Paper 1.5 Langford SR, Sims MR 1982. Root surface resorption, repair and periodontal attachment following RME in man. *Am J Orthod* 81:108-115
- Paper 1.6 Harry MR, Sims MR 1982. Root resorption in bicuspid intrusion. A scanning electron microscope study. *Angle Orthod* 52:235-258
- Paper 1.7 Wong RST, Sims MR 1983. Morphology of the enamel-cementum microvascular junction of the mouse incisor. *Aust Orthod J* 8:49-50
- Paper 1.8 Weekes WT, Sims MR 1986. The vasculature of the rat molar gingival crevice. *J Periodont Res* 21:177-185
- Paper 1.9 Weekes WT, Sims MR 1986. The vasculature of the rat molar periodontal ligament. *J Periodont Res* 21:186-194
- Paper 1.10 Freezer SR, Sims MR 1987. A transmission electron-microscope stereological study of the blood vessels, oxytalan fibres and nerves of mouse molar periodontal ligament. *Archs oral Biol* 32:407-412
- Paper 1.11 Wong RST, Sims MR 1987. A scanning electron-microscopic, stereopair study of methacrylate corrosion casts of the mouse palatal and molar periodontal microvasculature. *Archs oral Biol* 32:557-566
- Paper 1.12 Freezer SR, Sims MR 1988. Statistical correlations between cells, blood vessels, oxytalan fibres and nerves in normal mouse molar periodontal ligament using transmission electron microscopy. *Aust Orthod J* 14:227-230
- Paper 1.13 Lew KR, Sims MR, Leppard PI 1989. Tooth extrusion effects on microvessel volumes, endothelial areas and fenestrae in molar apical periodontal ligament. *Am J Orthod Dentofac Orthoped* 96:221-231
- Paper 1.14 Freezer SR, Sims MR 1989. Morphometry of neural structures in the mouse periodontal ligament mesial to the mandibular first molar. *Aust Orthod J* 11:30-37
- Paper 1.15 Cooper SM, Sims MR 1990. Evidence of acute inflammation in the periodontal ligament subsequent to orthodontic tooth movement in rats. *Aust Orthod J* 11:107-109
- Paper 1.16 Cooper SM, Sims MR, Sampson WJ, Dreyer CW 1990. A morphometric electron microscopic analysis of tissue channels by ionic tracer in normal and tensioned rat molar apical periodontal ligament. *Archs oral Biol* 35:499-507

- Paper 1.17 Lee D, Sims MR, Sampson WJ, Dreyer CW 1990. Stereo-pair three-dimensional imaging of microvascular architecture in primate dental tissues. *Aust Orthod J* 11:251-255
- Paper 1.18 Clark A.B, Sims MR, Leppard PI 1991. An analysis of the effect of tooth intrusion on the microvascular bed and fenestrae in the apical periodontal ligament of the rat molar. *Am J Orthod Dentofac Orthop* 99:21-29
- Paper 1.19 Lee D, Sims MR, Dreyer CW, Sampson WJ 1991. A scanning electron microscope study of microcorrosion casts of the microvasculature of the marmoset palate, gingiva and periodontal ligament. *Archs oral Biol* 36:211-220
- Paper 1.20 Tang MFP, Sims MR 1992. A TEM analysis of tissue channels in normal and orthodontically tensioned rat molar periodontal ligament. *Europ J Orthod* 14:433-444
- Paper 1.21 Tang MFP, Sims MR, Sampson WJ, Dreyer CW 1993. Evidence for endothelial junctions acting as a fluid flux pathway in tensioned periodontal ligament. *Archs oral Biol* 38:273-276
- Paper 1.22 Parlange LM, Sims MR 1993. A T.E.M. stereological analysis of blood vessels and nerves in marmoset periodontal ligament following endodontics and magnetic incisor extrusion. *Europ J Orthod* 15:33-44
- Paper 1.23 Chintakanon K, Sims MR 1994. Ultrastructural morphology of vascular endothelial junctions in periodontal ligament. *Aust Dent J* 39:105-110
- Paper 1.24 Foong KWC, Sims MR 1999. Blood volume in human bicuspid periodontal ligament determined by electron microscopy. *Archs oral Biol* 44:465-474
- Paper 1.25 Cameron J, Sims MR, Sampson WJ 2001. Ultrastructural changes in postcapillary-sized venule morphology in aged mouse periodontal ligament. *Aust Orthod J* 17:8-16
- Paper 1.26 Seto Boon H, Gotsopoulos H, Sims MR, Cistuli PH 2001. Maxillary morphology in obstructive sleep apnoea syndrome. *Europ J Orthod* 22:703-714

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APPENDIX 2. Listing and Published Abstracts of Research Presentations at National and International Conferences

Abstract 1.1	Bajada SB, Sims MR 1974. Demography and orthodontic care in South Australia. <i>J Dent Res</i> 54:643	Abstract No 4
Abstract 1.2	Dunstan IH, Sims MR 1977. Collagen fibre bundles in the alveolus of the mouse mandible. <i>J Dent Res</i> 56:D202	Abstract No 46
Abstract 1.3	Edwards RJ, Sims MR 1977. Perforating fibre bundles in the human mandible. <i>J Dent Res</i> 56:D202	Abstract No 47
Abstract 1.4	Harry MR, Sims MR 1977. A scanning electron microscopic study of orthodontic root resorption. <i>J Dent Res</i> 56:D202	Abstract No 48
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EOS, 59th Congress, Geneva Programme Abstract
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Europ J Orthod 15:344 *Abstract No 46
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Abstract No 64

Abstract 1.1
J Dent Res 54:643, 1974

4

Demography and Orthodontic Care in South Australia. S.B. BAJADA* and M.R. SIMS
Department of Dental Health, University of Adelaide, Adelaide, South Australia.

This study examines the demographic factors of population size, age groups and their distribution, which relate to the need for orthodontic care. Essential data was obtained from the Bureau of Census and Statistics and largely based on the 1971 Census. To ensure the comparability of this investigation, the Bureau's system of categorisation of data was adopted.

The populations in the 0 to 19 year age group, which represent current and future orthodontic patients in Adelaide and country divisions, were specifically studied. The Adelaide division had a large proportion of 0 to 19 year population. Considering the distribution of this population, future plans for urbanisation, socio-economic status and familism, areas in the Adelaide division showed varying requirements for orthodontic care. In the country divisions there was a significant number of centres which had large and stable populations in the ages requiring orthodontic attention.

Future orthodontic needs will be influenced by the projected increase in the populations in urban and rural areas and the development of the satellite city of Monarto.

Demographic factors play an important role in the pattern of organisation and delivery of dental health care.

Abstract 1.2
J Dent Res 56:D202, 1977

46

Collagen Fibre Bundles in the Alveolus of the Mouse Mandible. I.H. DUNSTAN* and M.R. SIMS. Department of Dental Health, University of Adelaide, South Australia.

Histological patterns of the collagen fibre bundles in the molar alveolar process of the mouse mandible were studied. These findings were compared with those of Cohn (1966, 1972) and Quigley (1970). These authors concluded that in the mouse and some other animals the principal collagen fibre bundles extended between adjacent teeth, coursing through the interdental bone where the bundles were described by Cohn as transalveolar collagen fibre bundles. In the present investigation, specimens were sectioned at eight microns in the axial mesio-distal, buccolingual and horizontal planes. Sections were stained with the silver method of Gordon and Sweet and studied with the light microscope. Many of the principal collagen fibre bundles could be traced from the cementum of one molar through the interdental alveolus to the cementum of the adjacent molar. Transalveolar fibre bundles were also seen passing through the buccal and lingual alveolus to the periosteum.

In the interdental and interradicular regions the principal collagen fibre bundles demonstrated localised variations in morphology. It was concluded that these morphological differences may be related to the migration of teeth.

Abstract 1.3
J Dent Res 56:D202, 1977

47

Perforating Fibre Bundles in the Human Mandible. R.J. EDWARDS* and M.R. SIMS
Department of Dental Health, University of Adelaide, South Australia.

A study by Cohn (1975) demonstrated transalveolar (perforating) fibre bundles wherever lamellated bone without Haversian systems was present in human jaws. The purpose of the present investigation was to examine the perforating fibre bundle pattern in the incisor, premolar and molar regions of normal human mandibles. Segments of mandible from autopsy subjects ranging in age from 11 to 68 years were examined histologically in the mesiodistal, buccolingual, and horizontal planes. Gordon and Sweet's Silver stain combined with Van Gieson proved to be the best method for the demonstration of the fibre bundles. Perforating fibre bundles were observed passing through the crestal third of the interdental, interradicular, buccal and labial bone in the mandibular molar, premolar, and incisor region of 19 to 21 year old males and females. Fibre bundles were also noted in the premolar interdental area of the alveolar bone crest of an 11 year old female. A theory is proposed to account for the presence and arrangement of these fibre bundles within the crestal regions. A morphological difference at the site of insertion of the fibre bundles into the alveolar bone on the mesial, distal, labial and lingual surfaces was observed. Age changes in the fibre bundles and their pattern were also noted.

Some findings of this investigation differed from those of Cohn. The perforating fibre bundles distribution may have significant orthodontic implications.

Abstract 1.4
J Dent Res 56:D202, 1977

48

A Scanning Electron Microscopic Study of Orthodontic Root Resorption. M.R. HARRY* and M.R. SIMS. Department of Dental Health, University of Adelaide, South Australia.

Recent scanning electron microscopic studies by Kvam (1972) on the effect of buccally directed orthodontic forces on human root surface topography have shown root resorption to occur in the cementum after approximately 10 days. The present project was undertaken to study the effects of the magnitude and duration of intrusive forces on the root surface of human premolar teeth.

Premolar teeth were obtained from patients who required extractions prior to orthodontic treatment. Maxillary and mandibular experimental premolars were intruded by means of a Begg type archwire. The contralateral premolar of each dental arch was used as a control. Extracted premolars of two patients who had worn a fixed rapid maxillary expansion appliance were also examined for changes in root surface topography. Resorption was observed in all teeth that had been intruded for longer than 14 days. Where heavy intrusive forces had been used resorption was more severe. Rapid palatal expansion appliances caused severe resorption of the buccal surfaces of the attached premolars. These findings suggest that some orthodontic forces adversely affect human teeth.

Abstract 1.5
J Dent Res 58:2240, 1979

37

Ultrastructure of Human Periodontal Blood Vessels. D.R. GILCHRIST*, Department of Dental Health, The University of Adelaide.

Many techniques have been used in the study of periodontal ligament vasculature. Electron microscopic investigations are, however, scant. This study examined vessels maintained in situ between the tooth root and a segment of the overlying alveolar plate. Specimens were obtained from orthodontic patients requiring first premolar extractions as part of their treatment. The surgical technique involved removal of the tooth with a small section of attached buccal plate. The tissue sections comprising a sandwich of tooth, periodontal ligament and attached bone were demineralized in 10% EDTA at 4°C in a phosphate buffer for periods of 10-17 days. Specimens were then further reduced, embedded in Spurr's resin and sectioned for the TEM using glass and diamond knife ultramicrotomy. Various types of vessels were identified. Their distribution was in accordance with generally held ideas. Larger vessels coursed apico-gingivally in the middle of the ligament. The smaller vessels projected towards the cementum from this central area. Ultrastructural associations that did not seem to have any parallel in reported studies of other vascular structures were found involving extracellular fibrous elements. It is proposed that this fibrous/vascular relationship is related to the specialized function of the periodontal ligament. Adopting the standards of vascular classifications for other tissues, the conclusion is reached that the vessels observed in this study were non-arterial.

Abstract 1.7
J Dent Res 60:1074, 1981

60

Endothelially Lined Lymphatics in Human Alveolar Bone and Periodontal Ligament. J.H. BARKER and M.R. SIMS*. Department of Dental Health, The University of Adelaide, South Australia.

Lymphologists report that there are no endothelially lined lymph vessels to be found in the cortex or medulla of bones (Deysine 1973; Olszewski et al 1977). However, during systematic qualitative and morphometric stereological analysis of human periodontium, we have found initial lymphatics within alveolar bone.

Six human maxillary first premolars were surgically extracted leaving biopsied buccal bone and periodontal ligament attached to the coronal two thirds of the roots. The intact specimens were processed for viewing in the transmission electron microscope (TEM). Horizontal serial sections 1µm thick were viewed using light microscopy. Where vessels of any type were seen, silver sections of the same area and orientation were mounted on grids and photographed in the TEM. Lymphatics were identified by various ultrastructural criteria including shape, size, wall structure, types of endothelial junctions, fibrillar insertions into the endothelial lining and the presence of fat globules (Casley-Smith 1980).

Both large and small cancellous spaces in the alveolar bone of each sampled specimen contained numerous vessels, all of which were ultrastructurally classified as 'initial lymphatic' vessels. These vessels protruded into the alveolar third of the periodontal ligament via foramina in the lamina dura. Thus, by contrast with other bones, human alveolar bone is, so far, unique.

Supported by: Australian Society of Orthodontists Foundation for Research and Education

Abstract 1.6
J Dent Res 58:2240, 1979

39

SEM Assessment of Rapid Maxillary Expansion on Premolar Root Topography. A. BARBER*, Department of Dental Health, The University of Adelaide.

Sutural responses to forces generated by rapid maxillary expansion (RME) have been clearly identified. In contrast, the effect of RME on dento-alveolar structures has been poorly defined. In this project the reaction to RME at the tooth periodontal ligament interface was examined. Six patients, aged 10-14 years, were treated with RME appliances fitted to first molars and either one first premolar (group one) or both first premolars (group two). Each of the three group one patients had both first premolars removed 1, 8 or 36 weeks after expansion. Group two patients had one first premolar removed immediately after treatment and the other 16, 24 or 32 weeks later. Each root specimen was rendered anorganic in sodium hypochlorite, alcohol dehydrated, air dried and coated with carbon and then gold-palladium before being examined in a Siemens Autoscan SEM. Untreated group one first premolars exhibited essentially normal root topography. The 1 and 8 week premolars showed sites of active resorption limited to the cervical third of buccal root surfaces. The 36 week premolar showed extensive, predominantly repairing resorption bays in the buccal root surface. Group two premolars extracted soon after treatment exhibited limited resorption of buccal root surfaces. The premolars extracted at 16, 24 and 32 weeks exhibited more extensive but mainly repairing bays. The typical response of anchor premolars to RME was extensive cementum and dentine resorption followed by cellular cementum repair.

Abstract 1.8
J Dent Res 60:10, 1981

4

An SEM Study of Cementum Repair in Humans. S.R. LANGFORD*, The Department of Dental Health, The University of Adelaide, South Australia.

Reports of root resorption associated with orthodontic treatment have generally centred on apical root loss. In contrast, recent studies reported by Wainwright (1973), Reitan (1974) and Barber (1978) have demonstrated extensive root surface resorption related to mechanical forces on teeth. Although repair of such defects has been reported, reattachment of principal periodontal fibres has not been demonstrated. The purpose of this study was to examine repair cementum on root surfaces to determine if reattachment could be observed in the SEM. Topographically, principal periodontal fibres inserting into cementum are characterized by either Sharpey fibre mounds or depressions (Boyd and Jones, 1972).

Extracted upper first premolar teeth, which had served as anchor teeth for rapid maxillary expansion and retention in orthodontic patients, were rendered anorganic and examined in the SEM. Extensive surface resorption was found on the buccal facing aspect of all these teeth. In most resorptive defects repair was seen to be occurring with cellular cementum while resorption was still active at the periphery of the defects, particularly with the shorter retention periods. Early repair occurred with rapidly mineralizing cellular cementum while later repair tissue appeared to be more slowly mineralizing cellular cementum. Depressions interpreted as periodontal fibre insertion sites were observed in the mineral front of repair cellular cementum. The nature and distribution of these depressions was variable and no regular pattern of occurrence was discernible. It was concluded that functional reattachment of principal periodontal fibres does occur in repair cementum.

Abstract 1.9
J Dent Res 62:409, 1983

58 Ultrastructural Associations of Periodontal Oxytalan Fibres. M.R. SIMS, Department of Dental Health, The University of Adelaide, South Australia.

Controversy surrounds the function of the oxytalan fibre system. The ultrastructure of mouse molar periodontal ligament was examined to establish the relationship of the oxytalan meshwork to other ligament structures.

Thirty day old male mice were perfused via the heart with a solution of 4% glutaraldehyde and 0.89% OsO₄ in Na cacodylate buffer. The mandibles were demineralized using 0.1M EDTA in cacodylate buffer at pH 6.9, containing 2.5% glutaraldehyde, cut into small molar blocks, postfixed and embedded in Epon 812. Ultra-thin sections in the silver-grey interference range were stained with uranyl acetate and lead citrate for TEM examination.

Oxytalan fibres were shown to approximate myelinated nerves, exposed unmyelinated axons and free nerve endings located alongside and within the walls of the microvascular bed. This periodontal model validates the hypothesis that the oxytalan meshwork forms part of a proprioceptor system.

Abstract 1.11
J Dent Res 64:652, 1985

24 Microfibril Populations in Mouse and Human Periodontal Oxytalan Fibres. M.R. SIMS, Department of Dentistry, The University of Adelaide, South Australia.

There are no reports which quantify or compare the microfibril content of animal and human oxytalan fibres in the periodontal ligament. For this TEM study four 30-day old male mice were perfused with 2% glutaraldehyde and 0.89% OsO₄ in 0.1M cacodylate buffer at pH 7.4. The mandibles were demineralized at 4°C with 0.1M EDTA in 2.5% cacodylate buffer at pH 7.4, post-fixed in OsO₄, and embedded in Epon 812. Human maxillary and mandibular periodontal ligament was obtained from the extracted premolars of two females aged 14 and 16 years. This tissue was similarly fixed, demineralized and embedded. Blocks were sectioned parallel to the occlusal plane in the silver-grey interference range, stained, and photographed in a JEOL 100-S. Magnifications were checked against a cross-grating replica. Forty true cross-sectional profiles of oxytalan fibres were selected from representative levels between the cervical and apical limits of the mice mandibular first molars. Forty comparable human oxytalan fibres were recorded in cross-section. From each pool of data, ten mouse and ten human fibres were randomly selected and digitized for total fibre area (TFA) and microfibrillar area (MFA). A third group of specific mouse oxytalan fibres (RER) was also evaluated.

Analysis of covariance showed that the structural relationship between MFA and TFA was linear for each of the three groups. Slopes for the mouse and human microfibril populations were the same ($p > 0.1$), but different ($p < 0.05$) for the RER group. Intercepts for all three groups were significantly different at the 1% level.

Abstract 1.10
J Dent Res 62:409, 1983

56 Vascular Architecture of Rat Molar Periodontium. W.T. WEEKES* and M.R. SIMS, Department of Dental Health, The University of Adelaide, South Australia.

Previous investigations into the vascular architecture of rat molar periodontium have been limited to describing "blood vessels" with no categorization into arteries or veins. Kindova and Matena (1962) provided such a delineation but their work has not been confirmed. Pilot studies showed that their model required revision.

Sprague-Dawley rats were perfused with methyl methacrylate using the method of Murakami (1971) modified by Gannon (1980). The tissues were corroded using KOH, HCL and enzymes. The casts were rendered conductive by osmification (Murakami et al. 1973). After sputter coating the casts were viewed in the SEM at 20kV. Stereopairs were taken.

Two patterns of blood vessels occurred in the periodontal ligament. (1) Around the perimeter of the socket, vessels coursed uninterrupted from the apex to the coronal region in a palisade arrangement. (2) At the interradicular septum, arterioles from the medulla perforated the socket wall, enlarged and coursed occluso-apically for a short distance before re-entering the medulla to anastomose with venules draining other similar vascular formations. In both arrangements the periodontal ligament vessels appeared to be venous in nature. The division of periodontal ligament vessels into definite arterial and venous networks could not be supported. In the interproximal col region there existed a number of large glomerular structures which have not been reported in the rat. The gingival crevicular plexus consisted of a dense network from which arose a band of twisted, vascular loops that extended towards the crown of the tooth. Vascular loops in the crevice region are part of the gingival plexus, not the periodontal plexus.

Abstract 1.12
J Dent Res 65:775, 1986

267 Gingival Vascular Architecture of the Common Marmoset (*Callithrix jacchus*). W.T. Weekes* and M.R. Sims, Department of Dentistry, The University of Adelaide, South Australia.

In previous investigations (Weekes and Sims 1985) the vascular architecture of the rat molar gingival crevice was described, utilizing the vascular casting SEM technique. This technique has been applied in the present project to view the microvasculature of the gingival tissues in the common marmoset (*Callithrix jacchus*), a model more closely resembling man.

Three marmosets were anaesthetised and perfused with physiological saline via both carotid arteries. Fluid egress was via the right atrium and perfusion continued until blood was eliminated from the vascular bed. Pre-polymerised methyl methacrylate was introduced into the vascular system under pressure. Following polymerisation, all tissues were corroded away using 20% KOH followed by 10% HCL. The casts were rendered conductive by gold coating and viewed in the SEM at 20kV and photographed as stereopairs.

Below the vestibular epithelium, vessels were aggregated into occluso-apically oriented ridges separated by deep clefts. Hairpin-like loop formations occurred at the crest of the vascular ridges and abundant anastomoses occurred between the loops. Supply and drainage was via vessels oriented at right angles to the epithelial surface. Few branches between these deeper vessels were observed. Vessels at the crest of each ridge were approximately 10 to 12 micrometres in diameter.

Immediately below the crevicular epithelium the vascular ridges became less distinct and, in some specimens, disappeared. The hairpin loops of the vestibular gingiva were replaced by densely packed vessels, 12 to 15 micrometres in diameter, arranged into glomeruli comprising 3 or 4 loops in each.

Arrangements of marmoset vasculature that differed from the rat were (1) vascular ridges beneath the vestibular epithelium and (2) a densely packed layer of glomeruli below the crevicular epithelium.

- 25 Scanning Electron Microscopy of Marmoset Periodontium and Palate Microvasculature using Corrosion Casts. D. LEE* and M.R. SIMS
The University of Adelaide, South Australia.

The aim of this study was to examine the microvasculature of the marmoset periodontium and palate using corrosion casts. Eight adult female marmosets were perfused with Mercox resin and the tissues were macerated. Cleaned casts were examined in a scanning electron microscope.

The palatal vasculature consisted of a subepithelial capillary network orientated sagittally. Capillary loops arose from this network, extending into the connective tissue papillae. The deeper venous network lacked a definite sagittal orientation. Arterioles were less common in the palate and tended to lie at a deeper level than venules.

Lying adjacent to the junctional epithelium around each tooth was a circular plexus of vessels. Just coronal to this plexus, on the crevicular side of the gingiva, lay crevicular loops, which also encircled each tooth. There was considerable variation in the crevicular loop patterns. Crevicular loops derived their blood supply from, and drained into, the circular plexus and the periodontal ligament plexus.

The periodontal ligament consisted of a network of occluso-apically orientated vessels. Arterioles were less commonly found, tended to have a constant diameter, ran a straighter course, and branched less often, compared to venules which had varying diameter, ran a more sinuous course, and branched more often. This investigation demonstrated distinct species differences with previous studies of the mouse and rat microvasculature.

This study was funded by the A.S.U. Foundation for Research and Education.

Abstract 1.13
J Dent Res 68:542, 1989

- 83 Tracer Molecule Passage Across Periodontal Ligament Vascular Endothelium. S.M.COOPER* and M.R.SIMS. Department of Dentistry, University of Adelaide, South Australia.

The labile water-rich tissue channels of connective tissue can be visualised directly by filling them with tracers as a form of negative staining. The purpose of this study was to examine the effect of an applied force on the tissue channels of the periodontal ligament of the rat.

Fourteen male rats were anaesthetised and a 1N tensile force was applied to the maxillary right first molar for a period of 30 minutes. The left first molar served as the control. In eight rats, sodium ferrocyanide was perfused through bilateral carotid arteries, and precipitated with a cobalt salt contained in the fixative to produce the ionic tracer. Six rats received one experimental ion only, to provide tracer controls. The maxillae were dissected out, demineralised and prepared for TEM analysis. Apical areas of the mesial root of the right and left sides were viewed at 10,000X magnification, and the number of tissue channels/ μm^2 connective tissue adjacent the blood vessels were counted. Immediately adjacent the endothelium, the mean \pm standard deviation density was 0.43 ± 0.05 on the left side, and 0.65 ± 0.13 on the right side. The density of tissue channels decreased away from the endothelium ($p < 0.01$). Extrusion resulted in a stat. sig. increase in tissue channel density adjacent to arterial and venous capillaries and post-capillary sized venules ($p < 0.01$). The size and density of the tissue channels concurred with tissue channels in other tissues (Reviewed by Casley-Smith, 1983).

Abstract 1.14
J Dent Res 69:943, 1990

- 46 PDL Neural and Microvascular Reconstitution Following Orthodontic Incisor Extrusion. A.P. WEIR* and M.R. SIMS. (Department of Dentistry, The University of Adelaide):

Four adolescent marmosets (*Callithrix jacchus*) were used as a model to mimic clinical treatment subsequent to subgingival incisor crown fracture. This regime involves endodontic therapy and orthodontic extrusion to allow crown restoration. The animals were anaesthetized with Saffan and the upper left central incisor sectioned at the gingival margin. Vital pulpectomy and immediate root canal obturation was carried out. A Samarium-cobalt magnet was cemented into the decoronated incisor root surfaces, and individually cast cobalt-chrome devices bonded to the maxillary anterior teeth with an identical magnet fixed in a housing over the treated incisor. Each left maxillary incisor was extruded 1.2mm and retained for 30 weeks. The animals were perfused via the common carotid arteries with 5.6% glutaraldehyde and 0.9% osmium tetroxide. The maxillary incisor blocks were demineralized in 0.1M EDTA in 0.06M cacodylate buffer and prepared for TEM examination. The apical region blocks were isolated and ultrathin sectioned to allow random selection of tissue for viewing and recording. Standard point counting techniques were used to morphometrically quantify the vascular and neural components. Statistical analysis revealed no significant morphological differences between the control and experimental microvascular beds following retention. Incisor extrusion and long term retention is accompanied by reconstitution of the PDL vascular and neural systems.

Abstract 1.15
J Dent Res 71:982, 1992

Abstract 1.16
J Dent Res 72:677, 1993

96

Ageing Changes in the Periodontal Ligament Microvascular Bed - Luminal Volumes. M.R. SIMS, W.J. SAMPSON¹, P.I. LEPPARD and C.W. DREYER, Orthodontic Unit, Department of Dentistry, The University of Adelaide.

There are no published quantitative electron microscopic data on ageing changes in the periodontal ligament (PDL) microvascular bed. It has been noted that the vascular volume decreases with age (Bradley, 1976). However, the validity of this statement has not been statistically tested. This investigation was undertaken to quantitatively analyse PDL microvascular bed luminal ageing changes. Four 35-day-old male ALCA mice and four colony related one-year-old male mice were anaesthetised and perfused via the ventricle with 2.5% glutaraldehyde containing 0.9% OsO₄, W/V and 0.06M cacodylate buffer. Mandibles were demineralised at 4°C with 0.1M EDTA in 2.5% glutaraldehyde. The first molar blocks were TEM processed and the PDL sectioned on the mesial aspect parallel to the occlusal plane at 150µm intervals from the alveolar crest to the apex. Sections were TEM stained and micrographs taken in the tooth, middle and bone circumferential thirds at each level (Freezer and Sims, 1987). Point count data were stereologically analysed (Nyengaard and Gundersen, 1988) and also evaluated using a repeated measures ANOVA. With ageing, the total vascular volume increased from $8.5\% \pm 1.37$ to $19.5\% \pm 2.14$ SEM. The collecting venule volume increased fourfold ($p < 0.001$). Ageing results in significant regional shifts in microvascular bed architecture, and a x2.3 luminal volume increase in the mouse molar PDL.

Abstract 1.17
J Dent Res 72:677, 1993

97

Stereological Changes in the Microvascular Bed of Mouse Molar Periodontal Ligament With Ageing. M.R. SIMS, C.W. DREYER¹, P.I. LEPPARD and W.J. SAMPSON, Orthodontic Unit, Department of Dentistry, The University of Adelaide.

On the basis of histological observations of animal and human periodontal ligament (PDL), it is claimed that ageing in the microvascular bed is characterised by the presence of arteriosclerotic changes (Grant and Bernick, 1970, 1972; Levy et al., 1972). Subsequently, Severson et al. (1978) refuted this finding following histological study of human and animal PDL. The present TEM study was undertaken to re-evaluate these conflicting claims. Four 35-day-old male ALCA mice and four 1 year old colony-related male mice were perfused for TEM examination (Freezer and Sims, 1987). The mandibular molar mesial PDL was sectioned parallel to the occlusal plane at 150µm intervals from the alveolar crest to the apex to sample the microvascular bed in the tooth, middle and bone circumferential thirds at each level. Vessels were stereologically analysed (Nyengaard and Gundersen, 1988) using a 140 point square lattice grid. No significant change occurred in the luminal volume of arterial capillaries between young and old mice. Luminal volume of grouped arteriovenous anastomoses and terminal arterioles, as a per cent of PDL volume, increased fourfold from 0.5% in young mice to 2.0% in old mice. Mean wall thickness of the grouped vessels in young and old mice was 2.78µm and 1.69µm respectively. Findings in this model do not reveal arteriosclerotic changes with ageing.

Abstract 1.18
J Dent Res 72:677, 1993

98

Microstructural Studies of Orthodontic Wires.

J. SINGH¹, J.V. BEE², and M.R. SIMS¹ (1Orthodontic Unit, Dept. of Dentistry, 2Dept. of Chemical Engineering, University of Adelaide, South Australia).

The mechanical properties of the relatively new α -titanium orthodontic wires have been studied for comparison with those of β -titanium and stainless steel. Since these properties and the performance of the wires are largely determined by their structures, an investigation has been carried out to characterise the microstructural features of each type of wire. Preliminary studies were performed using Scanning Electron Microscopy (SEM) and Energy-dispersive X-ray Analysis (EDS) to observe larger features and determine chemical composition data. In order to obtain meaningful microstructural and analytical information on these extremely fine structures it was necessary to carry out further studies using Transmission Electron Microscopy (TEM) techniques.

The purpose of this paper is to present the results of these electron microscopy investigations which reveal the detailed microstructures of orthodontic wires. Stainless steel, α - and β -titanium alloys all exhibited heavily dislocated, elongated grain structures, characteristic of cold-drawn material. The stainless steel wires had the finest microstructure with longitudinal grain thickness as small as 30nm. The titanium alloys were slightly coarser but still had typical dimensions of the order of only 1-2µm. It is clear, therefore, that the use of TEM techniques is essential both for the microstructural characterisation of orthodontic wires and the understanding of their mechanical properties.

This study was supported by A.J. Wilcock, Scientific and Engineering Equipment, Kinglake Road, Whittlesea, Victoria 3757, Australia.

Abstract 1.19
J Dent Res 72:677, 1993

- 95 Corrosion Cast SEM Stereopair Study of Marmoset PDL. C.M. STANLEY* and M.R. SIMS (Department of Dentistry, The University of Adelaide, S.A.):

The three-dimensional microvascular structure of the marmoset PDL is imperfectly understood. Most studies in the past have been limited to two-dimensional histologic or TEM sections. The resolution of the SEM combined with stereopair imaging of corrosion casts provides three-dimensional interpretation. Corrosion casts previously reported by LEE et al., (1991) were used to examine the unstudied anterior regions. The casts were immersed in double distilled water. Air bubbles around the casts were reduced through evacuation and the specimens frozen. Using a mini-motor with a diamond cutting wheel, the casts were sectioned midsagittally through the tooth sockets. Razor blades were used for final trimming. Significant vascular arrangements included: (1) An arterial supply to the mandibular labial gingiva originating in the apical PDL. (2) An arterial supply to the mandibular PDL from the alveolar mucosa. (3) Venous drainage from the mandibular PDL to the alveolar mucosa. (4) Large venules draining down the mesial mandibular PDL. (5) Different patterns of pulpal venous drainage into the apical PDL vessels. It is concluded that a knowledge of the normal microvascular structure of the PDL is important in understanding the vascular changes resulting from orthodontic tooth movement. Studies which have evaluated vessel type and vascular volume from one side of the tooth may not necessarily represent the other sides.

Abstract 1.20
J Dent Res 74:749, 1995

- 28 Stereology of Blood Vessels in the Periodontal Ligament (PDL) of Man. K.W.C. FOONG¹, M.R. SIMS*, W.J. SAMPSON¹ and C.W. DREYER¹ (Dental Faculty: The University of Adelaide¹ & University of Sydney):

Histological studies of human PDL report mean blood vessel volumes from 1.63% to 3.5% (Götz 1976, 1980). However, stereological TEM animal data show luminal volumes from 7.5% to 11.3% (Freezer and Sims 1987; Parlange and Sims 1993). Therefore the present TEM study was conducted to provide stereological blood vessel data in human PDL. Four premolars in the left and right mandibular segments of a 20 year old burns victim were immersion fixed in 2.5% glutaraldehyde and demineralized at 4°C in 0.1M EDTA. The teeth, bone, and PDL were processed for TEM. Stereological evaluation revealed PDL mean luminal blood volumes from $8.97 \pm 2.05\%$ (SE) to $9.52 \pm 2.28\%$. Wall volume added 3.39%. These findings show PDL blood volumes three to seven times greater than previously reported. Mean blood volume does not accurately define the regional distribution of the microvascular bed. This study was supported by a grant from the Australian Society of Orthodontists' Foundation for Research and Education.

Abstract 1.21
J Dent Res 74:756, 1995

- 81 A Comparison of Nickel-Titanium Wires by Tensile Test. G. STANTON*, T. MORI and M. SIMS (Faculty of Dentistry, University of Sydney, Australia).

Wires of various compositions are used in orthodontic treatment. The purpose of this study was to compare nickel-titanium wires by assessing their tensile properties. Eight commercial wires, 0.4 mm in diameter, were tested at a cross-head speed of 1 mm/min under ambient conditions. A stainless steel wire was included for comparison. At least five specimens were tested for each wire. After the initial linear relationship between load and elongation (modulus of elasticity), the nickel-titanium wires showed characteristic pseudoplastic behaviour (often called superelasticity) and then a second linear relationship in all but one (Nitinol). The values for modulus of elasticity ranged from 27-42 GPa for the first, including Nitinol. They were significantly reduced in the second in most wires. Other properties obtained with the nickel-titanium wires (except Nitinol) were 300-490 MPa for 0.1% yield strength, 8-10% elongation, and 1250-1500 MPa for ultimate tensile strength. Significant differences in force exist among nickel-titanium wires when utilising the pseudoplasticity, as evidenced by the yield strengths.

Abstract 1.22
J Dent Res 75:438, 1995

302 Age changes in the periodontal ligament endothelial junctions of mice.
J. CAMERON¹, M.R. SIMS, W.J. SAMPSON (Faculty of Dentistry, The University of Adelaide).

Vascular permeability is directly proportional to changes in microvasculature endothelial junction morphology (Bundgaard, 1988), and decreases with age (Hruza, 1977). An increased number of endothelial junction tight regions is related to a reduction in permeability of microvessels (Rippe and Haraldsson, 1994). The aim of this study was to assess endothelial junction morphology of postcapillary-sized venules (PCV) of four young ALCA (35 days) and four colony related old mice (365 days). The mice were anaesthetised and perfused with 5.6% glutaraldehyde and 0.9% osmium tetroxide W/V in 0.06M cacodylate buffer. Right and left mandibular first molar mesial roots with associated periodontal ligament (PDL) and bony socket were dissected out and demineralised at 4°C with 0.1M EDTA in 2.5% glutaraldehyde and embedded in resin. Blocks were sectioned at 160µm intervals from the alveolar crest to the tooth apex and stained for transmission electron microscope assessment. At each level, five PCV each with one complete endothelial junction were selected at random. Junction dimensions were measured on standardised micrographs magnified x 150K. The percentage of tight regions was 13.7% ± 3.5% higher in the old mice (p < 0.001) using a chi-square analysis. Tight regions (89.0%) were largely confined to the luminal side of the PCV. Other junctional dimensions (length, width and size) were not found to alter in aged PDL. These changes in endothelial junction morphology may lead to decreased permeability of the PDL microvasculature and may represent functional modification during ageing. Studies assessing permeability in aged PDL are needed to confirm this conclusion. (This study was supported by the Australian Society of Orthodontists Foundation for Research and Education)

Abstract 1.23
J Dent Res 74:461, 1995
also in
J Dent Res 75:272, 1996
Hatton Competition No 2038

2038 Morphometry of nerves in the human premolar periodontal ligament.
K.W.C. Foong¹ and M.R. Sims² (Faculties of Dentistry, National University of Singapore¹ and University of Sydney²)

Volumetric quantification and distribution of periodontal ligament (PDL) nerves have been documented in mice by Freezer and Sims (*Aust Ortho J* 11:30-37, 1989), and in marmosets by Parlange and Sims (*Eur J Ortho* 15:33-44, 1993). However, morphometric data on human periodontal innervation is lacking. Therefore, the present transmission electron microscopic (TEM) study was conducted to provide morphometric nerve data in human PDL. Four premolars in the left and right mandibular segments of a 20 year old burns victim were immersion fixed in 2.5% glutaraldehyde and demineralised at 4°C in 0.1M EDTA. The teeth, bone and PDL were processed for TEM. Ultrathin horizontal sections (Ag-Au interference range) were obtained from the distolingual and distobuccal ligaments from the alveolar crest to the root apex at predetermined levels, 1000 microns apart. Data collected using standard point counting procedures were analysed with ANOVA for main effects due to side of mouth, depth and zone across ligament. The mean axon volume was 0.52 ± 0.12% (SE) of the total PDL volume. Schwann cells and K-cells contributed an additional 0.48 ± 0.11% (SE). Unmyelinated axons were five to six times as common as myelinated axons. Statistically-significant differences (p<0.05) occurred between different lateral thirds, and between the vertical thirds. The greatest relative proportion of axons is located in the mid-lateral third (approximately 80 per cent), and in the apical third (± 70 per cent). These findings show that the neural composition of the human PDL is not uniform. The variable regional concentration of nerves suggests differential neural function and reaffirms the anisotropic nature of this connective tissue. This study was supported by a grant from the Australian Society of Orthodontists' Foundation for Research and Education.

Abstract 1.24
J Dent Res 74:461, 1995

471 Neurovascular constitution in marmoset periodontal ligament after long term retention.
S. CHINTAVAKORN¹, M.R. SIMS² and K.W.C. FOONG³ (Dental Hospital, Bangkok¹, University of Sydney² and National University of Singapore³).

Maxillary incisor fracture is a common dental injury, and is often treated by endodontic therapy, orthodontic extrusion, and a retention period. The aim of this study was to mimic this regime and test the hypothesis that vascular and neural reconstitution of the periodontal ligament (PDL) was complete after a long term non-human primate retention of 30 weeks in the marmoset, which approximated 3 years in human terms. Four male marmosets (*Callithrix jacchus*) were used as animal models to simulate this treatment regime. The experimental procedure involved the upper left central incisor crown removal, root canal therapy, followed by magnetic extrusion of 1.2 mm. The fractured incisor was retained in the extruded position for 30 weeks. The upper right central incisors acted as controls. Each animal was perfusion fixed with 5.6% glutaraldehyde and 0.9% osmium tetroxide mixture. The teeth, bone and PDL were processed for TEM. Horizontal sections at 150 micron levels from the alveolar crest to the root apex were obtained from the most mesial PDL regions. Data collected using standard point counting procedures were analysed using a generalised linear regression model for differences between control and experimental neural and vessel volumes. Differences in vessel diameters and vessel wall thicknesses were assessed using ANOVA. Stereological evaluation revealed that reconstitution of the PDL blood vessels and nerves were essentially complete after a 30 week retention period. However, wall thickness of postcapillary-sized venules in experimental central incisors were significantly smaller (p<0.05). These findings show that while physiological re-establishment of the vascular and neural system had occurred, the morphological reconstitution of postcapillary-sized venules was incomplete or represented a longer term or permanent change. Clinical implications of this study suggest a retention period of between 1 and 3 years in human terms in order to maximise restoration of both systems, and minimise orthodontic relapse. This study was supported by a grant from the Australian Society of Orthodontists' Foundation for Research and Education.

Abstract 1.25
J Dent Res 76:336, 1997

2581 Microvessel changes in aged mouse periodontal ligament. J. CAMERON*, M.R. SIMS, W.J. SAMPSON (Department of Dentistry, The University of Adelaide, South Australia, 5005).

The morphology of the microvasculature bed of periodontal ligament (PDL) provides information regarding PDL function, e.g., permeability and tissue perfusion. Age changes in mice PDL blood vessel luminal volumes and wall thickness have been reported (Sims, *J Dent Res* 70: 677, 1992). This study aimed to assess postcapillary-sized venule (PCV) morphology of four young ALCA (35 days) and four colony related old mice (365 days) using transmission electron microscopy (TEM). Anaesthetised mice were perfused with 5.6% glutaraldehyde and 0.9% osmium tetroxide W/V in 0.06M cacodylate buffer. Mandibular first molar mesial roots, with associated bony socket and PDL, were dissected out and demineralised at 4°C with 0.1M EDTA in 2.5% glutaraldehyde. Tissue blocks were resin embedded, sectioned at 160 µm intervals from the alveolar crest to tooth apex and stained for TEM. Dimensions of five PCV per level were measured on standardised micrographs magnified x1500. Age effects were tested using multiple regression. Age had no significant effect on PCV diameter or type. Numbers of PCV in the PDL tooth third were higher in old mice ($p < 0.01$). Of these PCV, significantly more were apericytic ($p < 0.001$). Numbers of PCV decreased significantly ($p < 0.001$) in young mice in the PDL middle circumferential third halfway down the molar root. These changes in PCV morphology with age may indicate functional modification of PDL microvasculature during ageing. Permeability studies in aged PDL are needed to further investigate this conclusion. (This study was supported by the Australian Society of Orthodontists Foundation for Education and Research)

Abstract 1.26
J Dent Res 76:944, 1997

80 Normal and Aged Microvascular Bed Volumes in the Periodontal Ligament. M.R. SIMS* (Discipline of Orthodontics, The University of Sydney):

Ultrastructural data comparing the normal and aged microvascular beds in periodontal ligament have not been established. This study tested the hypothesis that a reduction occurred in the total luminal volume in the aged ligament. Following vascular perfusion of thirty five day-old and one year-old male mice the mandibular first molars were sectioned for electron microscopy. The PDL circumferential third regions were examined at 150 µm intervals from the alveolar crest to the root apex. Blood vessel lumina were randomly sampled and the data analysed at the $P < 0.01$ level of significance for the ageing effect and differences across regions. The mean luminal volume of $8.63\% \pm 1.37SE$ of young PDL increased to $9.83\% \pm 2.14SE$ in aged ligament ($P < 0.01$). The mean PDL width of $119.9\mu\text{m} \pm 16.94SE$ in young mice decreased to $60.0\mu\text{m} \pm 10.58SE$ in aged mice. The collecting venules and the group comprising arteriovenous anastomoses plus terminal arterials demonstrated a twofold increase in luminal volume density ($P < 0.01$). Furthermore, the microvascular bed in aged ligament showed regional shifts for the different vessel profiles. In aged PDL the combined arteriovenous anastomoses and terminal arterial input bypassed the capillary bed to be shunted principally into the collecting venule segment. On the basis of these findings the hypothesis was rejected. This project was supported by NH&MRC Grant No. 880343 and the Australian Society of Orthodontists' Foundation for Research and Education.

Abstract 1.27
J Dent Res 76:937, 1997

26 A Model for the Response of Blood Vessels to Tensile Forces. P J LEWIS*, M R SIMS, D LAYMAN*, N HUNTER*. (Discipline of Orthodontics and ¹Institute of Dental Research, Faculty of Dentistry, University of Sydney and ²University of Louisiana):

AIMS: The microvasculature in the periodontal attachment is exposed to tensile forces during orthodontic manipulations. The blood vessel network could undergo both structural and functional changes in response to stretching and compression. A model is required to further investigate these possible changes. **METHODS:** Endothelial cells were collected by collagenase perfusion of human umbilical veins. The cells cultured from passage 3 to passage 6 were induced to form networks by exposure to a reconstituted basement membrane, Matrigel. The vessels form rapidly and are relatively stable within 4 to 6 hours of placement of the cells on Matrigel-coated flexible based petri dishes. These dishes were then stretched to place static loads on the networks. Time lapse photography using phase-contrast microscopy was used to study the vascular dynamics within a defined field. Mediators were tested using enzyme immunoassay techniques on the supernatants and the cell lysates. Internal cellular stress levels were tested by flow cytometric evaluation of heat shock protein (HSP 70). **RESULTS:** No gross reorganisation of the blood vessels was noted in response to tensile forces however, the vascular networks did undergo a degree of accelerated maturation and subjectively the vessels were of improved quality. Tension generated a rise in levels of Interleukin-1 β while compression induced a decrease. A reciprocal effect was noted for endothelin-1. HSP 70 levels were elevated by large tensional loads but not small loadings. **CONCLUSIONS:** From studies to date, the model of vascular dynamics using network manipulation in vitro will enable the evaluation of morphological, biochemical and cytoskeletal changes under tensile forces.

Abstract 1.28
J Dent Res 76:934, 1997

1 Thermal Effect on Bending Properties of Orthodontic Bonding Materials.
H. DORCHIN*, T. MORI and M. R. SIMS (Faculty of Dentistry, University of Sydney).

For the simulation of bonding situations information on the mechanical properties of the materials involved is required. The purpose of this *in vitro* study was to investigate the effect of three temperatures, 23°, 37°, 50°C on the bending properties of three orthodontic bonding materials. One hundred and twenty bar samples of two visible light-cured and one auto-curing bonding agent immersed in water, and dried at 23°C (control) were tested by the 3-point bending test. Bending properties investigated were modulus of elasticity, bending strength, deflection at fracture and fracture energy. The results were subjected to ANOVA and Student-Newman-Keuls' test. Statistically significant differences ($p < 0.05$) were noted in all flexural parameters in each material as a function of temperature. Modulus of elasticity reduced from 9.7 to 5.4 (GPa) in Sequence, 11.8 to 6.4 (GPa) in Transbond and 11.1 to 5.8 (GPa) in Concise. Average bending strength (MPa) reduced from 132.4 to 98.8, from 112.2 to 86.6 and from 110.1 to 80.3, respectively, when temperature increased from 23 to 50±1°C. The results of this study indicate significant brittle-ductile transition due to increased temperatures under wet immersion test conditions for all materials. In dry ambient conditions the materials remained brittle. The thermal effect on the mechanical properties of orthodontic bonding materials need to be taken into account in the correlation of *in vitro* and *in vivo* stress conditions. This study was supported by the Australian Society of Orthodontists' Foundation for Research and Education

Abstract 1.29
J Dent Res 76:937, 1997

24 A Comparison Of The Bending Behaviour of Nickel-Titanium Orthodontic Archwires. K. J. LESTER*, T. MORI and M. R. SIMS (Faculty of Dentistry, University of Sydney)

New generation nickel-titanium (Ni-Ti) orthodontic wires display a distinctive pseudoplastic plateau (PP), and a suitable marker for the onset of the (PP) is 0.1% pseudo yield stress (0.1% PYS) (Stanton 1995). In the present investigation a three point bending test was performed, at ambient temperature for Ni-Ti wires from the same batch as those tested in tension. Four replicate specimens of five commercially available brands of wire were deflected up to 4 mm at the middle of a 14 mm span. Results confirmed the presence of PP in three of the tested wires, but force levels for the start of the stress induced austenitic to martensitic phase transformation were substantially higher experimentally than those predicted by the 0.1% PYS data. In addition, no distinctive transition point was identified. These features can be explained by the complex stress distribution pattern and gradual increase in tensile stress from a concentrated area on the surface of the wire in the bending test. A reasonable estimate of the force produced by Ni-Ti wires as they unloaded across the PP was however, provided by the 0.1% PYS data. This feature was considered clinically relevant as it was the unloading of a wire that imparted a force to a malaligned tooth. The 0.1% PYS data can be used to give a reasonable estimate for this force in Ni-Ti wires in clinical use. This study was supported by the Australian Society of Orthodontists' Foundation for Research and Education.

Abstract 1.30
J Dent Res 76:939, 1997

38 Experimental Conditions for Stress Relaxation Test of Orthodontic Elastomeric Chains. H. CHAROENYING*, T. MORI and M.R. SIMS (Faculty of Dentistry, The University of Sydney)

Elastomeric chains have been widely used as a tooth moving device for the last 30 years. A variety of new generation chains has recently been introduced but little is known of their force delivery capabilities. These elastomers are known of their force decay or stress relaxation which takes place in relatively short periods of time, up to 74% of the initial force within 24 hr (Andreasen & Bishara, *Angle Orthod* 40:319, 1970). Therefore an initial force of about 1000 g (10 N) is required in order to use generally accepted forces of 150-300 g (1.5-3 N).

The present study aimed at finding a level of extension which would provide initial forces of approximately 10 N. Seven clear, closed space chains produced by five manufacturers were studied. Each product was cut in three different lengths (mm), short (6.3 - 7.5), medium (14.4 - 16.7) and long (29.9 - 35.3). They were tested at a cross-head speed of 50 mm/min under ambient conditions of 23 ± 1°C and 50 ± 10 % relative humidity.

Maximum extensions at fracture were in the range of 319 to 511 %, generating forces of 18.5 to 24.9 N. The force values were relatively constant despite variations in length (number of loops) for the extensions of up to 200 %. At this extension the force range of 6.2 to 11.3 N was recorded. A 200 % extension of long chains will be reasonable for the study of stress relaxation behaviour of orthodontic elastomers.

Abstract 1.31
J Dent Res 76:943, 1997

69

Maxillary Morphology of patients with Obstructive Sleep Apnoea.
B.H. Seto*, M.R. SIMS and P.A. CISTULLI^o, *Discipline of Orthodontics, Univ. of Syd.,
^oSleep Disorders Centre, St. George Hospital, Syd., Australia

The aim of this study is to compare the maxillary morphology of obstructive sleep apnoea patients with a group of matched normal controls. The hypothesis is that patients with obstructive sleep apnoea have different maxillary shape and archform configuration compared to normal controls. Forty patients with obstructive sleep apnoea confirmed by polysomnography were randomly chosen from the Sleep Disorders Centre, St. George Hospital. Twenty one normal controls were selected from volunteer employees of the United Dental Hospital by questionnaire screening to exclude heavy snoring and obstructive sleep apnoea. Dental casts were obtained for all patients and controls. The inter-canine, inter-premolar and inter-molar distances were measured with vernier callipers with centroids as landmarks according to Moyers et al. The maximum palatal height and maxillary depth were also recorded. All measurements were duplicated a month later. Index of reliability was more than 99% for all measurements. Fourth order polynomial equations were generated to describe the archforms. The results indicate that patients with obstructive sleep apnoea have narrower maxillary arches compared to normal controls. These findings were statistically significant at the canine ($p < 0.005$), first premolar ($P < 0.005$) and first molar ($p < 0.05$) region. Similarly, patients have higher palatal indices than controls, at the canine ($p < 0.005$), first premolar ($P < 0.05$) and molar ($p < 0.05$) region. In addition, the quadratic (x^2) coefficients derived from the polynomial equations suggest a more tapered archform in the patients compared with the controls. These results support the hypothesis that patients with obstructive sleep apnoea have narrower tapered arches and higher palatal indices compared to normal controls. This project has been supported by the Aust. Soc. of Orthodontists' Foundation for Research and Education.

Abstract 1.32
J Dent Res 76:943, 1997

71

Craniofacial Features of Binder's Syndrome and Chondrodysplasia Punctata: Profile Analysis. K.TITUS*, M.R.SIMS, M.D.SNOW, L.J.SHEFFIELD, A.M.HOWE, (Discipline of Orthodontics, University of Sydney, N.S.W):

The multifactorial aetiology of Binder's Syndrome (BS) and Chondrodysplasia Punctata (CDP) is linked to arrested nasal septal development, with resultant characteristic facies. Whilst most cephalometric studies have focussed on hard tissues (HT), few have quantified soft tissue (ST) profiles or compared relatives with BS/CDP patients. This cephalometric study of HT and ST compared craniofacial features of 11 patients affected by CDP/BS with norms and midfacial profile proportions of 23 patients with relatives using photographs. Three midfacial ST ratios derived from anthropometric norms were assessed photographically. Student t-test and Pearson's Correlation Coefficients provided statistical analysis set at $p < 0.05$. There were significant findings for 12 out of 29 HT and ST variables. CDP/BS subjects had reduced anterior cranial base length (S-N), maxillary length (PNS-A and Co-A), angle of facial convexity (N-A-Pog), but had increased mandibular to maxillary differential (Co-Gn to Co-A), lower anterior face height (ANS-Men) and upper to lower anterior face height ratio. Holdaway ST analysis showed: CDP/BS subjects had reduced nose prominence, increased upper lip thickness and "strain", and lower lip prominence. A Normal nasolabial angle was present in 55% of subjects. Differences existed between patients and norms for ratio Sn-C:Sn-Pru, indicating either shorter columella length (Sn-C) or nasal tip protrusion (Sn-Pru), or both. HT findings confirmed that the nasomaxillary complex was affected in BS and CDP. ST analyses confirmed nasal retraction and convex lip morphology resulting in a Class III skeletal pattern. Parents had normal ratios. There was no correlation between patients and parents for these ratios. These findings do not support inheritance of the facies. This project was supported by the Australian Society of Orthodontists' Foundation for Research and Education.

Abstract 1.33
J Dent Res 79:1050, 2000

36

Endothelin-1 Expression in Normal and Continuously Loaded Rat PDL Microvasculature. MILTON R. SIMS. (Microcirculation Laboratory, Flinders University; Orthodontic Unit, University of Adelaide, South Australia).

The endothelin-1 (ET-1) peptide is the most potent vasoconstrictor known. It occurs in a variety of tissues and is synthesized by the endothelial cells of arteries and veins in animals and man. This study tested the hypothesis that short term, continuous molar loading would cause upregulation of ET-1 in the PDL vasculature. Five male Sprague-Dawley rats, 350 \pm 50 gm, were anaesthetised IP with Nembutal (0.6mg/100gm). A head frame applied an external load of 100 \pm 20gm for 3 hours, transmitted via a randomly allocated inter-occlusal rubber pad between the left or right molars; controls were the contralateral molars. The rats were perfused with 5% paraformaldehyde and the jaws sectioned sagittally at \approx 150 μ m. Sections were immunolabelled with ET-1 (Peninsula) and α -smooth muscle actin (Maxim Biotech) and secondary antibodies conjugated with CY3 and CY5 (Jackson). Serial images were captured at a constant signal to noise ratio with a BioRad MRC-1000 confocal microscope. In control PDL, the ET-1 labelling occurred sporadically in all types of blood vessels. Loaded PDL revealed strong upregulation of ET-1 expression in cementoblasts and a greater number of vessels, such as apical arcades of postcapillary-sized venules, and those adjacent to alveolar walls. Upregulation affected blood vessels with and without smooth muscle, including those in alveolar bone. The study confirmed the hypothesis and demonstrated that ET-1 quiescence is not the PDL vascular endothelial cell default state. Funded by a grant from the Australian Society of Orthodontists' Foundation for Research and Education.

Abstract 1.34
J Dent Res 79:1054, 2000

C-19 Confocal Laser Scanning Microscopic Study of Marmoset Periodontal Ligament Microvasculature.

J. ASHWORTH and M. SIMS (The University of Adelaide, South Australia).

Knowledge of normal periodontal ligament (PDL) microvascular physiology is required to determine its biological response to orthodontic forces. This investigation examined normal and continuously loaded marmoset paradental microvasculature using antibodies to endothelin-1 (ET-1) and alpha smooth muscle actin (α -SMA). Four animals were anaesthetised for 2 hours. An external loading device applied a continuous load of 120-200gms against the mandible. An inter-occlusal rubber pad was randomly placed between the left or right molars. The contra-lateral molars acted as controls. Undemineralised mandibular sagittal sections 150-200 μ m thick were incubated in anti-ET-1 (Peninsula) and IgG/Cy3TM (Jackson) and anti-human α -SMA (Maxim Biotech) and IgG/Cy3TM (Jackson) antibodies, respectively. A confocal laser scanning microscope (Bio-Rad) and CoMOS software programme provided three-dimensional images by computer stacking of optical sections. The microvasculature showed a scattered distribution of ET-1 immunolabelling and a more generalised vascular smooth muscle cell presence in both the experimental and control PDL. These findings demonstrate the presence of vasodilatory and vasoconstrictive elements in the unloaded and loaded marmoset PDL microvascular bed. This research was supported by the Australian Dental Research Fund, Grant No. RDR 000773 and the Australian Society of Orthodontists Foundation for Research and Education.

Abstract 1.35
EOS Congress, GENEVA, 1983

ULTRASTRUCTURAL AFFILIATIONS OF OXYTALAN FIBRES, NERVES AND THE MICROVASCULAR BED IN THE MOUSE PERIODONTAL LIGAMENT.

M.R.Sims, Faculty of Dentistry, University of Adelaide, Australia

Controversy surrounds the function of the of the oxytalan fiber system. Mouse molar periodontal ligament was investigated to determine if its ultrastructure validates the hypothesis that the oxytalan fibre meshwork forms part of a proprioceptor system for the regulation of vascular flow.

Throughout the ligament oxytalan fibres approximate myelinated nerves, unmyelinated exposed axons and free nerve endings. In the cervical and apical regions accumulations of vessel-related simple and complex mechanoreceptors comprising Schwann cell-axon units were associated with collagen fibrils and fibres of the oxytalan system. The various receptors and nerve endings penetrated to the abluminal surface of the endothelial wall in the different categories of vessels constituting the microvascular bed. Periodontal receptors with oxytalan fibres were also present in the septal wall of dividing vessels and related to endothelial protrusions into the lumen of microvessels.

Similarities existed between the periodontal mechanoreceptors and baroreceptors. The oxytalan fibre meshwork provided coupling between the the various mechanoreceptor units in the microvascular bed. This periodontal model has morphological characteristics which support the proprioceptor hypothesis.

46 AGEING CHANGES IN THE PERIODONTAL LIGAMENT
MICROVASCULATURE, M R Sims, W J Sampson,
C W Dreyer, University of Adelaide, Australia

Abstract 1.36
Europ J Orthod 15:344, 1993

AIM: To evaluate arterial luminal volume and distribution in young and aged periodontal ligament.

MATERIAL: 4 male ALCA mice 35-days-old and 4 colony related 1-year-old males.

METHOD: After anaesthesia with IP nembutal, the mice were perfused via the ventricle with 2.5% glutaraldehyde containing 0.9% OsO₄ w/v and 0.006M cacodylate buffer. The mandibles were demineralised at 4°C and the first molar blocks processed for electron microscopy (TEM) to section the mesial PDL parallel to the occlusal plane at 150µm intervals from the alveolar crest to the apex. Sections were TEM stained and micrographs taken at x3000 in the tooth, middle and bone circumferential thirds at each level. Point count data were stereologically analysed and statistically evaluated using a repeated measures ANOVA.

RESULTS: The luminal volume of arterial capillaries comprised 0.2% of young PDL at 2.4% of the total microvascular bed volume. In aged PDL the arterial capillaries formed 0.4% of PDL volume and 74% of the total vascular volume, which had increased x2.3.

Arterio-venous anastomoses (AVA) had a luminal capacity of 0.5% of the PDL volume in young mice and provided 6.0% of the vascular volume. By contrast, in aged PDL the AVA luminal volume increased significantly ($P < 0.01$) to form 2.0% of the PDL and 10.2% of a doubled vascular volume.

CONCLUSIONS Arterial capillary bed luminal volume did not show a major change in the aged PDL. The statistically significant AVA increase provided the additional arterial avenue to supply the enlarged vascular volume in aged PDL.

The extra AVA input bypassed the capillary bed and confined a higher proportion of the blood within thick-walled, less physiologically active vessels. These changes, and regional vascular shifts, may help to account for the slower orthodontic tooth movement in adult patients.

5 PERIODONTAL (PDL) MICROVASCULATURE
FOLLOWING MAGNETIC INCISOR

EXTRUSION AND LONG-TERM RETENTION

S Chintavalakorn, M R Sims, W J Sampson, University of Adelaide, South Australia, and University of Sydney, New South Wales, Australia

Abstract 1.37
Europ J Orthod 17:331, 1995

AIM: This investigation was undertaken to quantify PDL blood vessel reorganization in an animal model after a relatively long-term retention period of 30 weeks. TEM studies of marmoset PDL after incisor decoronation, endodontics and magnetic root extrusion (Parlange and Sims, 1993) showed incomplete microvascular bed (MVB) reconstitution after a short-term retention period of 8 weeks. The present duplicate study extended the retention time by 4.

MATERIALS AND METHODS: Four male marmoset, aged from 17 to 36 months, were used to repeat the Parlange and Sims (1993) study. After 30 weeks retention, the marmosets were anaesthetized, perfused with 5.6 per cent glutaraldehyde and 0.9 per cent OsO₄. Maxillary experimental and control central incisors were isolated for TEM processing and analysis. Log-likelihood ratio statistics were calculated using a generalized linear model (McCullagh and Nelder, 1983) for vessel volumes and regions with Genstat v5, 2.2. An ANOVA was performed of luminal and abluminal diameters, and wall thickness, for each vessel type.

RESULTS: Luminal and abluminal MVB volumes were not significantly changed. For post-capillary-sized venules (PCV) there was an interaction between regions ($P < 0.05$) and treatment ($P < 0.05$). In experimental PDL the PCV wall thickness was reduced ($P < 0.05$), as was total MVB wall thickness ($P < 0.05$).

CONCLUSIONS: Total PDL blood volume was essentially unchanged. However, PCV reconstitution was still incomplete, or had undergone a permanent anatomical change. Extrapolation of these findings to human PDL suggests retention periods of 2-3 years for MVB recovery.

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64 ORTHODONTIC TOOTH MOVEMENT AND THE ROLE OF THE MICROVASCULAR BED OF THE PERIODONTAL LIGAMENT

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(KEYNOTE ADDRESS)

Abstract 1.38

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Without the presence of the periodontal ligament (PDL) microvascular bed (MVB), orthodontic tooth movement could not occur. A feature of this vascular system is its essentially venous structure that hold over 90 per cent of the PDL blood volume.

The PDL blood system provides the source reservoir for the exchange of metabolites and cells across the endothelium between the vascular and connective tissue compartments. This endothelium is an active structure in the physiological exchange mechanisms, generating an array of regulator molecules to control both internal and external changes.

Orthodontic tooth movement can reposition a tooth 1 mm per month. Consequently the total PDL structure must relocate over a distance of more than twice its width each 30 days of treatment. Clearly the associated MVB reconstitution must involve rapid angiogenesis.

In vitro model systems provide growth of endothelial cells under conditions that mimic an *in vivo* situation. Angiogenesis in the laboratory provides an insight into the formation of lumens and the anastomoses of adjacent sprouts to form vascular loops and planar networks.

Some morphological, functional, and ageing features of the endothelium will be discussed in this presentation.

LIBRARY NOTE:

The following articles have been removed due to copyright.

APPENDIX 3. CD-ROM: Movies of ET-1 and α -SMA immunolabelling

(Minimum System: PC, Windows 98SE, P4 processor 2.0GHz, 256MB RAM)

VIEWING MOVIES ON CD

1. Open Confocal Assistant Program (CAS40)



2. From the menu open the file of the selected movie



3. Press the Play button  and the control panel appears



Movie 1.	Paper 18	Figure 7a	Stack Images 4-26	CY3,CY5	70MB
Movie 2.	Paper 18	(from Fig 7b)	Stack Images 4-26	CY5	36MB
Movie 3.	Paper 18	Figure 11b	Stack Images 1-13	CY3,CY5	36MB
Movie 4.	Paper 18	Figure 12b	Stack Images 6-20	CY3,CY5	70MB
Movie 5.	Paper 19	Figure 3	Stack Images 3-10	CY3,CY5	36MB
Movie 6.	Paper 19	Figure 6	Stack Images 1-11	CY3,CY5	36MB
Movie 7.	Paper 19	Figure 7	Stack Images 1-18	CY3,CY5	36MB
Movie 8.	Paper 19	Figure 8	Stack Images 1-11	CY3,CY5	36MB
Movie 9.	Paper 20	Figure 4	Stack Images 3-20	CY3	36MB
Movie 10.	Paper 20	Figure 5	Stack Images 1-17	CY3,CY5	36MB
Movie 11.	Paper 20	(from Fig 5)	Stack Images 8-15	CY5	36MB
Movie 12.	Paper 20	(from Fig 5)	Stack Images 3-15	CY3	36MB
Movie 13.	Paper 20	Figure 10	Stack Images 1-13	CY3,CY5	36MB
Movie 14.	Paper 20	(from Fig 10)	Stack Images 1-13	CY5	36MB
Movie 15.	Paper 20	(from Fig 10)	Stack Images 1-13	CY3	36MB