REFEREED SCIENTIFIC ARTICLE

Journal of the Dental Association of South Africa, **51**, 754-758

Nerve degeneration within the dental pulp after segmental osteotomies in the baboon *(Papio ursinus)*

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Keywords: degeneration; pulp; osteotomy.

SUMMARY

Following dentofacial surgical procedures, teeth in segments often do not sense thermal or electric stimuli. This study was undertaken to assess changes in the neural component of the dental pulp after posterior maxillary and mandibular segmental osteotomies, with or without interpositional autogenous bone grafting, in 26 Chacma baboons. Innervation was assessed histologically immediately after operation, and at 3, 6, 12 and 18 months. Statistically significant differences were present between the experimental and control groups. Even after 18 months no nerves were present in any of the mandibular teeth. In maxillary teeth, 50 per cent had demonstrable nerves in the graft group and 40 per cent in the no graft group. As nerve degeneration was present in the experimental teeth, patients should be warned of possible change in tooth sensibility, following these operations. Careful post-operative follow up for long periods in humans following dentofacial surgical procedures is thus essential.

OPSOMMING

Dikwels toon tande in segmente geen gevoel vir termiese of elektriese stimuli na dentofasiale chirurgiese prosedures nie. Hierdie studie is onderneem om veranderinge in die neurale komponent van die tandpulpa na posterior maksillêre en mandibulêre segmentele osteotomies te bepaal, met of sonder interposisionele outogene beenoorplanting, in 26 Chacma bobbejane. Senuweevoorsiening is histologies bepaal onmiddelik na chirurgie en na 3, 6 en 18 maande. Statisties-betekenvisvolle verskille was teenwoordig tussen die eksperimentele en kontrolegroepe. Selfs na 18 maande was geen senuwees teenwoordig in enige van die mandibulêre tande nie. In 50 persent van maksillêre tande was daar bewyse van senuwees in die eksperimentele groep, en in 40 persent van mandibulêre tande in die nie-oorplantingsgroep. Aangesien senuweedegenerasie in die eksperimentele tande aanwesig was, behoort pasiënte gewaarsku te word dat veranderinge in tandgevoeligheid op hierdie operasies mag volg. Na dentofasiale chirurgiese prosedures is dit noodsaaklik dat pasiënte vir lang periodes sorgvuldig opgevolg moet word.

INTRODUCTION

The nerves in the dental pulp, both myelinated and non-myelinated, follow the course of blood vessels. Somatic afferent nerves are myelinated and branch into smaller fibres as they reach the periphery of the pulp to form a dense neural plexus in the subodontoblastic region. Here they lose their myelin sheath to become free nerve endings (Avery, 1987; Berkowitz, Holland and Moxham, 1992). The terminal branching into free nerve endings in the subodontoblastic layer is only a histological feature once root formation has been completed (Mjör and Fejerskov, 1979). Non-myelinated nerves are from the autonomic nervous system. They supply the pulp vessel walls and are associated with vasocontrol (Avery, 1987). The nerves enter the teeth at the apex and can be damaged during surgery.

The Le Fort I osteotomy has become a widely used technique for correcting deformities in the maxilla in either the horizontal or vertical planes. (Summers and Booth, 1975; Vedtofte and Nattestad, 1989). Nerve supply to the pulps of teeth in these segments must be damaged because it is well known that teeth in the mobile segments do not respond to thermal or electrical stimuli immediately after segmental osteotomy (Leibold, Tilson and Rask, 1971; Summers and Booth, 1975). It is assumed that in most cases this is temporary and that the teeth do not show any signs of a compromised blood supply as assessed by colour change in the crown of the tooth (Daniel, White and Proffit, 1971; Pepersack, 1973, Kohn and White, 1974).

Various methods are available to clinicians to assess the sensibility of pulpal tissue. These include a clinical examination of the teeth with emphasis on coronal discolouration (Hutchinson, Robinson and MacGregor, 1972; Kohn and White, 1974), thermal assessment (Theissen and Guernsey, 1976. De Jongh, Barnard and Birnie, 1986), electrical stimulation (Reynolds, 1966; Bhaskar and Rappaport, 1973; Tajima, 1975; Kahnberg and Engstrom, 1987; Vedtofte and Nattestad, 1989), radiographic examination to assess pulpal pathology (Pepersack, 1973; Kohn and White, 1974), and more recently laser Doppler flowmetry (Ramsay, Artun and Bloomquest, 1991; Aanderud-Larsen *et al.*, 1995). Currently the vitalometer or electric pulp tester has become the accepted diagnostic aid in the majority of papers reporting sensibility changes after segmental surgery (Johnson and Hinds, 1969; Daniel *et al.*, 1971; Kohn and White, 1974; Tajima, 1975; Theissen and Guernsey, 1976; Kahnberg and Engstrom, 1987; Vedtofte and Nattestad, 1989; Shehab, Coghlan and Magennis, 1996). Papers published over the past two decades in which sensibility has been assessed with the methods listed have all reported diminished sensation in teeth following Le Fort I and segmental alveolar procedures (Johnson and Hinds, 1969; Leibold *et al.*, 1971; Hutchinson *et al.*,1972;Theisen and Guernsey 1976; Kahnberg and Engstrom 1987; Shehab, Coghlan and Magennis, 1996).

Published research on histological changes in human pulp with emphasis on nerve degeneration after surgery is sparse. In a study by MacGregor (1971), nerves were absent in teeth 6½ months after anterior segmental surgery, whilst Summers and Booth (1975) found that, teeth removed 4 to 10 weeks post-operatively showed that only non-myelinated nerves survived. In contrast, Di *et al.*, (1988) reported normal pulp in third molar teeth removed 6 to 18 months following Le Fort I osteotomies.

Information from histological studies on nerve degeneration of the pulp following experimental orthognathic surgery is also scarce. Degeneration of nerves and odontoblasts, as well as intrapulpal bone formation was reported after anterior maxillary osteotomies in two monkeys followed up for 8 to 24 weeks post-operatively (Poswillo, 1972). Similar findings were reported by Bailey *et al.*, (1993), whilst Holland and Robinson (1986) noted that the total number of nerves found at the apices of canine teeth following mandibular osteotomies in cats was only 36 per cent of those found in unoperated animals.

In contrast to these studies, Sugg *et al.*, (1981) showed that although nerve fibres were absent at 6 weeks after segmental osteotomies in monkeys, nerve regeneration had occurred after 3 months. Similar results were reported following Le Fort I osteotomies on the same species by Browne, Brady and Frame (1990).

Literature concerning the effect of bone grafting on the pulp of teeth in mobilized segments is even more sparse. In one report (Di *et al.*, 1988), 3 out of 10 patients who had undergone Le Fort I osteotomies to correct occlusal discrepancies had inter-positional autogenous grafts placed. However, no specific mention of nerve degeneration was investigated.

There is no doubt that accurate surgical placement of the horizontal bone cuts as well as the nearness of vertical interdental cuts to the roots of adjacent teeth are important. The proximity of intra-osseus wires or bone plates to the root apices also play a role in the return of sensibility to teeth (Johnson and Hinds, 1969; Leibold*et al.*,1971; Hutchinson, Robinson and MacGregor, 1972; Pepersack, 1973; Kohn and White, 1974; Summers and Booth, 1975; Theisen and Guernsey, 1976; Kahnberg and Engstrom, 1987; Vedtofte and Nattestad, 1989).

The objectives of the present study were firstly to assess the nerve degeneration of the pulp following posterior segmental maxillary and mandibular osteotomies and secondly to assess the effect of inter-positional bone grafting on nerve degeneration.

MATERIALS AND METHODS

Prior to undertaking this research, the protocol was approved by the Animal Ethics Committee of the University of the Witwatersrand, Johannesburg.

Twenty-six adolescent female baboons were selected for this study and were divided into 4 groups of 6 animals each and one group of 2 animals. The baboons were anaesthetized with intermittent intravenous sodium pentobarbitone sodium and spontaneously breathed room air through an oro-tracheal tube. After cleaning, shaving and draping, a maxillary segmental osteotomy was performed by exposing the buccal surface of the maxilla and by using a fine fissure bur making the necessary osteotomy cuts (Fig. 1). The osteotomized maxillary segment containing the two premolars plus first and second molar teeth was then fully mobilized but remained pedicled on the palatal mucosa. A gap measuring 10mm x 5mm was cut in the residual basal bone above the two premolar teeth to create a defect into which autogenous bone harvested from the iliac crest was placed. The dentoalveolar segment was then immobilized into its original position using direct intra-osseus wiring.

A similar procedure was carried out in the mandible, however, the osteotomized segment contained the two premolars and only the first molar tooth (Fig. 2). The horizontal bone cut was placed above the neurovascular bundle. As for the maxilla, autogenous bone was placed below the apex of the first premolar tooth. A control operation was performed on the non-osteotomized side of each maxilla and mandible. During this, the buccal aspect of the bone was exposed by raising a mucoperiosteal flap, but no bone cuts were made. All wounds were sutured and the animals were placed on a soft diet with adjunctive antibiotic and analgesic therapy. Immediately after operation 2 baboons were killed with an intravenous lethal dose of sodium pentobarbitone, then 6 baboons were killed at each post-operative period of 3, 6, 12 and 18 months. All were perfused with a mixture of physiological saline plus fine barium sulphate followed by 10 per cent buffered formalin plus barium sulphate. The entire maxilla and mandible were removed, trimmed and all teeth in both the osteotomized and control segments prepared for histological examination. Each tooth with associated alveolar bone was decalcified in a Hydrochloric acid/Formic acid solution then embedded in Medim-Plast wax. Teeth were all sectioned longitudinally at 6μm. Step serial sections containing the entire pulp chamber, radicular as well as coronal pulp were selected and stained with a modified Protogral peroxide technique (Loots *et al.*, 1979) for axons. This technique was controlled by staining neural tissue harvested from rats. Only sections from teeth with vital pulp tissue were selected for assessment. Any teeth with pulp necrosis or where the pulp chamber had been obliterated by osteodentine and/or secondary dentine formation were excluded from the study. Each section was examined under a Univar microscope

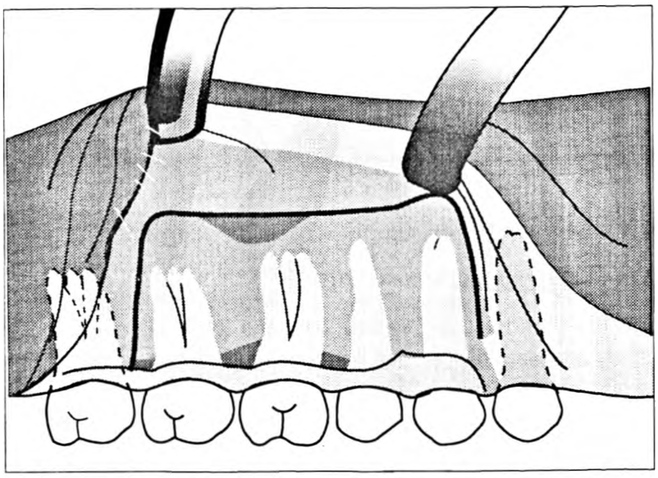

Fig. 1: **Diagram of osteotomy cuts in the maxilla.**

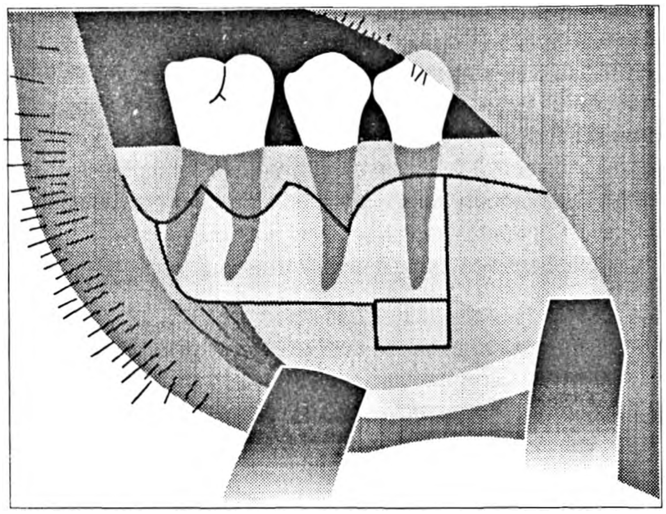

Fig. 2: **Diagram of osteotomy cuts and graft recipient site in the mandible.**

(Reichert, Vienna, Austria) with a 10x eyepiece. The presence of nerve fibres was recorded as — in the apical 1/3 only, in the entire root pulp or in the entire pulp chamber. Assessment of the presence or absence of nerve fibres was made at 3 specific areas on each slide namely the junction of the apical 1/3 and remaining radicular pulp, the junction of the radicular and coronal pulp and as close as possible to the odontoblast layer at the highest point in the coronal pulp (Fig. 3). The observations were entered on computer coding forms designed for the study, transferred into an IBM 3081K32 mainframe computer via magnetic tape and analysed using SAS (1989). The histological observations were discrete random variables which were recorded for each section of tooth examined. The number of sections varied from 12 to 25 per tooth depending on the size of the tooth. In order to avoid inappropriate, inflated sample sizes for intergroup comparisons, mean scores were determined for each tooth. The mean values were then rounded off into discrete values. A linear logistic analysis (Proc CATMOD) was carried out with the presence of nervous tissue at each tooth level as dependent variable and individual baboon, post-operative time, experimental group and jaw as independent

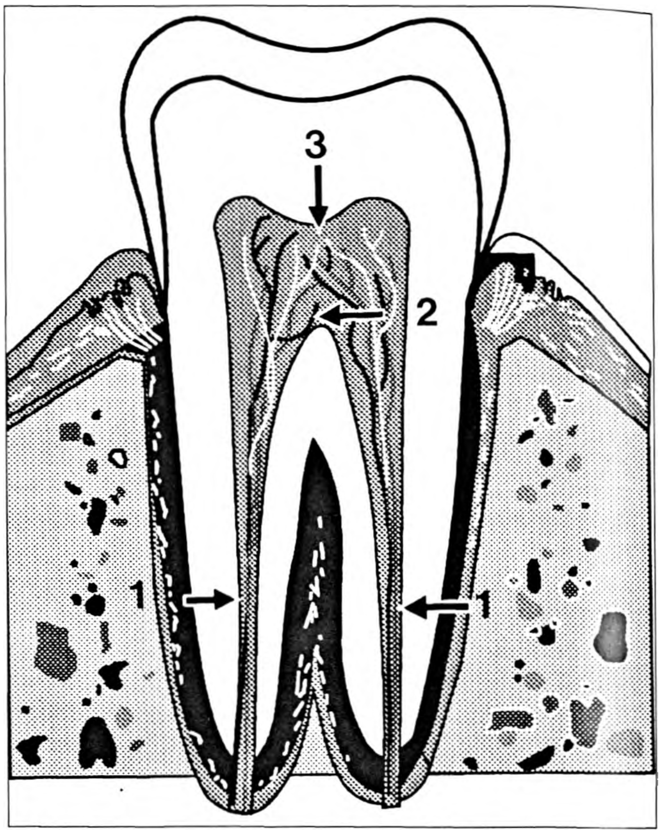

Fig. 3: **Diagram of areas of the pulp examined for nerve presence. 1=apical 1/3, 2=entire root, 3=entire pulp.**

variables. The critical level of statistical significance was $p<0,05$.

RESULTS

A total of 210 teeth comprising 93 experimental and 26 control maxillary teeth as well as 65 experimental and 26 control mandibular teeth was prepared for examination. Of these 21 (10 per cent) were discarded as unsuitable for histological examination. Five were ruined by a malfunctioning tissue processor, the remaining 16 had either unacceptable cutting artifacts or the pulp chamber was obliterated by osteodentine. A total of 3 600 sections was examined from 189 teeth. Because the numbers of teeth in each experimental group, i.e. graft, no graft and control were small for each jaw, all teeth were combined in order for the statistical analysis to be relevant. In the presentations of results X^2 indicates the chi-squared value for each dependant variable included in the linear logistic analysis, df indicates the degrees of freedom and is followed by the probability value.

The percentage teeth with nerve fibres in the pulp tissue of the entire root pulp for mandibular teeth is shown in Figure 4. Immediately after operation all but one tooth had nerves. Thereafter nerve fibres in control teeth remained relatively constant except for a decrease to 83 per cent at the 12 month post-operative period. In both the graft and no graft groups there was a rapid decline in nerves, none were present after 6 months in the no graft group and after 12 months in the graft group.

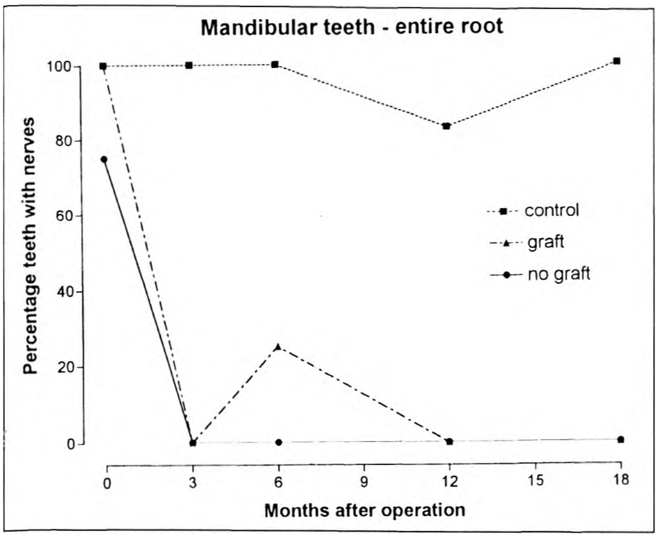

Fig. 4. **Percentage of teeth with nerves present in the entire root pulp of mandibular teeth by experimental group.**

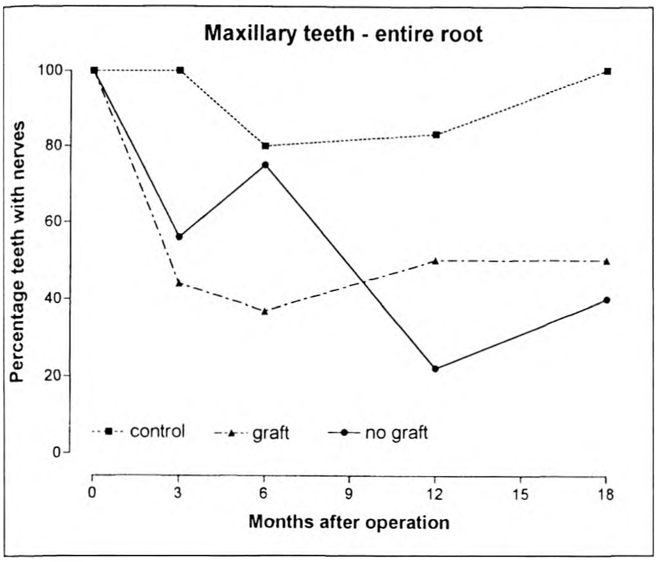

Fig. 5. **Percentage of teeth with nerves present in the entire root pulp of maxillary teeth by experimental group.**

At the end of the experiment, at 18 months, no nerve fibres could be identified in any of the experimental mandibular teeth. The results were identical for the entire pulp chamber.

The percentage teeth with nerve fibres in the entire root pulp for maxillary teeth is shown in Figure 5. A less dramatic pattern was seen than in the mandibular teeth in that some nerves were present in some teeth at each time period but were less in the graft and no graft groups than in the control group. 50 per cent of experimental teeth had nerve degeneration after 18 months. Nerves were present in the entire pulp chamber in only 20 per cent of teeth after 12 months (Fig. 6).

Linear logistic analysis for the presence of nerve tissue, in the root pulp showed statistically significant differences between all 3 experimental groups i.e., graft, no graft and control for apical 1/3 (X^2=23,85, df=2, P=0.001) and complete root pulp

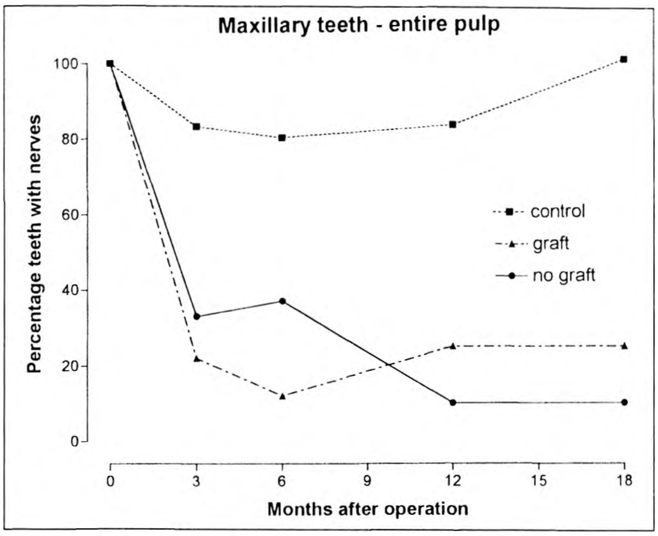

Fig. 6: **Percentage of teeth with nerves present in the entire pulp chamber of maxillary teeth by experimental group.**

(X^2=27,12, df=2, P=0,001). As far as post-operative timing was concerned, however, no statistically significant difference was apparent. Concerning the entire pulp chamber, there were statistically significant differences between all three experimental groups (X^2=23,61, df=2, P=0,001) as well as for post-operative time (X^2=17,11, df=4, P=0,001).

DISCUSSION

Both myelinated and non-myelinated nerves are present in the pulpal tissue. Myelinated axons transmit sharp pain and non-myelinated nerves are associated with vascular channels (Avery,1987). In this study no differentiation was made between the two types of fibres.

The fact that nerve fibres were present in all but one tooth, which demonstrated pre-existing fibrosis of the pulp immediately post-operatively, confirms that the histological technique was well controlled as no time was available for nerve degeneration to occur.

The overall results of this study were similar to those reported by Holland and Robinson (1986) who showed that the nerve fibres in the apical portion of teeth following segmental osteotomy in cats was only 36 per cent of that found in control teeth. Bailey *et al.*, (1993) have reported that regeneration of nerve fibres does not occur following surgical transection of roots of teeth in experimental animals. The results of the present study support this finding for mandibular teeth, however, some maxillary teeth showed innervation throughout the entire pulp chamber. The explanation for this difference is that because the apices of the mandibular teeth are closer to the inferior alveolar neurovascular bundle, the horizontal bone cut is closer to the apices of the mandibular teeth. There is more space above the maxillary teeth, so the horizontal bone cut may be further from the periapical area. In the mandibular teeth in the current study some of the apices could easily have been damaged by the rotary instruments during an attempt to avoid the neurovascular bundle. This would cause degeneration for the reasons described by Bailey *et al.*, (1993).

The observation that in some sections of control teeth nerve fibres could not be positively identified requires comment. The exact reason is not clear. There is considerable anatomical variation between animals in the proximity of the apices of the buccal roots of the maxillary second premolar and first molar to the surface of the cortical bone. It is postulated that damage to the apices of these teeth might have occurred as a result of raising the mucoperialial flap, even although no bone cuts were made.

A similar anatomical variation could account for the one instance when this occurred related to one mandibular tooth 12 months post-operatively.

That the number of nerves present in the graft group of maxillary teeth was greater than in the no graft group is probably because the horizontal bone cut was closer to the apices of the molar teeth in the no graft group with a greater likelihood of apical damage. This emphasizes the need to place horizontal bone cuts in Le Fort I osteotomies, and all segmental procedures, as far above the apices of the teeth as possible — certainly at least 5 mm (Bell, 1969).

The prevalence of nerve fibres being greater in the entire root than in the entire pulp chamber indicates that the degenerative process was self limiting. It can only be speculated that the reason for the fluctuating percentages of teeth with nerve fibres present is possibly variation between the experimental animals. Since it is unlikely that experimental animal studies will exceed the 18 months of the current investigation, clinicians need to do long-term follow-ups of tooth sensibility in patients who have had segmental osteotomies.

CONCLUSIONS

This primate study has shown that degeneration of nerve fibres in the pulp occurs following segmental surgical procedures. The number of nerve fibres present in experimental teeth is always less than those in control teeth even after relatively long follow up periods. The operative procedures in man corresponding to the experimental procedures in this study, would be anterior and posterior segmental osteotomies in both jaws as well as the Le Fort I osteotomy and total mandibular subapical osteotomy. There must be careful planning of sites of bone cuts, particularly in the mandible, in individual patients to minimize damage to nerves entering the pulp. In addition, patients need to be told that tooth sensibility is likely to change after operation. Long term follow up of patients undergoing these procedures is necessary to establish whether sensibility lost or diminished after operation will return.

ACKNOWLEDGEMENTS

The authors wish to thank the staff of the Central Animal Service, University of the Witwatersrand, Johannesburg for their assistance with the operative procedures and post-operative care of the animals. Also to staff of the Dental Research Institute of the Medical Research Council and the University of the Witwatersrand, Johannesburg for assistance in preparing the histological sections. We are indebted to Dr H Coleman of the Department of Oral Pathology for his guidance with the histological assessment. And finally to Mrs Carmen Mulder for typing the manuscript.

REFERENCES

Aanderud-Larsen, A, Brodin, P, Aars, H & Skejelbred, P (1995) Laser Doppler Flowmetry in the assessment of tooth vitality after Le Fort I osteotomy. *Journal of Cranio-Maxillo-Facial Surgery.* **23**, 391-395.

Avery, JK (1987) *Oral Development and Histology.* Baltimore: Williams and Wilkins, 164-178.

Bailey, LJ, Sadowsky, PL, Nelson, C, Hassanali, JB & Fox, CF (1993) Patterns of nerve regeneration in dental pulps of monkeys following surgical transection at 1 year. *International Journal of Adult Orthodontics and Orthognathic Surgery,* **8**, 48-54.

Bell, WH (1969) Revascularization and bone healing after anterior maxillary osteotomy: a study using adult rhesus monkeys. *Journal of Oral Surgery.* **27**, 249-255.

Berkowitz, BK, Holland, GR & Moxham, BJ (1992) *A Colour Atlas and Textbook of Oral Anatomy, Histology and Embryology.* 2nd ed. London: Wolfe Medical Publications Limited, 146-154.

Bhaskar, SN & Rappaport, HM (1973) Dental vitality tests and pulp status. *Journal of the American Dental Association,* **86**, 409-411.

Browne, RM, Brady, CL & Frame, JW (1990) Tooth pulp changes following Le Fort I osteotomy in a primate model. *British Journal of Oral and Maxillofacial Surgery,* **28**, 1-7.

Daniel, HT, White, RP & Proffit, WR (1971) Anterior maxillary osteotomy in dental treatment. *Journal of the American Dental Association,* **83**, 338-343.

De Jongh, M, Barnard, D & Birnie, D (1986) Sensory nerve morbidity following Le Fort I osteotomy. *Journal of Maxillofacial Surgery,* **14**, 10-13.

Di, S, Bell, WH, Mannai, C, Seale, NS, Hunt, WC, Taylor, J & Waite, DE (1988). Long term evaluation of human teeth after Le Fort I osteotomy. A histological and developmental study. *Journal of Oral Surgery.* **65**, 379-386.

Holland, GR & Robinson, PP (1986) Reinnervation of teeth after segmental osteotomy. *International Journal of Oral and Maxillo Facial Surgery,* **15**, 437-443.

Hutchinson, GR, Robinson, PP & MacGregor, AJ (1972) Tooth survival following various methods of subapical osteotomy. *International Journal of Oral and Maxillo Facial Surgery,* **1**, 81-86.

Johnson, JV & Hinds, EC (1969) Evaluation of teeth vitality after subapical osteotomy. *Journal of Oral Surgery,* **27**, 256-257.

Kahnberg, KE & Engstrom, H (1987) Recovery of maxillary sinus and tooth sensibility after Le Fort I osteotomy. *British Journal of Oral and Maxillofacial Surgery,* **25**, 68-72.

Kohn, MW & White, RP (1974) Evaluation of sensation after segmental alveolar osteotomy in 22 patients. *Journal of the American Dental Association,* **89**, 154-156.

Leibold, DG, Tilson, HB & Rask, KR (1971) A subjective evaluation of the re-establishment of the neurovascular supply of teeth involved in anterior maxillary osteotomy procedures. *Journal of Oral Surgery.* **32**, 531-534.

Loots, GP, Loots, JM, Brown, JMM & Schoeman, JL (1979) A rapid silver impregnation method for nervous tissue: a modified protogral-peroxide technic. *Stain Technology,* **54**, 97-100.

MacGregor, AJ (1971) Histology of a pulp following segmental alveolotomy. *British Journal of Oral Surgery,* **8**, 292-293.

Mjör, IA & Fejerskov, O (1979) *Histology of the Human Tooth.* 2nd ed. Copenhagen: Munksgaard, 43-74.

Pepersack, WJ (1973) Tooth vitality after alveolar segmental osteotomy. *Journal of Maxillofacial Surgery,* **1**, 85-91.

Poswillo, DE (1972) Early pulp changes following reduction of open bite by segmental surgery. *International Journal of Oral Surgery,* **5**, 39-48.

Ramsay, DS, Artun, J & Bloomquest, D (1991) Orthognathic surgery and pulpal bloodflow: a pilot study using Laser Doppler flowmetry. *Journal of Oral and Maxillofacial Surgery,* **49**, 564-570.

Reynolds, RL (1966) The determination of pulp vitality by means of thermal and electrical stimuli. *Journal of Oral Surgery,* **22**, 231-240.

SAS/STAT (1989) Users guide, version 6, 4th ed, Vol 2. Cary N.C: SAS Institute Inc.

Shehab, AD, Coghlan, M, Magennis, P (1996) Sensory disturbance following Le Fort I osteotomy. *International Journal of Oral and Maxillo Facial Surgery,* **25**, 13-19.

Summers, L & Booth, DR (1975) The early effects of segmental surgery on the human pulp. *International Journal of Oral Surgery,* **4**, 236-241.

Sugg, GR, Fonesca, RJ, Leeb, IJ & Howell, RM (1981) Early changes after anterior maxillary osteotomy. *Journal of Oral Surgery,* **39**, 14-20.

Tajima S (1975) A longitudinal study on electrical pulp testing following Le Fort type osteotomy and Le Fort type fracture (A preliminary report) *Journal of Maxillo Facial Surgery,* **3**, 74-80.

Theissen, FC & Guernsey, LH (1976) Postoperative sequelae after anterior segmental osteotomies. *Journal of Oral Surgery,* **41**, 139-151.

Vedtofte, P & Nattestad, A (1989) Pulp sensibility and pulp necrosis after Le Fort I osteotomy. *Journal of Cranio-Maxillo-Facial Surgery,* **17**, 167-171.

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