The effects of local anaesthetics on gingival blood flow in humans

MOHAMMAD KETABI (DDS)

Department of Dentistry

The University of Adelaide

A research report submitted as partial fulfilment
of the requirements for the degree of
Master of Dental Surgery-Periodontics

May 1996

TABLE OF CONTENTS

DECLARATION i

ACKNOWLEDGMENTS ii

SUMMARY iii

INTRODUCTION 1

REVIEW OF THE LITERATURE 3

Different techniques for recording gingival blood flow (GBF) 3
Vital microscopy 3
Infusion of plastic microspheres into the internal carotid arteries 4
Impedance plethysmography 5
Heat clearance 6
Electrolytic hydrogen clearance method 6
Laser Doppler flowmetry (LDF) 7

Measuring blood flow in oral tissues by LDF 10
Effects of local anaesthetics on pulpal blood flow (PBF) measured by LDF 10
LDF measurement of inflamed gingiva 14
Orthognathic surgery and its influence on PBF as measured by LDF 16
Effect of orthodontic forces on blood flow in human gingiva 16
Laser Doppler readings from gingival sulci 17
Aging and microcirculatory dynamics in human gingiva 18

Source and regulation of blood flow in the gingiva 19
Blood supply to the gingiva 19
Regulation of gingival blood flow 21

The pharmacological effects of cigarette smoking on gingiva 24

MATERIALS AND METHODS 26

Ethical approval for the project 26

Subjects 26

LDF operating principles 26
Depth of penetration of laser beam in gingival blood vessels 29
Performance of laser Doppler flowmetry in model systems 29
Movement Artefacts 30

Construction of stent to immobilise LDF probe 30

Dose Response Curve (DRC) 32
Definitions 32
Effective dose (Therapeutic Index) 32
Lethal dose 33

Steps in establishing the DRC in this study 35
Development of tolerance to adrenaline 39

Controls 39

Procedure for main part of the study 40

RESULTS 42

Dose Response Curve: findings 42

Tolerance: Findings 43

Controls 44

Results of injection of lignocaine with adrenaline 49
Pattern of LDF signals before injection 49
Baseline values 49
Reduction in LDF signal after injection 50
Period of maximum depression of LDF signals 53
Recovery of LDF signals to baseline 54

DISCUSSION 57

Lignocaine 57

Adrenaline 58

Measurement of gingival blood flow 59

Difficulties encountered during the study 60

Dose response curve 61

Controls 64

Injection of lignocaine with adrenaline 64

BIBLIOGRAPHY 71

APPENDIX 82

Table of abbreviation 82

Recording sheets 83

Consent forms 84

Subject's recruitment 85

Declaration

This research report is submitted in partial fulfilment for the Degree of Master of Dental Surgery in Periodontics in the Department of Dentistry, University of Adelaide. This research report contains no material which has been accepted for the award of any other degree or diploma in any university. To the best of my knowledge, this report contains no material previously published or written by another person, except where due reference is made in the text of this report.

I consent to this research report being made available for photocopying and loan if it is accepted for the award of the degree.

Mohammad Ketabi

May 1996

Acknowledgments

I am especially indebted to Dr Robert Hirsch who supervised my study and whose advice, patience, guidance and criticism are appreciated.

I would also like to express my thanks to Dr Trevor Bridges for his advice about pharmacological aspects of this project.

I also wish to thank Mrs Afgei for photographic assistance and Mr Rezai for assisting with the statistical analysis.

Special thanks to my wife and my daughter for their understanding, help and encouragement.

Summary

This study comprised a series of investigations into the responses of the gingival vasculature when challenged by local anaesthetics. Laser Doppler flowmetry (LDF) using the Perimed flowmeter and Perisoft program was used to measure gingival blood flow. In brief, a helium-neon laser beam (632.8nm) was applied adjacent to the gingival surface; laser light which strikes moving red blood cells in the gingiva undergoes a frequency shift according to the Doppler effect. The flowmeter's output signal was a relative blood flow flux. Forty male volunteers, aged between 18-35 with healthy gingival tissues were recruited. Half of the subjects were habitual smokers, consuming a minimum of 15 cigarettes per day. Subjects with a known sensitivity to adrenaline or known adverse response to the administration of local anaesthetics were excluded.

Informed consent was obtained from all subjects.

A custom made mouthguard was used to hold the laser probe in place.

The desired dose of local anaesthetic (0.5 ml) was calculated after constructing a dose response curve (DRC) for 3 subjects (2 non-smokers & 1 smoker). This amount was chosen because it caused more than 30% reduction in LDF signals and was in the range of therapeutic dose. It was also chosen because it was considered to be acceptable to subjects.

Plain lignocaine and normal saline were used as controls. Saline injection was given to rule out any possible influence of psychological factors (eg fear of injection) on LDF readings. Plain lignocaine was injected to compare the effects of local anaesthetics with and without vasoconstrictor on LDF values.

Blood flow through the gingival tissues (interdental gingiva between upper left central and lateral incisor) was monitored for 15 minutes. Then 0.5 ml of a local anaesthetic (lignocaine hydrochloride 2% with adrenaline 1:80,000) was infiltrated into the labial fold between the upper left central and lateral incisors (usual place for injection of local anaesthetics for dental procedures). Blood flow was monitored for another hour.

The injection of lignocaine containing adrenaline had little effect on LDF signals when different doses were injected at frequent short intervals. The results showed that tolerance developed to adrenaline in the receptors of gingival blood vessels. Although the mechanisms of this effect are incompletely understood, modification of adrenergic receptors is the most likely explanation.

Following injection with normal saline or plain lignocaine, the gingival blood flow did not change significantly in any subject although there was a slight increase in LDF readings in 3 subjects after injection of plain lignocaine. Injection of 0.5 ml of 2% lignocaine with 1:80,000 adrenaline consistently resulted in a marked and significant drop (average:46.1%, sd;13.5) in the LDF output signals from gingiva. Using Student's t-test (t value=0.13, $p>0.05$), no statistically significant differences were observed between smokers and non-

smokers in the percentage reduction in LDF signals. The reduction in LDF values lasted on average 67.9 minutes (sd ;24.3) in all subjects.

The significant finding of this study was that the recovery of LDF signals to baseline took considerably longer in smokers (average 80.2 minutes; sd 24.2) than non-smokers (average 56.6 minutes; SD 18.8). Using Student's t-test, the difference was statistically significant (t value=3.44, $p<0.05$).

In summary this study has shown the ability of adrenaline to produce a significant reduction in LDF signals from interdental papilla. This reduction was of a significantly longer duration in smokers than non-smokers. As little is known about the effects of smoking on gingival blood flow, more work is required to clarify this area, particularly in relation to vascular changes.

INTRODUCTION

The gingiva is heavily vascularised. This heavy vascularisation is necessary to support the tissue's high turnover rate and its defensive activities. The blood supply to the gingiva is very important for the health and maintenance of the periodontium.

There is little information in the literature about the vascular dynamics of the gingival circulation in health and disease. Recent evidence suggests that fundamental differences exist in the development of gingival and periodontal diseases in smokers versus non-smokers (Haber *et al* 1993). For example, smokers are known to be susceptible to severe forms of periodontitis but generally have a less pronounced gingival reaction to plaque than non-smokers. These differences could be partly related to vascular events in the periodontal tissues.

Various methods have been used for measuring gingival blood flow (GBF) in the past. However, most of them were invasive, indirect and inaccurate and could not be used in humans.

The invention of laser Doppler flowmetry (LDF) however, now permits non-invasive measurement of blood flow in humans and opens possibilities for investigating patterns of blood flow and the effects of pharmacologically active agents.

Aims of this study

This study comprised a series of investigations into the responses of the gingival vasculature to a standard challenge with local anaesthetic containing adrenaline. Furthermore, the responses of smokers were compared with non-smokers in an attempt to elicit information about the different gingival vascular responses of habitual smokers and non-smokers.

Review of the literature

Different techniques for recording gingival blood flow (GBF)

A variety of methods have been used for measuring GBF. However, most of these methods had great difficulty in measuring actual blood flow in the gingival tissues without disturbing their physiological status. In this chapter different techniques used to measure GBF, their advantages and limitations will be discussed.

Vital microscopy

A microscopic technique for direct observation of capillary blood flow in gingiva was first developed in 1947 (King 1947). In 1953, Forsslund (1953) used a stereoscopic microscope to photograph peripheral blood vessels in human and animal studies. He looked at the constancy of the vascular bed, and the effects of injecting adrenaline and histamine. He noted that adrenaline caused a vasoconstriction while histamine caused a vasodilation of the terminal gingival capillaries. In a later report, Forsslund (1959) noted that the vessels of the gingiva are formed into loops, anastomoses and central canals. Staple and Copely (1959) also used a capillaroscopy technique to observe GBF. They concluded that the gingival circulation was primarily regulated by changes in the diameter of arterioles, which in turn increased the blood volume of the gingival area. Hock and Nuki (1976) used high speed cinematography in which erythrocyte velocity in the free gingival margin was estimated by tracing the path of individual red blood cells.

These techniques suffered from certain drawbacks

A Vital microscopy and red blood cell velocity estimation could show only superficial capillary flow and was confined to visible vessels only (Forsslund 1959; King 1947).

B Vital microscopy requires bulky and expensive optical equipment, and only a small sample of capillaries may be observed (Forsslund 1959).

C In order to estimate red blood cell velocity, in the high speed cinematography technique, one must trace the complicated path of individual cells in successive film frames past blood vessel landmarks (Hock and Nuki 1976).

D Since the velocity of the blood cells is only one factor in blood flow, the possibility of quantifying changes in flow using vital microscopy is limited.

Infusion of plastic microspheres into the internal carotid arteries

In this method, plastic microspheres were infused into the internal carotid arteries and the relative number of spheres per unit volume of gingival tissue was used as an indicator of blood flow (Vandersall and Zander 1967).

Blood flow has also been calculated by measuring the radioactivity in oral tissues (Hock and Kim 1983; Kaplan *et al* 1982). Similarly, a single radioisotope, Xenon-133, has been injected into dog gingiva and the clearance rate has been measured by external monitoring of the radioactivity (Hock *et al* 1980).

Microsphere infusion and radioactive techniques have also certain limitations

A Microsphere infusion permits flow measurements on only one occasion and can not be used in human subjects (Kaplan *et al* 1973; Vandersall and Zander 1967).

B Microsphere infusion and radioactive techniques lack the ability to continuously follow rapid changes in blood flow.

C Radioisotope clearance methods are similarly limited and also suffer from difficulties in data analysis (Hock and Nuki 1980).

Impedance plethysmography

Electrical impedance plethysmography was used to study blood flow in tooth pulp and oral soft tissues (Neidle and Liebman 1964). This technique has also been used to estimate gingival blood flow; electrodes were placed against the gingiva using a plastic stent (Kinnen and Goldberg 1978). The procedure depends upon the relatively low electrical impedence of a volume of blood compared to a similar volume of blood-free soft or hard tissue with higher electrical impedence. Impedence plethysmography is applicable to human studies, but requires that metal electrodes touch the tissue being monitored (Kinnen and Goldberg 1978).

This technique has two major limitations

A Impedence changes at the electrode tissue interface can not easily be distinguished from those in the bulk of the tissue (Kinnen and Goldberg 1978).

B While measuring GBF, the electrodes have to touch the tissue. This contact itself may alter capillary blood flow.

Heat clearance

Temperature measurements and heat clearance have been used for the assessment of vascular changes in oral mucosa (Clarke and Shephard 1984). In this technique heat diffusion transducers were placed into the gingival sulcus, heated by a known current, and the voltage across it was continuously monitored. The diffusion of heat from the device was altered by the change of blood flow through adjacent tissue.

The heat clearance method has also 2 major limitations

A Heat clearance is a very indirect measure of blood flow.

B Heat itself can alter the blood flow (Kinnen and Goldberg 1978).

Electrolytic hydrogen clearance method

In this technique an electrode is inserted into the tissue and the velocity of diffusion of ionised hydrogens are measured for short and interrupted periods. This method is invasive and may alter blood flow (Kvietys *et al* 1986).

Other techniques

Giddon *et al* (1963) used a photo-electric method for continuously monitoring gingival vascular reactions to vaso-active drugs in the gingiva of dogs. Both local and systemic administration of small amounts of adrenaline were shown to reduce the vascular activity in the gingiva. Dorman and Bishop (1964) used oxygen tension of the extra-cellular fluid of the gingiva as a criterion for the adequacy of the local circulation. They noted that an intra-vascular injection of 10 μg adrenaline caused a temporary reduction of the GBF. They concluded that gingival capillary flow was regulated so that the oxygen tension in venous blood remained stable.

Laser Doppler flowmetry (LDF)

LDF is a non-invasive instantaneous and continuous technique which is used widely in medicine to measure blood flow in different organs of the body. It is based on the Doppler-shift of back-scattered laser light (Stern 1975).

History: The introduction of the laser by Townes (1958) made a source of truly monochromatic light available. As stable lasers were built in the early 1960's, many experiments in classical optics took on a completely new importance (Levine 1963). In 1964, Yeh and Cummins demonstrated the feasibility of optical velocimetry, using a helium-neon laser to measure the velocity of polystyrene spheres in a solution.

LDF was initially developed to assess blood flow in microvascular systems, eg in the retina, gut mesentery, renal cortex and skin. Riva *et al* (1972) employed this technique to measure blood flow in glass capillary tubes and in rabbit retinal vessels. Tanaka *et al* (1974) repeated this measurement in human retinal vessels and Stern (1975) used LDF for observing changes in skin blood flow.

In 1980, LDF was used to study blood flow in the oral tissues (De Rink *et al* 1980). This flowmeter allowed immediate measurement of the erythrocyte flux in approximately $1mm^3$ of the capillary bed without touching the tissues.

Laser Doppler flowmetry has certain advantages over other methods for measuring blood flow

A It is simple and non-invasive.

B Produces a direct and continuous readout of the average blood flow of the gingival tissues.

C There is no contact between probe and measuring area. Therefore, there is no possibility of blood flow alteration by pressure of the probe on the tissues.

Limitations of Laser Doppler Flowmetry (Vongsavan and Matthews 1993)

A The principal disadvantage of laser Doppler flowmetry measurements is that it is impossible to calibrate the signals generated in absolute units; the output may not be linearly related to blood flow. This is due to the fact that the signal derived from any one moving cell will depend upon the distance of that cell from the recording probe, and that distance is not known. LDF therefore produces a relative, rather than an absolute value of blood cell flux. For example if the output signals increases by 100%, it cannot be assumed that the blood flow rate has increased by 100%. The non-linearity arises from the effects of multiple collisions of photons with moving cells, the probability of which increases with increasing red cell concentration in the tissue. Non-linearity becomes a problem when the red cell volume fraction of tissue exceeds 1%. This figure is likely to be exceeded in dental pulp and most other tissues (Vongsavan and Matthews 1993).

B Inherent variability in LDF value in longitudinal recordings in the same subject and between subjects. Therefore it may not be a reliable method for longitudinal or discontinuous recordings.

C LDF is extremely sensitive to movement.

D The probe tip and optical fibre line are easily damaged. The operating procedure of LDF will be discussed in detail in the next section.

Measuring blood flow in oral tissues by LDF

LDF has been used in dentistry to study the effects of local anaesthetics on pulpal blood flow (Gazelius *et al* 1986; Odor *et al* 1994b; Pitt Ford *et al* 1993), inflammation on GBF (Hock and Kim 1983; Kaplan *et al* 1982), smoking on GBF (Baab *et al* 1986), orthodontic forces on GBF (Yamaguchi *et al* 1991b), orthognathic surgery on pulpal blood flow (Ramsay *et al* 1991b) and blood flow in luxated and traumatised permanent teeth (Olgart *et al* 1988; Heithersay and Hirsch 1993).

Effects of local anaesthetics on pulpal blood flow (PBF) measured by LDF

The addition of vasoconstrictor to a local anaesthetic potentiates and prolongs the anaesthetic effect. Vasoconstrictor-containing local anaesthetics such as 2% lignocaine with adrenaline 1:100.000 are employed in dentistry to induce profound anaesthesia. However, these agents are capable of profoundly altering blood flow. Olgart and Gazelius (1977) reported that supra-periosteal infiltration of lignocaine containing adrenaline caused almost complete cessation of blood flow in the pulp.

Kim *et al* (1984) evaluated effects of lignocaine with adrenaline (1:100,000) administered by various local anaesthetic techniques (infiltration, mandibular block, and intraseptal injection) on PBF in dogs. Using the 15 um radioisotope-labelled microsphere injection method, the pulpal blood flow decreased significantly with all three techniques. However, the most dramatic reduction occurred in the molar teeth with the intraseptal injection. When 2% lignocaine

without adrenaline was used in the intraseptal injection, pulpal blood flow increased significantly. In this study the PBF was not measured by LDF. However, as the effect of local anaesthetics on PBF was investigated, it was therefore mentioned here.

Gazelius *et al* (1986) used LDF to study pulpal blood flow. In intact incisors which responded to pulp testing, the level of the output signal from the flowmeter was clearly distinguishable from that obtained in adjacent non-sensitive or pulpectomized teeth. In 5 subjects, a slow injection (1.0 ml) of lignocaine (20mg/ml) with adrenaline (12.5μg/ml) was made in the apical region in the buccal alveolar mucosa. Within a few minutes, the output signal voltage decreased by 70 (±13)% (mean±SD) and remained at this low level throughout the 20 minute recording period. The fluctuations occurring in the signal before injection were also reduced.

Pitt Ford *et al* (1993) investigated the effects of dental anaesthetic solutions containing either 2% plain lignocaine or 2% lignocaine with 1:80,000 adrenaline. The duration of anaesthesia and the measurement of the reduction in blood flow together with its duration were carried out in the dental pulps of maxillary central incisor teeth in 10 human subjects. The local anaesthetic solution was injected into the soft tissues adjacent to the apex of the teeth. The blood flow in the dental pulp was assessed by LDF, and the effectiveness of pulpal anaesthesia was determined by an electric pulp tester. The injection of 1-2 ml of 2% plain lignocaine had no significant effect in blood flow of the pulp of the incisor tooth in 8 of 10 subjects. In the other two, there

was a small but significant increase in pulpal blood flow. The duration of pulpal anaesthesia was 25.1 minutes on average. Following injection of 1 ml of 2% lignocaine with 1:80,000 adrenaline, there was a significant reduction (31%) of pulpal blood flow ($p<0.05$). The duration of reduced blood flow was 68.5 minutes. The duration of pulpal anaesthesia was 100 minutes, four times the duration of anaesthesia induced by plain lignocaine. The anaesthetic solution with vasoconstrictor produced far longer anaesthesia more consistently at lower dose than the plain solution.

Odor *et al* (1994a) investigated the effects of inferior alveolar nerve block anaesthesia using 2% lignocaine with 1:100,000 or 1:80,000 adrenaline on PBF in mandibular molar and canine teeth in 10 human subjects by LDF. The injection of 2 ml of 2% lignocaine with 1:100,000 adrenaline caused a decrease in PBF in both teeth in every subject. The mean PBF in the canine teeth at 15 minute was 58% of the baseline value whilst that in the molar was 76%. These values were not significantly different from the reduction in PBF produced by 2% lignocaine with 1:80,000 adrenaline. Both solutions produced a reduction in blood flow that was of shorter duration than pulpal and soft tissue anaesthesia, and of shorter duration in molar tooth compared with the canine. When 2% lignocaine with 1:100,000 adrenaline was injected, the mean reduction of blood flow was of shorter duration (canine, 60 min; molar, 42 min) than following 2% lignocaine with 1:80,000 adrenaline (canine, 93 min; molar 72 min); these differences in blood flow reductions were statistically significant. Using 2% lignocaine with 1:100,000 adrenaline, the mean duration of pulpal

anaesthesia was 76 minutes in the canine tooth compared with 58 minutes in the molar tooth. Full soft tissue anaesthesia lasted for 117 minutes. These values reduced significantly when compared with the lignocaine solution containing 1:80,000 adrenaline.

In another related study, Odor *et al* (1994b) investigated the effects of PBF and anaesthesia in mandibular teeth during inferior alveolar nerve block anaesthesia using 2% lignocaine with 1:80,000 adrenaline in 10 adults human subjects. Local anaesthetic solution was delivered close to the inferior alveolar nerve at the mandibular foramen using a standardised nerve block technique. The PBF of the canine and first permanent molar was monitored by a laser Doppler flowmeter. At selected times, pulpal anaesthesia was tested with an electric pulp tester. The injection of 2 ml of solution caused a decrease in pulpal blood flow in both teeth in every subject. The reduction in PBF was of shorter duration than pulpal and soft tissue anaesthesia. A period of increased blood flow following the period of reduction was noted in several subjects. The mean reduction in PBF in the molar at 15 minutes was 25% and of 72 minutes mean duration. The mean duration of pulpal anaesthesia was 114 minutes in the canine compared with 88 minutes in the molar. Full soft tissue anaesthesia lasted for a mean of 151 minutes, while complete recovery took 255 minutes. This study indicated that lignocaine and adrenaline in block anaesthesia acted not only at the site of injection but also within the individual teeth.

In conclusion, these studies showed that there was a significant drop in PBF as a result of injection of local anaesthetics containing adrenaline and therefore addition of adrenaline to the LA solution increased the duration of anaesthesia.

LDF measurement of inflamed gingiva

Histologic and anatomical changes occur in the gingival microcirculation during the development of gingivitis (Barnett *et al* 1989; Page and Schroeder 1976). Prospective studies of the gingival vasculature have demonstrated that, in the absence of inflammation, the vascular network is arranged in a regular, repetitive and layered pattern (Nuki and Hock 1974).

In contrast, the gingival vasculature is altered by naturally occurring or experimental gingivitis such that the vascular plexus assumes an irregular pattern, with the microvessels exhibiting a looped, dilated and convoluted appearance (Nuki and Hock 1974).

Most recent studies have used LDF to study changes in the gingival microvasculature following the development of partial mouth experimental gingivitis. LDF has been used by Baab & Öberg (1986) , Baab *et al* (1987a, 1990) , to study the human healthy gingival microcirculation, an animal model of gingival inflammation, and in humans with a history of periodontitis.

Baab *et al* (1987b) compared the blood flow rate in treated and untreated gingivitis in dogs by LDF. Four adult mongrel dogs with generalised mild gingivitis were fed a dental-plaque-inducing diet. Teeth on the left were scaled and polished ; teeth on the right were left untreated. Test teeth were the

upper canines through to the fourth premolars, and lower second premolars through to the first molars. LDF indicated that blood flow increased slightly with time on the untreated side, but there was no significant decrease in blood flow with the resolution of inflammation on the treated side.

Matheny *et al* (1993a) studied the changes that occurred in the gingival microcirculation during the development of experimental gingivitis in humans. Alterations occurring in the microcirculation were monitored during the development of gingivitis in 10 (18-30 year old) healthy male humans when they suspended oral hygiene procedures for 12-16 days. A partial mouth, experimental gingivitis was employed. Gingival health was evaluated before and after the experimental period by assessing gingival and plaque indices and gingival crevicular fluid volume. Gingival vascular monitoring included measurement of regional blood flow using LDF and stereomicroscopy. The number of vessels visible in a given microscopic field in a given subject and the number of vessels exhibiting flow were also determined from the videotapes. Gingivitis developed in all subjects; significant increases were seen in plaque index, gingival index, bleeding on probing and crevicular fluid volume. There was no change in superficial capillary blood velocity but a significant decrease in gingival regional blood flow was seen with gingivitis. A significant increase in the number of vessels visible in microscopic field and a decrease in the percentage of vessels exhibiting flow were observed.

In general, studies with LDF have shown that gingival microcirculation exhibited a dynamic change in response to the development and progression

of gingivitis. Chronic gingival inflammation resulted in a net increase in gingival blood flow (Hock and Kim 1983; Kaplan *et al* 1982). As few studies with limited number of subjects have used LDF to study changes in the gingival circulation with onset of gingivitis, further investigations using a larger number of subjects are required.

Orthognathic surgery and its influence on PBF as measured by LDF

LDF has been also used to study the effects of orthognathic surgery (Le Fort I osteotomy) on pulpal blood flow by Ramsay (Ramsay *et al* 1991a). Pulpal blood flow of maxillary right and left central incisors and randomly selected mandibular canine in 14 volunteers was measured prior to surgery and at various intervals during the 6 months following surgery. Custom-made splints allowed accurate and reproducible positioning of the measurement probe. The data showed a significant reduction in vascular supply at the final observation. However, a high variability of individual blood flow patterns was found. In some patients, transient periods of ischaemia were observed shortly after surgery. Also, numerous teeth demonstrated hyperaemia at later intervals. This study relied on LDF readings of the same tooth on different days being reproducible, but there is evidence of a high degree of variability of consecutive readings that is not related to actual changes in blood flow.

Effect of orthodontic forces on blood flow in human gingiva

Yamaguchi *et al* (1991a) used LDF to study the relationship of GBF to the magnitude and duration of applied orthodontic forces in humans. The sample consisted of five adult volunteers with interdental spaces between their

maxillary central incisors. A buccal tube was bonded to the labial surface of each central incisor and a spring force was applied to close the space. The forces applied were between 50-250g. Each force was applied between 30 seconds and 10 minutes. The blood flow signals were recorded continuously using a pen recorder. Measurements indicated that blood flow was negatively correlated to the amount of force applied. The duration of reactive hyperaemia was positively correlated to the duration of force.

It was concluded that measurements of gingival blood flow may provide a means of estimating physiological orthodontic forces by correlating the relationship of blood flow changes to the magnitude and duration of orthodontic forces.

Laser Doppler readings from gingival sulci

The size of early laser Doppler probes used were too large in diameter (>0.85mm) to insert to the base of the gingival sulcus. Recently a new laser Doppler probe has been developed in which the light emitting and collecting optical fibres are 0.4 mm apart. This probe has an external diameter of 0.5 mm, which is similar to that of conventional periodontal probes. Hinrichs *et al* (1995b) used this probe to investigate intrasulcular laser Doppler readings. Nine adult volunteers with clinically healthy gingiva were studied for 30 seconds intervals at 5 sites. Baseline LDF readings were obtained twice at each site using an acrylic stent to stabilise the probe and once without the stent. All LDF readings were repeated at one month and again two months after baseline. One additional reading per subject was obtained following the

administration of a local anaesthetic with a vasoconstrictor. Results from this study demonstrated that:

A Intrasulcular LDF readings were reproducible at 1 and 2 month intervals.

B Trauma associated with probe placement resulted in increased LDF readings.

C A highly significant reduction in LDF signals occurred following the administration of local anaesthetic with vasoconstrictor.

Intrasulcular recordings of LDF have also been used as a method for monitoring the response to periodontal therapy (Hinrichs *et al* 1995a). LDF readings and clinical measurements were obtained from 2 healthy and 2 diseased sites in 30 systemically healthy adult volunteers with localised moderate to advanced periodontitis. All 30 subjects were re-examined 1 month following root planing while 10 subjects were re-examined at approximately 1 year after treatment. One month following root planing, the diseased sites had undergone a significant reduction in LDF readings and pocket depth with an accompanying gain in periodontal attachment. It was concluded that LDF readings from gingival sulcus was an unbiased non-invasive method for monitoring the response to periodontal therapy.

Aging and microcirculatory dynamics in human gingiva

Matheny *et al* (1993b) compared the gingival vascular dynamics in 60 healthy male humans ranging in age from 18-75 years [young, 18-25 years(Y), middle, 35-45 years(M), old, 65-75 years(O), 20 subjects/group. Both videomicroscopy of individual microvessels and LDF were used to assess the dynamic of the

marginal gingival circulation. The number of gingival vessels visible in a microscopic field was higher and the number of microvessels exhibiting active flow was lower in M and O compared to Y. There were no differences among age groups in LDF values (tissue blood flow) or in red blood cell velocity in individual vessels. Although not statistically significant with age there was a trend towards decreasing blood flow velocity with increasing age in both the LDF and videomicroscopic measurements.

Source and regulation of blood flow in the gingiva

Blood supply to the gingiva

The maxillary gingiva is supplied by postero-superior alveolar, the infra-orbital, the greater palatine, and the sphenopalatine arteries. The mandibular gingiva is supplied by branches of the inferior alveolar artery, including the mental, the sublingual, and the buccal arteries (Grant *et al* 1988).

The blood supply to the gingiva is derived from 3 sources (Figure 1):

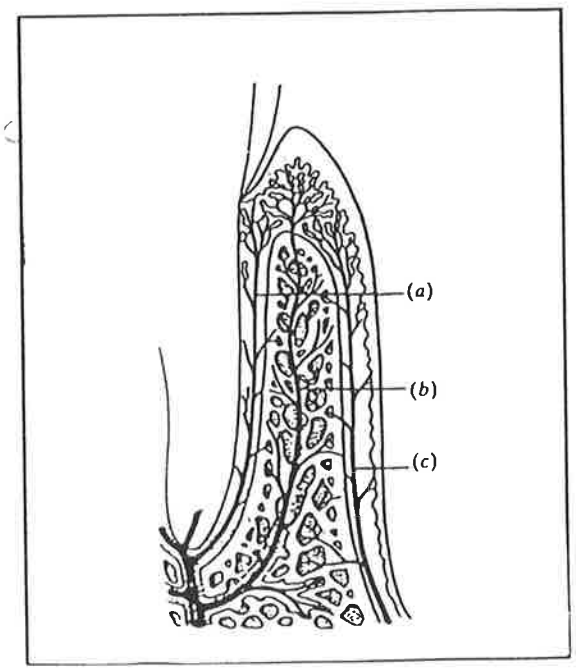


Figure 1: Diagrammatic representation of the 3 principal sources of gingival blood supply (Manson and Eley 1995).

(a) **Vessels of the periodontal ligament**

Vessels of the periodontal ligament which extend into the gingiva and anastomose with capillaries in the sulcus area.

(b) **Branches of the alveolar arteries**

Branches of the alveolar arteries penetrating the interdental septa or emerging from the periodontal ligament contribute to the gingival blood supply. These branches anastomose with the periosteal ones and form the vascular bed of the gingiva.

(c) **Supraperiosteal vessels**

Supraperiosteal vessels are the main source of gingival blood supply originating from the lingual, mental, buccal, and palatal arteries.

Gingival epithelium is supplied by capillaries terminating in groups immediately below the basement membrane. It has been shown that there are approximately 50 capillaries/mm^2, each of which terminates jn a loop in the peripheral part of the connective tissue papilla adjacent to the epithelial border (Karring and Loe 1969). The terminal blood vessels form a plexus that extends under the epithelial surface from the gingival margin to the apical extension of the junctional epithelium. Most of the vessels in the gingival connective tissue are arterioles, capillaries, and small veins (Genco *et al* 1990).

Kindlova and Matena (1962) showed that two groups of capillary networks were present in the gingiva. The first group gave rise to slender loops extending into the apex of the papillae from the facial and lingual surfaces of

the gingiva. Vessels of the second group gave rise to capillaries resembling renal glomeruli which may be arterio-venous anastomoses. Kindlova and Matena also showed that the gingival margin has a double blood supply; periodontal ligament vessels frequently connect with those arising from the gingiva.

Regulation of gingival blood flow

The blood flow in the gingiva is controlled by central nervous system mechanisms and local factors.

Central nervous system mechanisms

The size of the arterioles and pericapillary sphincters are regulated by autonomic nervous system which determines the quantity of blood flowing into the gingiva. This regulation is brought by peripheral vasomotor control and medullary vasomotor centre control.

Peripheral vasomotor control

Sympathetic control

The gingiva and dental pulp are supplied with post-ganglionic sympathetic noradrenergic fibres from the superior cervical ganglion (Avery *et al* 1974; Parker *et al* 1986). Functional experiments suggest that noradrenaline plays a primary role in the sympathetic control of gingival, periodontal and pulpal blood flow. Noradrenaline seems to act on post-synaptic alpha-adrenoreceptors that induce vasoconstriction to reduce blood flow in these tissues (Kim 1985). Reflex excitation of the sympathetic nervous system by experimental hypotension (haemorrhage and nitroprusside infusion) or decrease in oxygen

transport (by hemodilution or hemoconcentration) causes a reduction in PBF (Kim 1985). No data are available to support the hypothesis of parasympathetic control of blood flow in the gingiva, dental pulp and periodontal ligament.

Medullary vasomotor centre (vasoconstricting centre)

Uvnas (1960) reviewed the specific location (medulla oblongata) and function of the medullary vasomotor centre. He suggested that the tonicity of the vasoconstricting centre is influenced by a number of factors such as:

A Chemoreceptors, located in the carotid and aortic bodies.

B Pressoreceptors, located in the carotid sinus and aortic arch.

C Afferent fibres that convey pain information and also send collaterals to vasoconstricting centres.

D Descending fibres that originate in the hypothalamus and cortex sending collaterals to the vasomotor centre.

Rhythmicity of vasomotor discharge is influenced by the chemical composition of the blood, its temperature and pressure on the vasomotor centre. The frequency of discharge of the vasomotor centre at any one time is thought to be the algebraic sum of all factors stimulating the centre.

Local neurogenic control (axon reflexes)

The smallest blood vessels have an inherent muscular activity that is independent of blood-borne substances or nervous influences. Their activity can be altered by stimulation of nerve fibres, local electrical stimulation, or by

changing the physical and chemical environment. The mechanism of this local neurogenic control seems primarily to involve inhibition of sympathetic vasoconstriction due to release of various vasodilatory agents, including substance P. The vasodilation results from an increased vascular permeability, including protein leakage from capillaries, for example gingiva and skin (Lundberg *et al* 1984).

The vascular system of the oral tissues appears to be functionally similar to that found in most other body regions. Smooth muscle sphincters guard the opening of the capillaries in the majority of tissues, including those in the oral region. Characteristically, these vessels are intermittently controlled. Capillaries of the marginal gingiva may be an exception to this general rule because the capillaries that loop toward the outer edge of the gingiva always contain red blood cells.

The pharmacological effects of cigarette smoking on gingiva

Baab and Öberg (1987a) studied the effects of cigarette smoking on GBF. This is the only controlled study of its type in humans. The acute effects of cigarette smoking on blood flow to the gingiva was studied in 12 young smokers. Relative gingival blood flow was measured by a laser Doppler fibre-optic probe placed 1 mm in to the buccal sulcus of upper left first molar. The probe continuously measured the flux of red blood cells in the gingival crest. Relative skin blood flow (SBF) to the forearm and heart rate were also monitored continuously; blood pressure was assessed at 5-minute intervals. After resting for 5 minutes, subjects sham smoked an unlighted cigarette for 5 minutes, then smoked the cigarette, and finally rested for 25 minute afterwards. Mean changes from resting for all variables were co-paired to sham smoking for each 5 minute block. Mean GBF rose significantly above sham smoking values during smoking, and remained elevated during the first 5 minutes after smoking ($p<0.05$). Mean SBF decreased slightly during and after smoking, but the changes were not significantly depressed compared to sham smoking ($p>0.05$). During smoking, the blood pressure and heart rate increased significantly over sham smoking ($p<0.05$).

This study showed that smoking increased GBF in young subjects with healthy gingival tissues. The result from this study failed to confirm previous findings in rabbits (not using LDF) in which nicotine was injected intravenously (Clarke *et al* 1981; Clarke and Shephard 1984). In those studies, gingival circulation initially increased, then dropped to below baseline levels. The transient

increase in GBF seen in Baab and Öberg's (1987a) study may have been the same initial rise in gingival blood flow reported earlier by studies, but GBF never fell below baseline level afterwards.

Important findings from this study

Cigarette smoking caused a significant increase, rather than a decrease, in human gingival circulation. Therefore, the theory that smoking impairs an already compromised gingival blood flow may not be true in humans. However, the chronic effects of smoking on GBF is unknown particularly in relation to gingival inflammation. Circulation to the gingival margin is not "end-arterial without collateral support", instead this area has a double blood supply; periodontal ligament vessels frequently connect with those arising from the gingiva (Kindlova and Matena 1962). The steep rise in heart rate and blood pressure seen in this and other studies, could have increased the gingival circulation during smoking.

Smoking also induces a significant rise in plasma vasopressin that does not occur when nicotine is given intravenously (Rowe *et al* 1980). It seems that differences in measurement techniques, delivery of nicotine, and experimental subjects (humans vs animals) prevent direct comparison between different studies which have investigated the role of smoking on GBF. As Baab's study has been the only controlled study of its type in humans, more work is required to clarify the effect of smoking on GBF, particularly in relation to gingival inflammation.

Materials and Methods

Ethical approval for the project

Approval for investigation of the effect of local anaesthesia on gingival blood flow using LDF was granted by the Committee on the Ethics of Human Experimentation, the University of Adelaide (approval # H/43/90).

Subjects

Forty healthy males volunteered as participants in the study; of these 20 were smokers and 20 were non-smokers. The subjects' ages ranged from 18-35 years. Most of the smokers were dental students who had habitually smoked cigarettes (10 to 25 per day) for at least one year. A full medical history was taken for each subject and those with hepatic, renal or cardiac disease, pace-makers, known infectious diseases, thyrotoxicosis and those on medications for high blood pressure were excluded. Subjects with any type of periodontal disease were excluded. Female subjects were not selected due to the possible influence of their menstrual cycles on gingival blood flow.

LDF was used as the method for measuring GBF in this study. Therefore aspects of the operating principles of LDF will be discussed below:

LDF operating principles

(Baab and Oberg 1987a; Vongsavan and Matthews 1993)

LDF instruments measure the microvascular blood cell perfusion through tissue. To do this, they utilise the Doppler shift, ie the frequency change that

light and other radiations of a wave nature undergo when reflected by objects in motion.

All wave movements are characterised by the relation

$$V = f \times \lambda$$

where **V** is the propagation velocity

f is the wave frequency

λ is the wavelength

Therefore, when the frequency changes, the wavelength will also change, as will the quality of the radiation.

Blood flow is measured by a specifically designed, laser Doppler fibre-optic probe (outside diameter of 0.85mm). The probe carries 3 optical fibres of 0.25 mm diameter, one for transmitting light, and two for receiving. All the fibres are arranged in parallel within a single probe. The operating principal of LDF is based on the specific shift of monochromatic laser light when scattered by moving red blood cells (Stern 1975). In the Periflux PF-2b flowmeter (Perimed, Stockholm, Sweden) which was the instrument used in this study, light from a 2 mW He-Ne laser was guided to the tissue by one optical fibre. Some of the light waves strike non-moving structures in the tissue and are returned to the surface at the same frequency as the incident light. Other light waves, which strike moving blood cells are shifted in frequency (or colour) according to the Doppler principle. The mixture of lights, both shifted and un-shifted in frequency, is received by two optical fibres that lead the light photodetectors (Figure 2) .

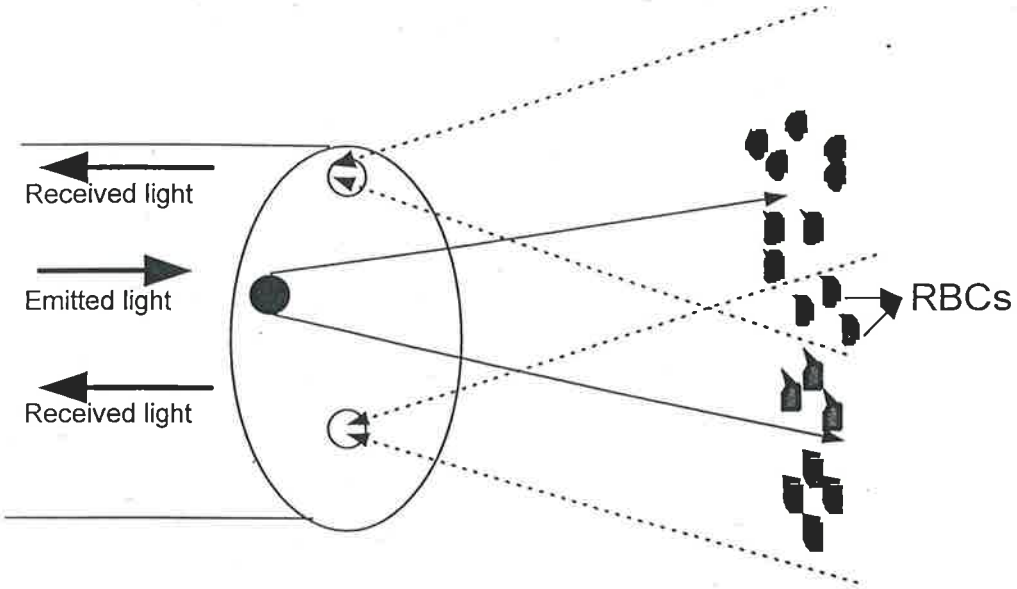


Figure 2 shows a diagrammatic representation of the LDF probe tip

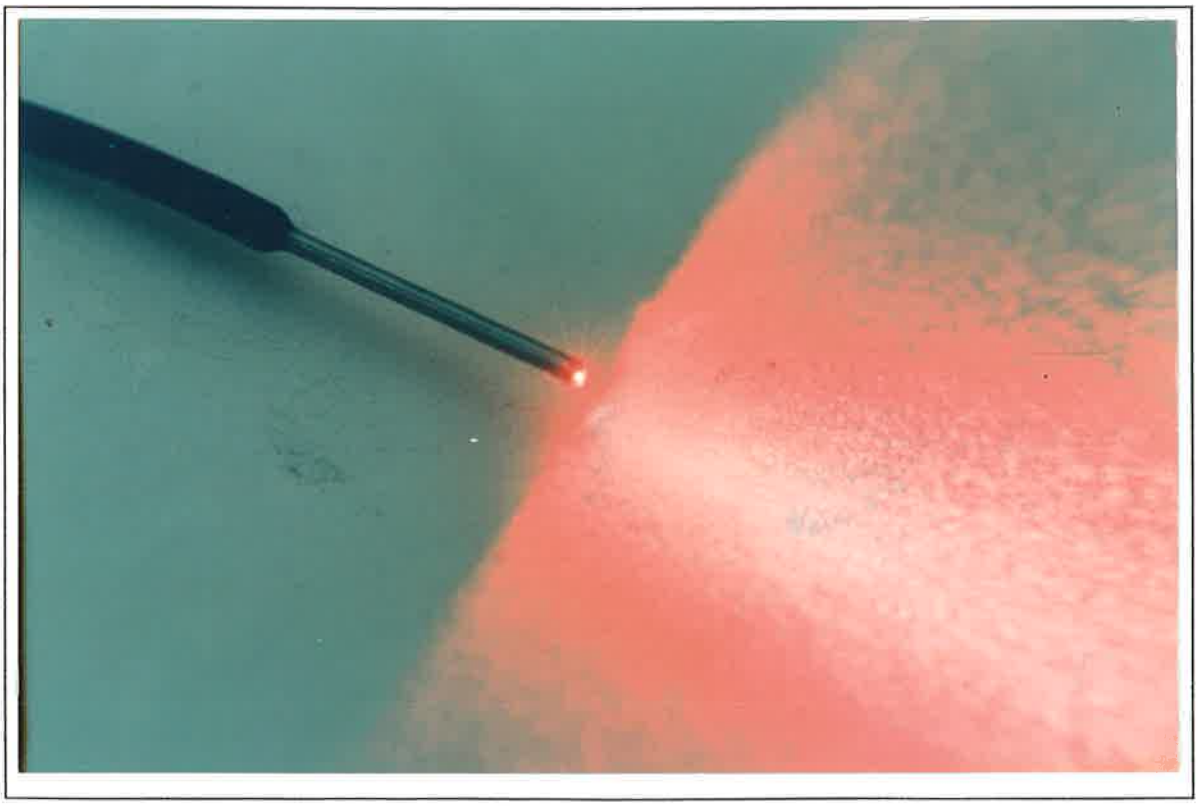


Figure 3 shows scattering of laser light from the probe tip

Two photodetectors are arranged in a differential configuration. This arrangement is essential for use with He-Ne lasers to reduce the noise in the photodetector output to acceptable levels. In the photodetectors, components of the light with different frequencies are mixed, and give rise to an electrical signal (the photocurrent), the frequency of which is related to the difference between the two light beam frequencies. This difference represents the Doppler shift frequency and therefore is a measure of the velocity at which the red blood cells are moving. The flowmeter output signal is a function of the product of red cells' volume fraction and their mean velocity.

Depth of penetration of laser beam in gingival blood vessels

Since laser light of 632.8 nm wave length penetrates skin to approximately 0.6 mm (Anderson and Parrish 1981), the measurement volume is from a radius from the probe tip to about 1 mm in the tissue. Therefore the measurement area includes capillaries, arterioles, and venules in the interdental papilla and gingival margin.

Performance of laser Doppler flowmetry in model systems

The performance of these instruments in estimating red cell flux has been assessed using model systems perfused at different rates with diluted blood (Barnet *et al* 1990; Nilsson 1984). Under certain conditions, they have been shown to perform very satisfactory in that the blood flow signal was found to be linearly related to the flux of red cells through the model. However the ranges of red cell volume fractions and flow velocities employed in these tests do not

cover all the combinations likely to be encountered in the tissues which are accessible to examination with LDF.

Movement Artefacts

All LDF instruments that employ flexible optical fibres for signal transmission are affected by movement artefacts to varying degrees, ie false signal spikes not related to perfusion if the fibre line is suddenly displaced. These artefact signals are superimposed upon the flux signal. In the PeriFlux records they appear as steep, upward spikes and are always directed toward higher perfusion. They are related to the speed by which the fibre line is displaced and rise steeply with increasing speed. They disappear when the fibre movement stops. It is therefore essential to reduce movement between the probe tip and the underlying tissue to a minimum, otherwise structures other than blood cells generate Doppler shifts and the signal produced can be indistinguishable from that caused by blood flow. In order to overcome the problem of movement artefacts, the PF 2b has been equipped with a movement artefact filter which can be engaged or disengaged by a switch on the instrument's rear panel. This artefact filter was switched on during recording the GBF in this study. More information regarding LDF operating procedure can be obtained from Peri Flux handbook (PeriFlux PF 2 and PF 2B User's Handbook; 1986).

Construction of stent to immobilise LDF probe

A custom stent was made for each subject to allow for accurate placement and repositioning of the LDF probe. It was made from dental impression material.

Initially type I putty (very highly viscosity) polyvinylsiloxane (Extrude, Kerr, USA) impression material was mixed and directly applied around upper teeth from upper left second premolar to upper left second premolar. When initially set, the material was withdrawn from the mouth and was placed into hot water to accelerate the setting reaction. The inner surface of the stent was then filled with medium viscosity type I polyvinylsiloxane (Extrude, Kerr:USA) and was re-inserted around the teeth. The material was allowed to set in the mouth. The stent was then withdrawn from the mouth. Using a scalpel blade, excess material was cut off and with a long burr, a hole was drilled in the stent, adjacent to the interdental papilla between upper left central and lateral incisor. The stent allowed accurate positioning and re-positioning of the LDF probe at 90° to the gingival surface (Figure 4).

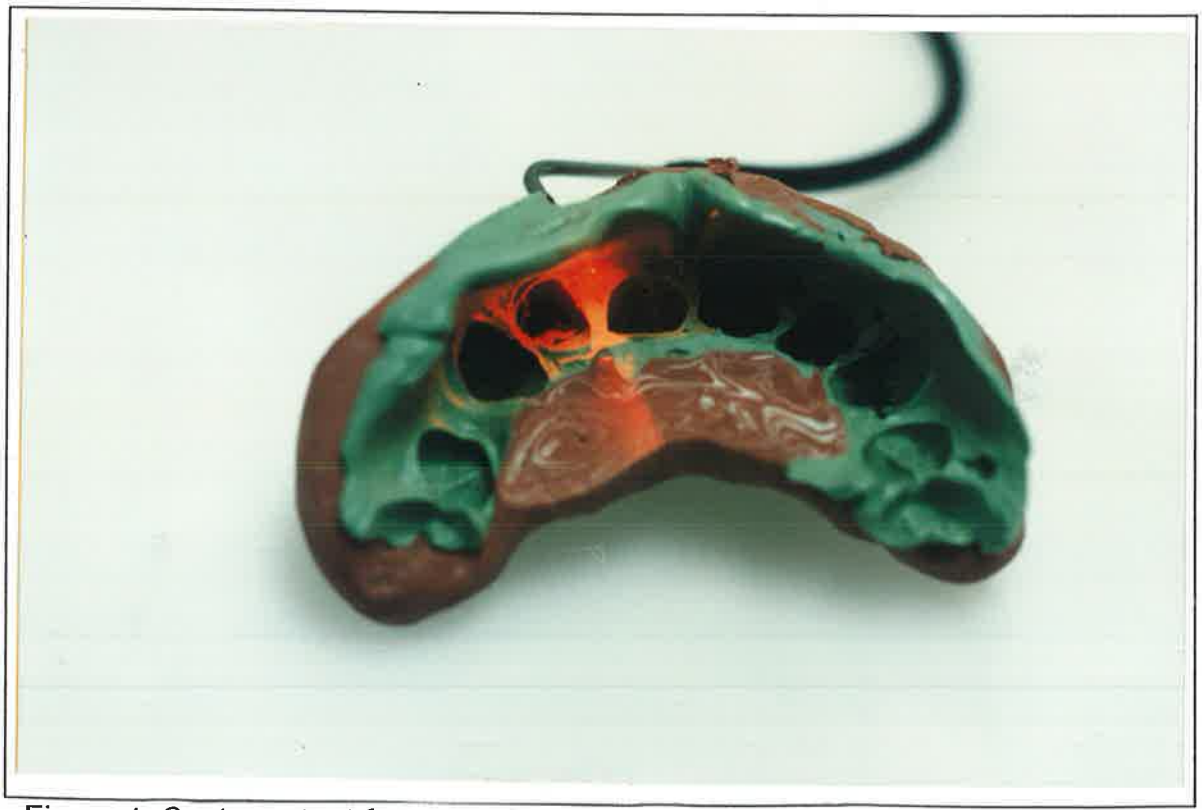


Figure 4: Custom stent for stabilising and accurately positioning probe. The green polyvinylsiloxane material (Kerr Extrude) partially absorbs scattered light and minimised light scattering to and from the gingiva.

Dose Response Curve (DRC)

There are four studies in the literature which investigated the effects of local anaesthetics on pulpal blood flow with LDF (Gazelius *et al* 1986; Odor *et al* 1994a; Odor *et al* 1994b; Pit Ford *et al* 1993). In all of these studies, a fixed amount of 1-2 ml of local anaesthetic had been injected. This is the amount which is usually injected clinically to achieve infiltration or nerve block anaesthesia during dental procedures. However, in any trial in which the response of the body to a drug is investigated, it is useful to establish a DRC. In order to understand the importance of DRC, its definition and some of the related terminology will be discussed.

Definitions

The degree of effect produced by a drug is generally a function of the amount (dose) administered. In other word, responses to drugs are graded. That is, the response continuously increases as the administered dose is continuously increased. This relationship is expressed in terms of a DRC. In general, the DRC represents estimates of the frequency with which each dose elicits the desired response in the population (Craig and Stitzel 1990).

Effective dose (Therapeutic Index)

The establishment of a DRC is done through the calculation of the effective therapeutic dose(ETD) or ED_{50} (ie, the dose that produced 50% of the maximum response). Effective dose or ED_{50} is the dose of a drug required to produce a specified effect in 50% of the population (Craig and Stitzel 1990).

This is the most wanted dose of any particular drug for producing the desired pharmacological actions. This value can usually be obtained from the DRC.

Lethal dose

Another important characteristic of a drug's activity is its toxic effect. The ultimate toxic effect is death. In pre-clinical trials of drugs, the median lethal dose, as determined in experimental animals, is abbreviated as LD_{50}. from the DRC, one can calculate the LD_{50} (lethal dose, 50%). Since the degree of safety associated with drug administration depends on an adequate separation between doses producing a therapeutic effect (eg, ED_{50}) and doses producing toxic effects (eg, LD_{50}), comparison of these two doses can be used to estimate drug safety. Thus, one estimate of a drug's margin of safety is the ratio of LD_{50}/ED_{50}. This is referred to as the therapeutic index, which is a statement of how selective the drug is in producing its desired effects (Craig and Stitzel 1990). The DRC has 5 different parts (see next page, Figure 5).

In conclusion, it is important to establish the DRC in any trial in which a dose of drug is administered because pharmacodynamic variations or dose of a drug required to produce a particular effect in most of the population may overlap the concentration required to produce toxicity in some of the population, even though the drug's therapeutic index may be large. In simple terms, a fixed dose of a drug may evoke different responses in different individuals. It would therefore be ideal to find the DRC for all subjects in any trial in which the role of particular drug or medication is investigated. However, the construction of a quantal DRC requires that data be obtained from many individuals and

obtaining the data for complete dose response curves in humans is generally difficult and sometimes dangerous.

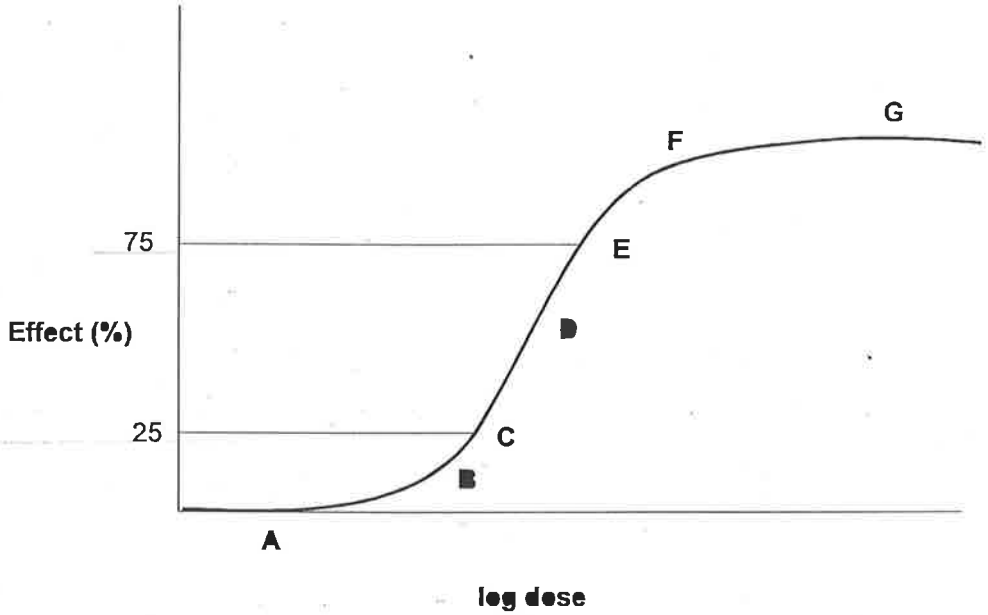


Figure 5 shows different parts of a DRC.

A non-effective area: Up to certain dose, no effect is evoked.

B threshold area: Initial response to increased dose.

C-E Therapeutic Index area: an area between 25-75% of evoked response. After completion of DRC, the dose administered is usually chosen from this area.

F Sub-maximal threshold area

G Maximal effect area: Maximum response is evoked and additional dose will arouse same response.

In this study, due to the difficulty in finding enough volunteers, a DRC for lignocaine with adrenaline (1:80,000) was constructed in three subjects (two

non smokers and one smoker). The aim was to study the changes in LDF readings from gingiva in response to different doses of lignocaine containing adrenaline injected in the muco-buccal fold between upper left central and lateral incisors.

Steps in establishing the DRC in this study

Measurement of the desired dose of local anaesthetic before injection:

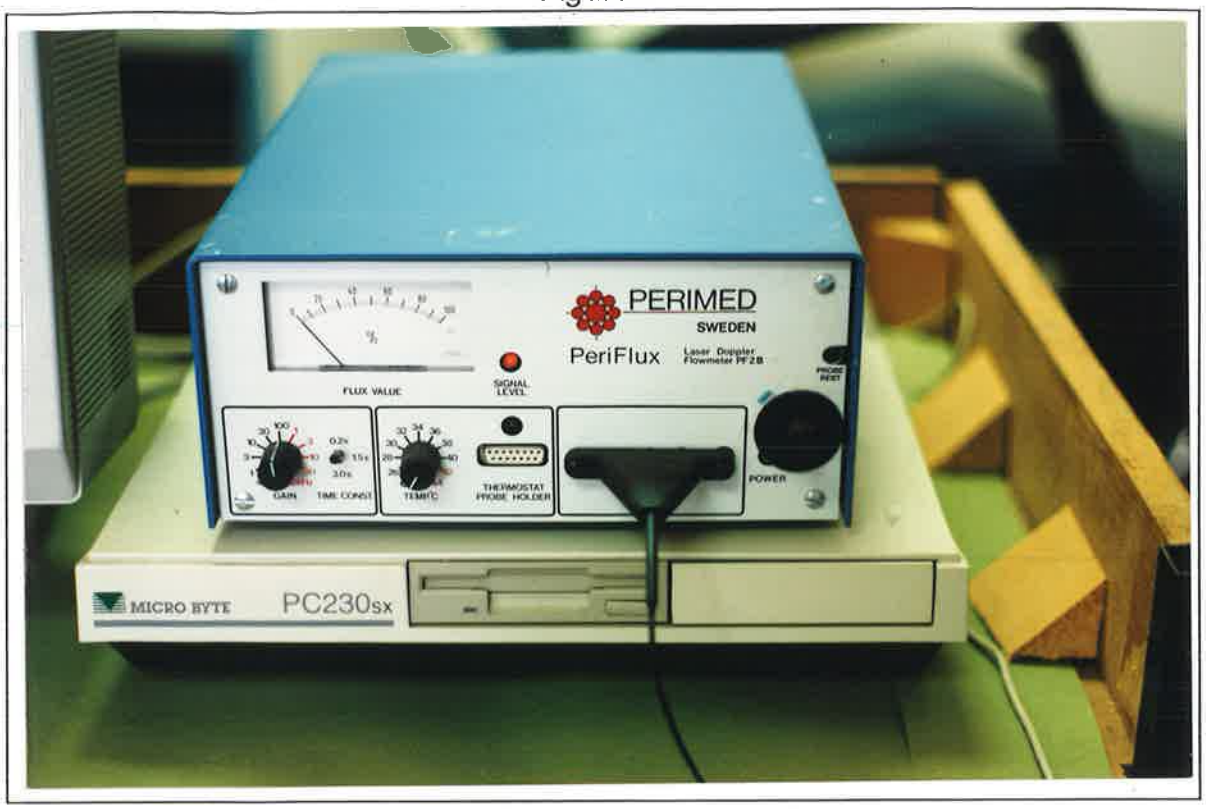
The length of a 2.2 ml carpule of local anaesthetic solution is 6.3 cm. The different lengths of carpule corresponding to different doses of local anaesthetic was established (Table 1). With the help of a coloured pencil, a marker was put corresponding to the required dose. For eg for 0.5 ml injection, a marker was put on the carpule corresponding to 1.4 cm. Excess local anaesthetic was removed and 1.4 cm (0.5 ml) of carpule was injected.

Carpule measurements	
Length in cm	volume in ml
0.5	0.2
0.8	0.3
1.1	0.4
1.4	0.5
1.6	0.6
1.9	0.7
2.2	0.8
2.5	0.9
2.8	1
4.1	1.5

Table 1 shows different lengths of carpule corresponding to different doses of LA.

LDF readings were recorded with a PeriFlux 2b machine connected to computer for data acquisition (Figure 6). The LDF machine was turned on for 20 minutes before commencing recording to allow the laser to warm up.

Figure 6



Each subject's personal details were entered using the Perisoft ® program (Figures 7 and 8).

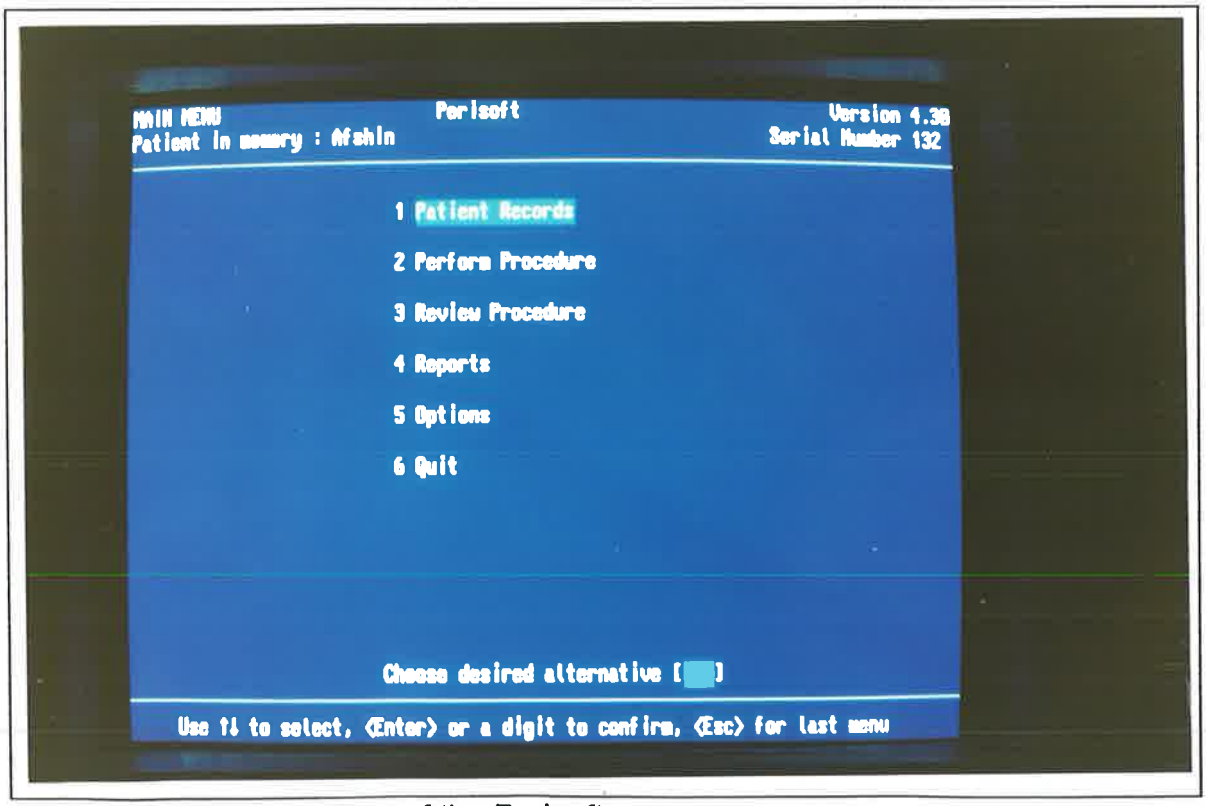


Figure 7: Opening menu of the Perisoft program.

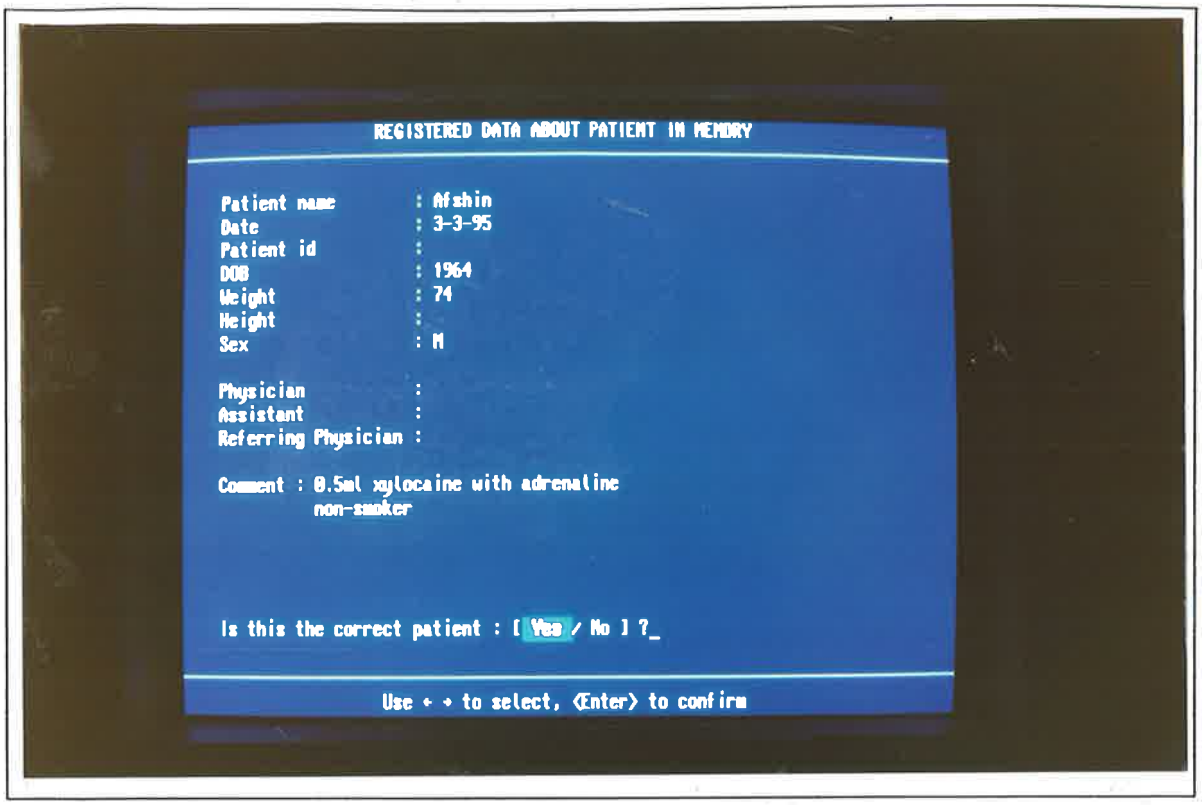


Figure 8: Subject's data being entered into Perisoft program.

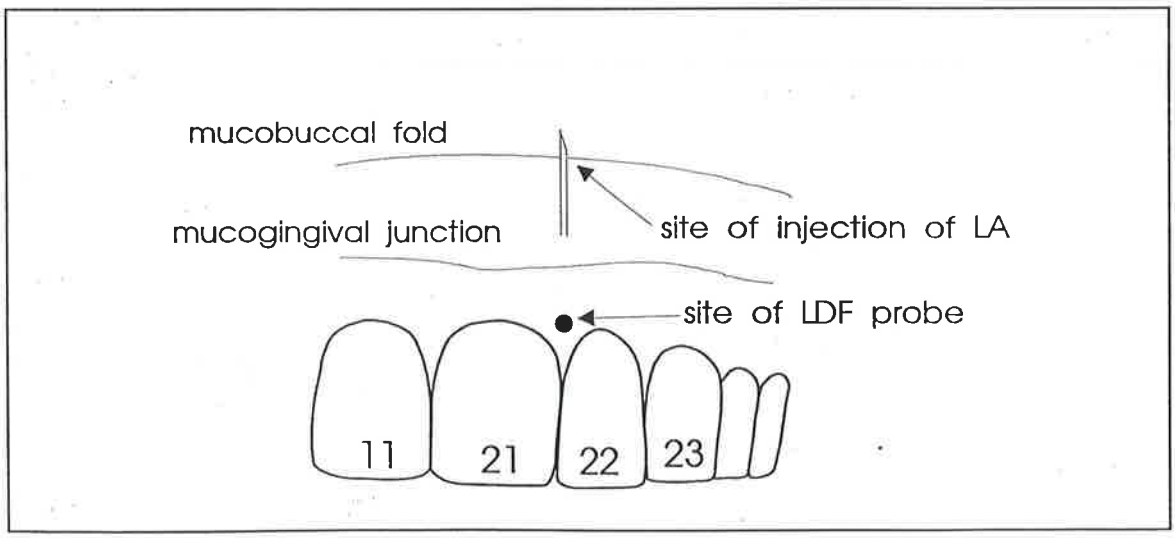


Figure 9 shows the site of injection and the site of placement of LDF probe.

With the stent in the mouth, LDF readings of the interdental gingiva between upper left central and lateral incisor (Figure 9, page 37) were recorded for 10-15 minutes, until the signal level stabilised.

Keeping the stent in place, 0.2 ml of lignocaine with adrenaline (ASTRA) was injected in to the labio-mucosal (normal infiltration) fold in the area between upper left central and lateral incisors. The solution was injected over a 20 second period. In order to direct the needle tip accurately, a V shaped notch had been cut in the stent corresponding to the area of injection (Figure 10).

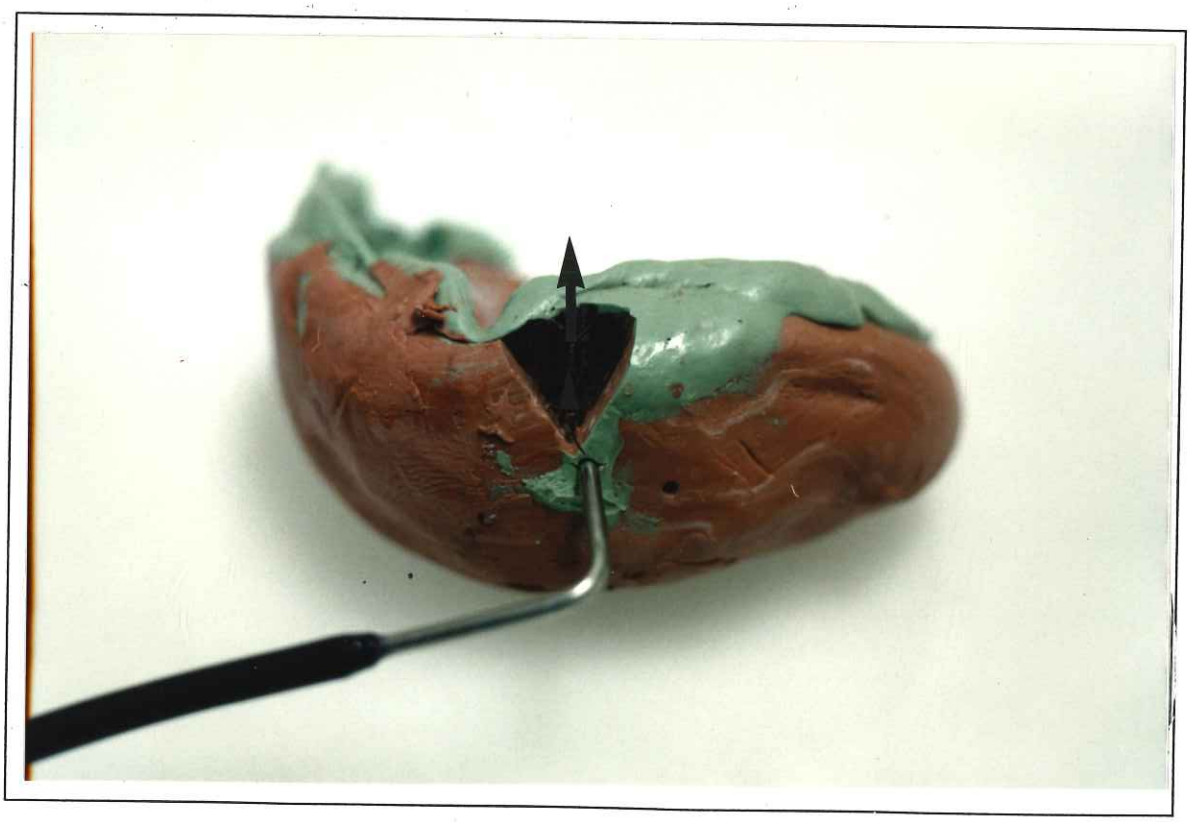


Figure 10 shows the notch cut in the stent; the arrow indicates the direction of the needle.

Just before the injection, an electronic marker was inserted on to the computer tracings by pressing the <Enter> key.

Immediately after the injection, another marker was put into the tracing. The recording was continued for half an hour after injection. The procedure was recorded and saved on the computer's hard disk. During the procedure, the subjects were requested to remain as still as possible to avoid movement artefacts.

The initial dose of local anaesthetic (for calculating DRC) was 0.2 ml of lignocaine with adrenaline. At intervals of 10 days, between each injection, the doses of 0.3, 0.5, 0.7, 1.0 and 1.5 ml of lignocaine with adrenaline were injected.

After completion of the dose response procedure (refer to the results section page 42), 0.5 ml of lignocaine with adrenaline was chosen as the standard dose to be injected in all the subjects participating in the trial. This amount was chosen because it caused more than 30% reduction in LDF signals and was in the range of therapeutic index dose. It was also chosen because it was considered to be an acceptable volume for injection by the subjects (ie a little less than that used to obtain pulpal anaesthesia by infiltration).

Development of tolerance to adrenaline

During the process of establishing the DRC, it was noticed that injection of lignocaine containing adrenaline had no effect on LDF signals when different doses (0.1-0.6 ml) were injected for 6 consecutive days (see results section page 43). This effect was investigated in another subject in which, 0.5 ml of lignocaine with adrenaline was infiltrated for 5 consecutive days.

Controls

Saline (sodium chloride 0.9%) and plain lignocaine were used as controls. In 4 subjects, plain lignocaine (ASTRA) and normal saline injection were administered at intervals of 10 days. In the first visit, 0.5 ml of sterile saline was infiltrated in the labio-buccal fold of the area between upper left central and lateral incisor. After 10 days the procedure was repeated with 0.5 ml of plain lignocaine being injected. At the third visit, 0.5 ml of lignocaine with adrenaline was injected. Saline injection was given to rule out any possible influence of psychological factors (eg fear of injection) on LDF readings. Plain lignocaine was injected to compare the effects of local anaesthetics with and without vasoconstrictor on LDF values.

Procedure for main part of the study

Blood flow through the gingival papilla between upper left central and lateral incisor was monitored for 15 minutes, until the signal level stabilised (The detailed procedure of recording blood flow was explained earlier, see page 38). Then 0.5 ml of a local anaesthetic (lignocaine hydrochloride 2% with adrenaline 1:80,000) was infiltrated into the labial fold between the upper left central and lateral incisors (the usual place for injection of local anaesthetics for dental procedures). The changes in LDF signals were monitored on the computer screen. After injection, an immediate and considerable reduction in LDF signals occurred in most of the subjects. The recording was continued until LDF values reached the level before the injection. The procedure was recorded and saved on the computer's hard disk.

Statistical Analysis

The Student t-test was used to determine statistical significance between the data for smokers and non-smokers because:

A Data were approximately normally distributed.

B Data could be described in terms of means and variances (parametric data).

Results

Dose Response Curve: findings

Percentage reduction in LDF signals corresponding to each dose of local anaesthetic are shown in Tables 2, 3, 4 and Figures 11, 12 and 13.

Table 2

Dose in log	Dose in ml	% reduction in LDF signals
-0.69	0.2	0
-0.52	0.3	27.1
-0.30	0.5	36.3
-0.15	0.7	37.6
0	1	43.7
0.17	1.5	52.2

Figure 11

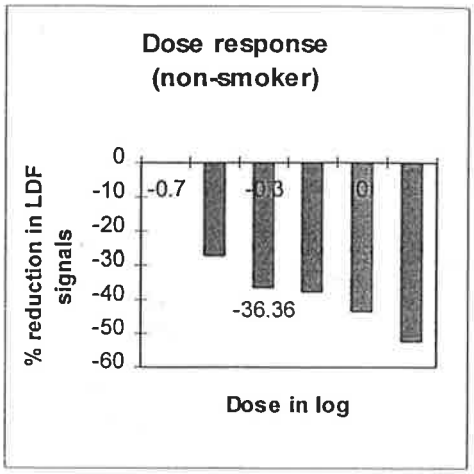


Table 3

Dose in log	Dose in ml	% reduction in LDF signals
-0.69	0.2	0
-0.52	0.3	34.3
-0.30	0.5	38
0	0.7	57.1
0.17	1.5	66.4

Figure 12

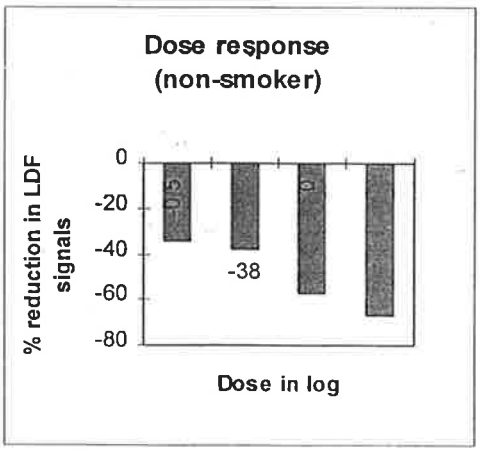
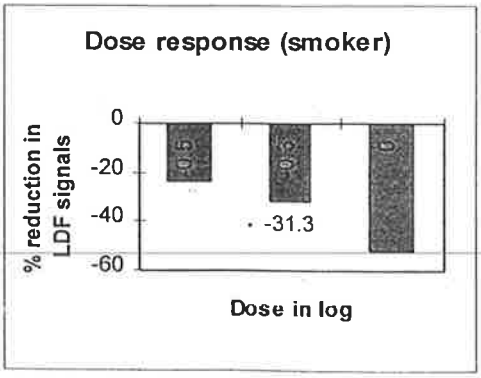


Table 4

Dose in log	Dose in ml	% reduction in LDF signals
-0.69	0.2	0
-0.52	0.3	23.8
-0.30	0.5	31.3
0	1	51.7

Figure 13



Reference to these data show that 0.2 ml of lignocaine with adrenaline was a non-effective dose in all the 3 subjects. Initial response (reduction in LDF signal) was observed with 0.3 ml of lignocaine with adrenaline. This response continuously increased as the administered dose was increased up to 1.5 ml (the maximum dose that was acceptable to the subjects).

Tolerance: Findings

Table 5 shows that there was no reduction in LDF signals after the injection of lignocaine with adrenaline when different doses were injected at the same site for 6 consecutive days.

Frequency of Injection DAY	Dose of lignocaine with adrenaline (ml)	% of reduction in LDF signals
1	0.1	0
2	0.2	0
3	0.3	0
4	0.4	0
5	0.5	0
6	0.6	0

Table 5: Effects on LDF signals of increasing doses of lignocaine with adrenaline injected daily.

As there was no reduction in LDF signals with 0.6 ml of lignocaine with adrenaline (a dose that is known to cause at least 30% reduction in LDF readings when injected in *de novo*), it was apparent that the gingival blood vessels were no longer reactive to adrenaline (ie no vasoconstriction occurred to reduce GBF and consequently, LDF readings). After 10 days, 0.5 ml of lignocaine with adrenaline was infiltrated in labio-buccal fold at the same location in the same subject; it resulted in a 30.5 % reduction in LDF signals.

The phenomenon of tolerance to adrenaline was investigated in another subject. The result is shown in Table 6 and Figure 14.

Tolerance	
DAY	% of reduction in LDF signals
1st	68
2nd	28
3rd	16
4th	13
5th	0

Table 6

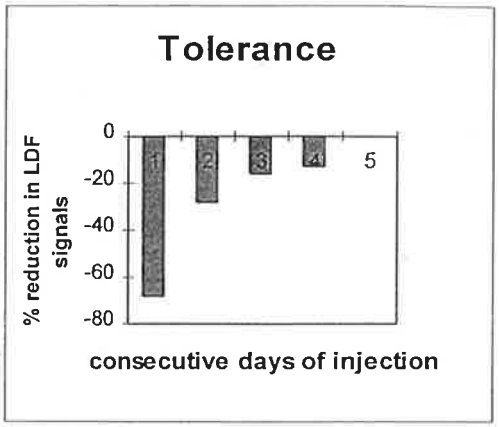


Figure 14

As it can be seen from table 6 and figure 14, daily injection of the same dose of lignocaine with adrenaline (0.5 ml) resulted in diminished response of gingival blood vessels to adrenaline.

Controls

Anaesthesia was achieved in all the 4 subjects following infiltration of 0.5 ml plain lignocaine and lignocaine with adrenaline (subjects reported feeling numbness in the gingiva around the area of injection). Following injection with normal saline and plain lignocaine, the LDF signals did not change significantly in any subject, although there was slight increase in LDF signals in 3 subjects after injection of plain lignocaine.

The results are given in Table 7 and shown in Figure 15

Controls		
Subject	Solution	Change in LDF signals
1	Saline lignocaine Lignocaine with adrenaline	0 3.3% rise 44.4% reduction
2	Saline lignocaine Lignocaine with adrenaline	0 15.4% rise 54.5% reduction
3	Saline Pure lignocaine Lignocaine with adrenaline	0 13.3% rise 45.8% reduction
4	Saline lignocaine Lignocaine with adrenaline	0 0 40% reduction

Table 7

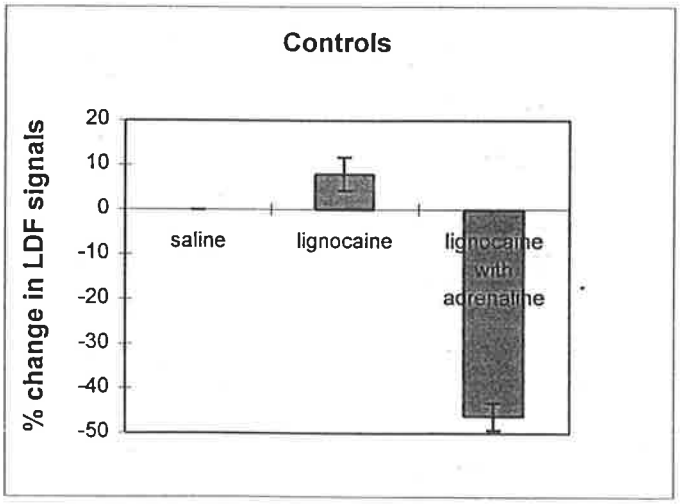


Figure 15

Table 7 and Figure 15 show that following injection with normal saline, the LDF signals did not change but there was slight increase in LDF signals in 3 subjects after injection of plain lignocaine. The bars in Figure 15 are Standard Error of the Means (SE's).

Figures 16, 17 and 18 are photographs of the computer monitor showing the entire procedure of recording LDF signals before and after injection of saline, plain lignocaine and lignocaine with adrenaline in the same subject (non-smoker).

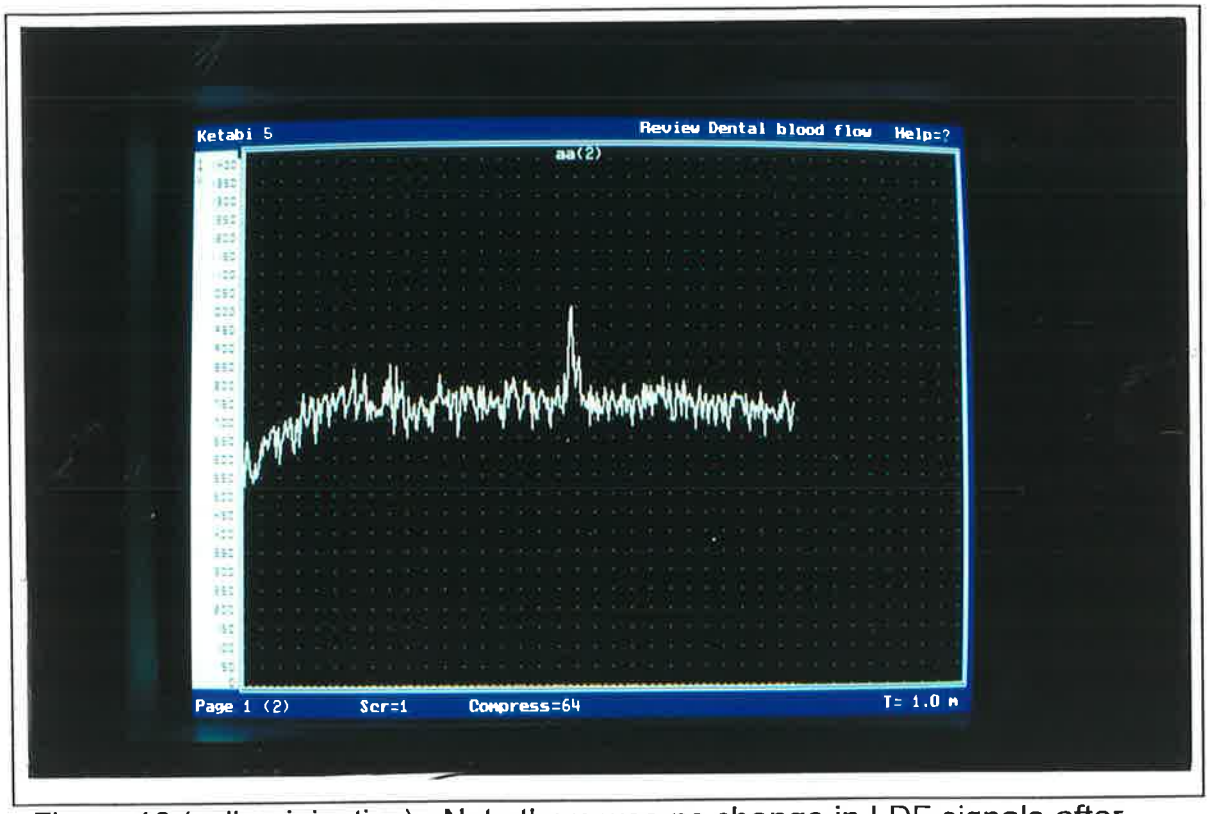


Figure 16 (saline injection): Note there was no change in LDF signals after injection of normal saline. Immediately after injection of saline (0.5 ml, at the point marker aa) the spike of the LDF readings was due to movement artefact that occurred as a result of the injection. The X axis corresponds to time (interval between grid dots=1 minute). The Y axis shows LDF readings (arbitrary units); the maximum value possible was 1000 units. The initial rise in LDF signals to baseline (before injection) was a consistent finding.

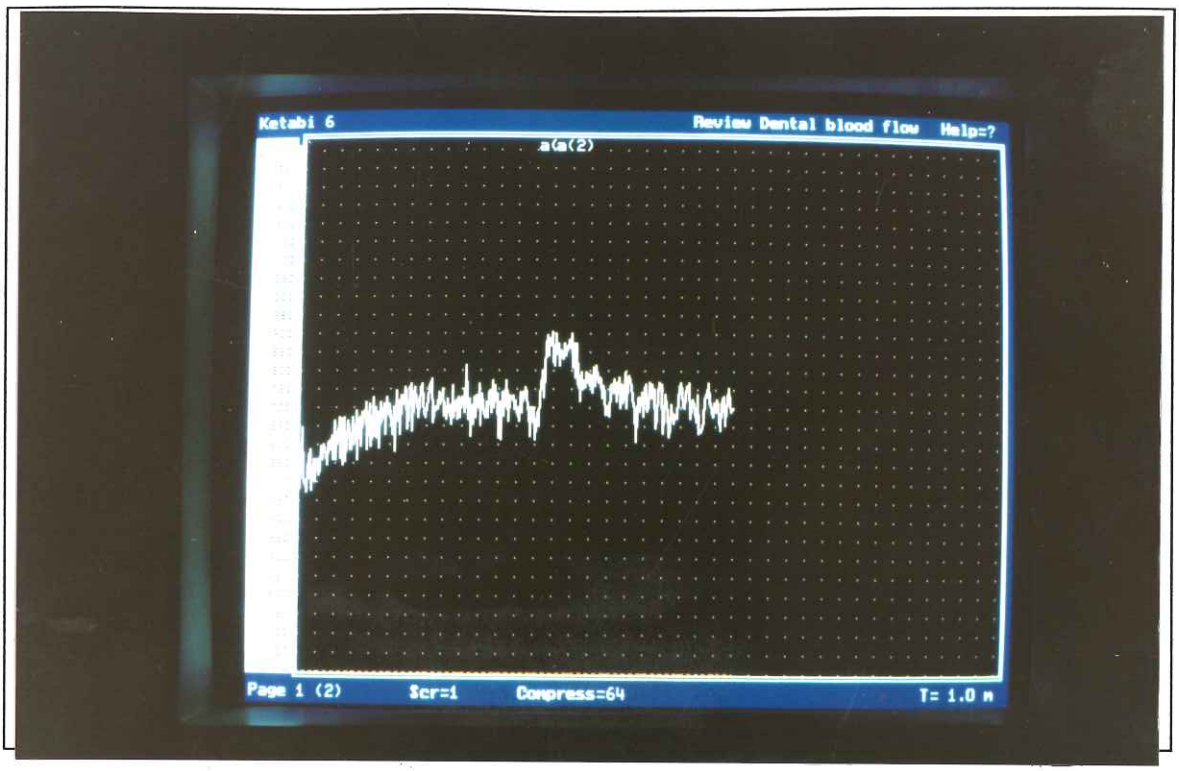


Figure 17 (Plain lignocaine): Plain lignocaine (0.5 ml) was injected (at point marked a(a); Once again, the injection resulted in a significant disturbance to LDF readings which persisted for about 2 minutes. Overall there was a slight increase in LDF signals after injection of plain lignocaine. This trace highlights the inherently labile nature of LDF readings.

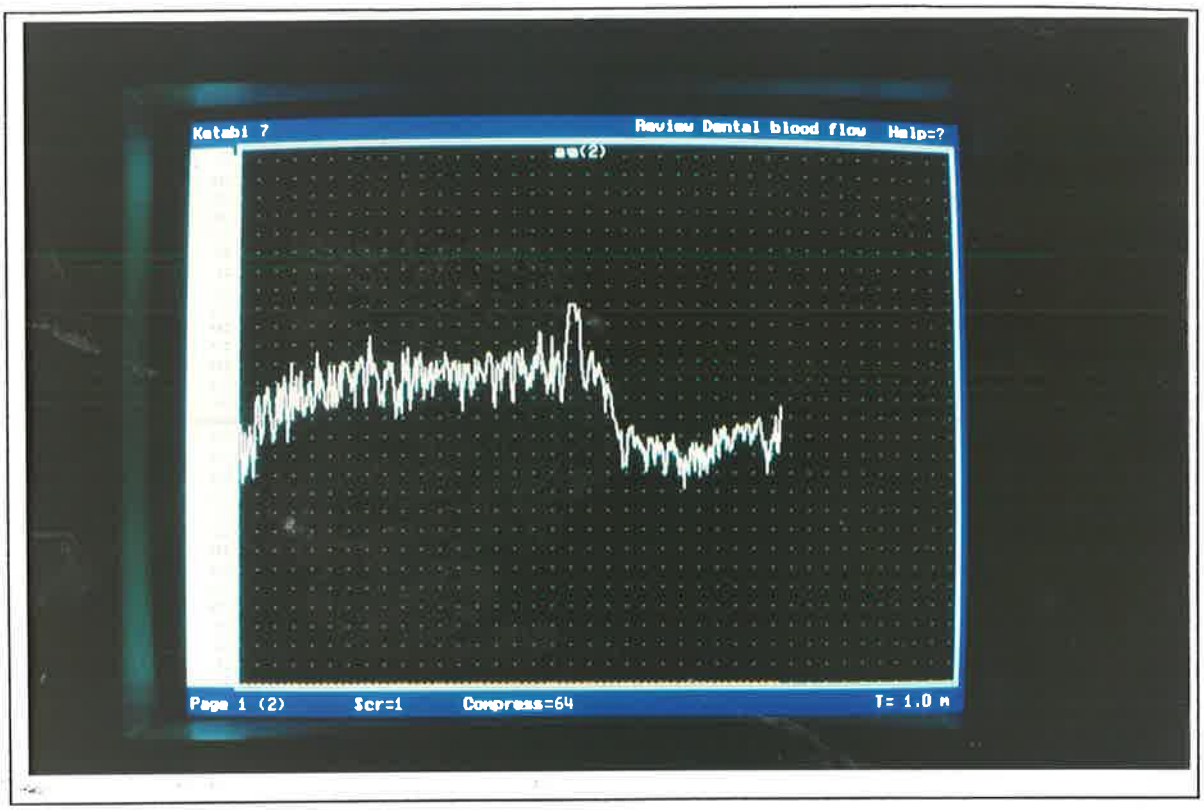


Figure 18 (lignocaine with adrenaline): Lignocaine with adrenaline (0.5 ml) was injected (at point marked aa), causing a transient increase in LDF signals due to movement. After the signals had stabilised, there was a considerable reduction in LDF signals. Signals had not returned to baseline 11 minutes after injection, when the procedure was stopped.

Results of injection of lignocaine with adrenaline

Pattern of LDF signals before injection

An initial increase of 21.8 % (on average) was observed in 80% of the subjects before the signal level stabilised. This corresponds to section A of the schematic representation in Figure 20 (page 50). This pattern occurred in both smokers and non-smokers.

Baseline values

In each subject, LDF readings were recorded for 15 minutes or until the signal level stabilised. The stabilised signal level was considered as baseline value. This corresponds to section B of the schematic representation in Figure 20 (page 50). The average baseline readings in smokers and non-smokers is given in Table 8 and Figure 19.

Baseline readings	
smokers	non-smokers
average: 685	average: 613
SD: 203	SD: 167

Table 8

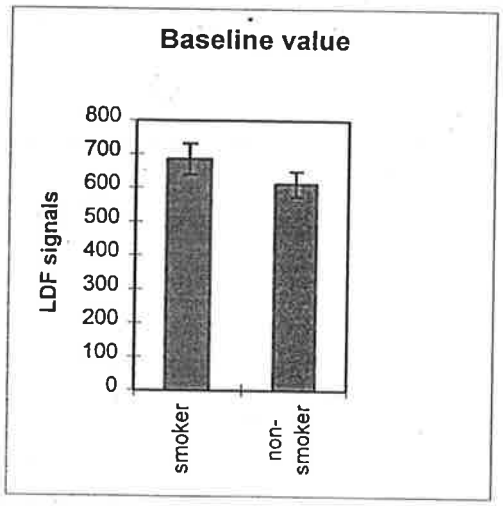


Figure 19

Table 8 and Figure 19 show average baseline LDF readings in smokers and non-smokers. The bars in the Figure 19 are SE's.

As it can be seen from Table 8 and Figure 19, the average base line readings for smokers is higher than non-smokers. Using, Student's t-test (t value=1.22, P> 0.05) this difference was not statistically significant.

Reduction in LDF signal after injection

After injection of lignocaine with adrenaline, an immediate reduction in LDF signals usually occurred (figure 20 part C).

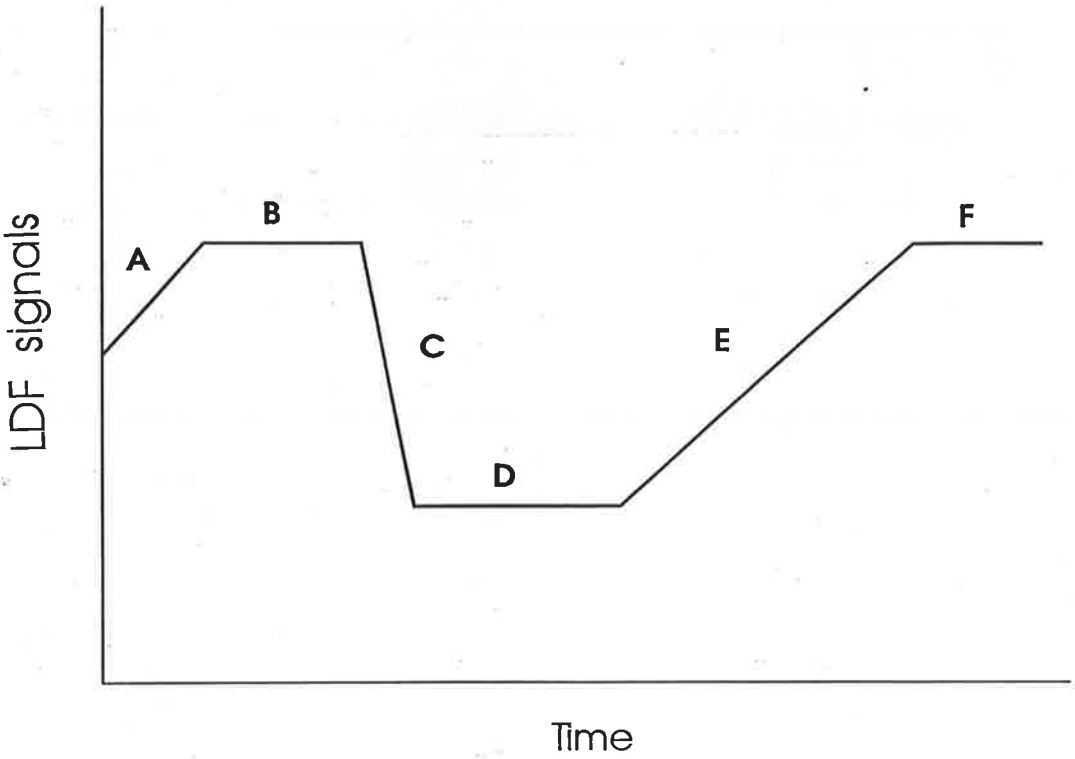


Figure 20 is a schematic representation of LDF readings before and after injection of lignocaine with adrenaline.

A Initial increase of LDF signals before stabilisation

B Baseline

C Reduction in LDF signal after injection

D Period of maximum depression of LDF signals

E Gradual return of LDF signals

F LDF signal returned to baseline

Figure 21 is a photograph of computer displays the LDF signals obtained before and after injection of lignocaine with adrenaline in one of the subjects (non-smoker).

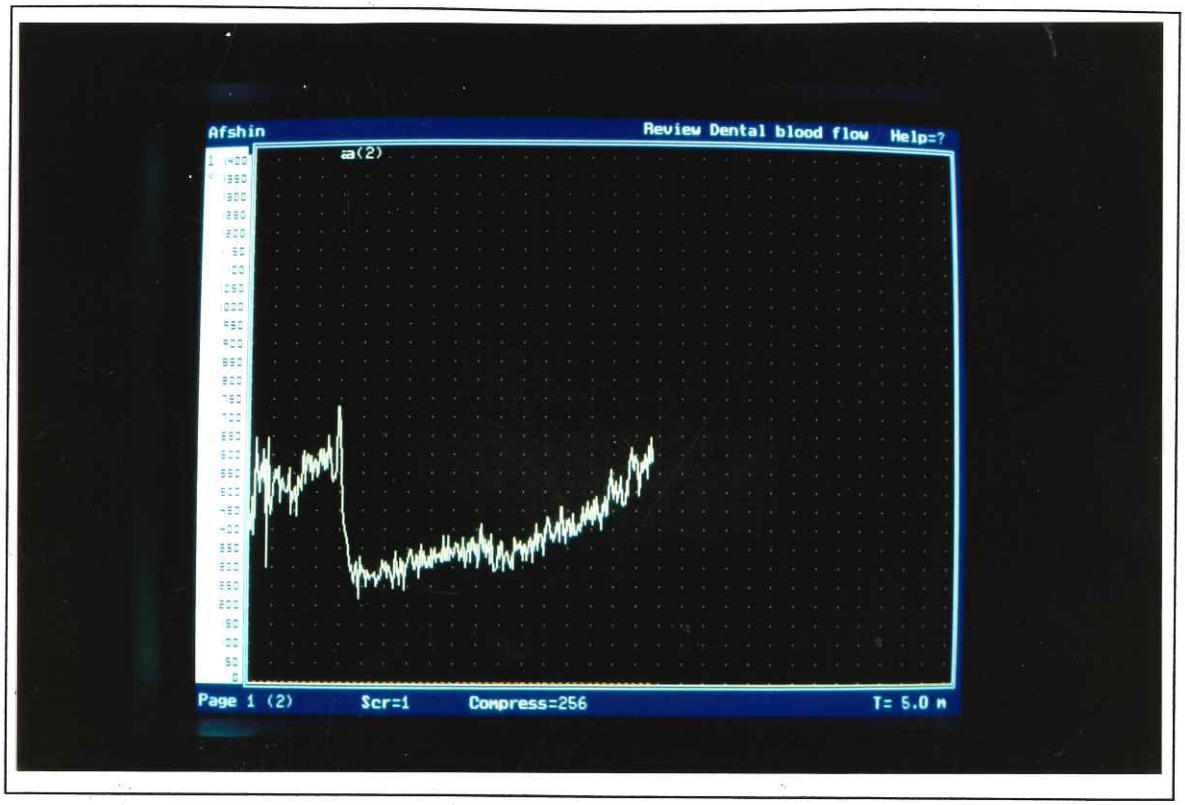


Figure 21: Injection of 0.5 ml of 2% lignocaine with 1:80,000 adrenaline consistently resulted in a marked and significant drop in LDF signals. The 'time' between the grid dots in this figure correspond to 5 minutes.

The mean percentage of reduction in LDF recording signals is given in Table 9.

Percentage reduction in LDF signals	
Reduction in LDF values	Percentage change
minimum reduction	25.3
maximum reduction	80.6
average	**46.1**
SD	13.5

Table 9 show mean percentage of reduction in LDF recording signals.

There was no statistically significant difference between smokers and non-smokers in the percentage reduction in LDF signals using Student's t-test (t value=0.13, P>0.05). The data are given in Table 10 and Figure 22.

Percentage reduction in LDF signals	
smokers	non-smokers
average: 45.8	average: 46.4
SD : 12.9	SD : 14.5

Table 10

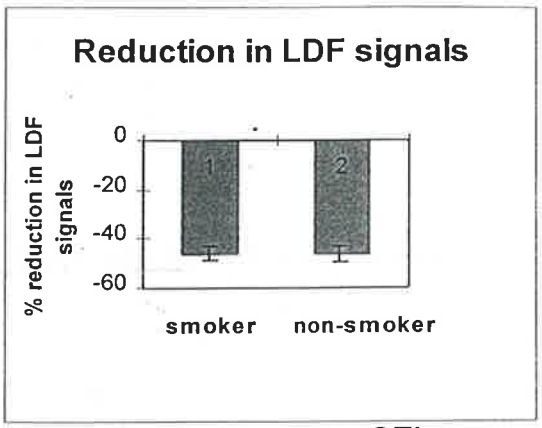


Figure 22 The bars are SE's

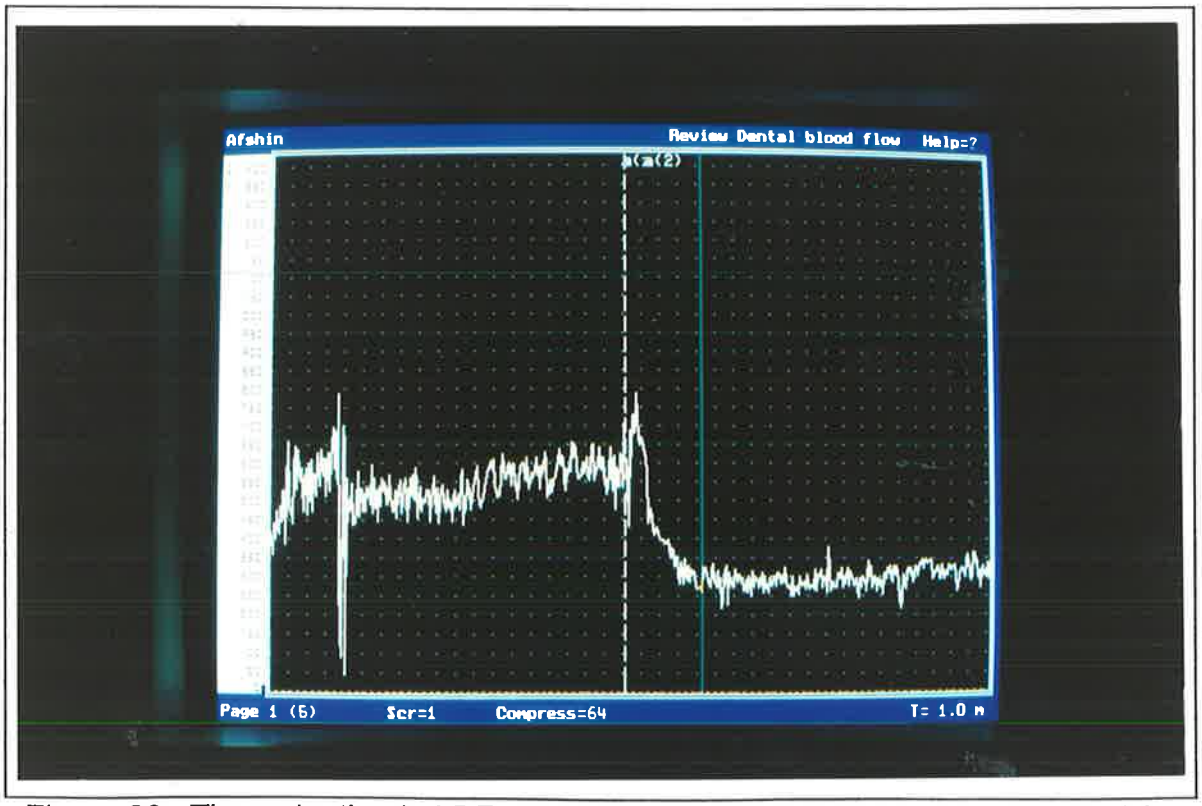


Figure 23: The reduction in LDF signals in one of the subjects (non-smoker) is shown in Figure 23 (area between vertical cursors). Time between grid dots is 1 minute. In this subject, the percentage reduction in LDF signals was 50.8%.

Period of maximum depression of LDF signals

Reduction in LDF readings generally remained low for an average of 21.8 minutes in all the subjects after which LDF readings started to increase. This corresponds to section D of the schematic representation in Figure 20 page 50. This time was on average of 25.31 minutes in smokers and 19.76 minutes in non-smokers. The difference was not statistically significant using Student's t-test (t value=1.34, p>0.05). The data are given in Table 11 and shown in Figure 24.

Period of maximum depression of LDF signals			
	Duration that flow remained low in all subjects (minutes)	Duration that flow remained low in smokers (minutes)	Duration that flow remained low in non-smokers (minutes)
Average	21.8	25.31	19.76
SD	12.2	15.57	10.07

Table 11

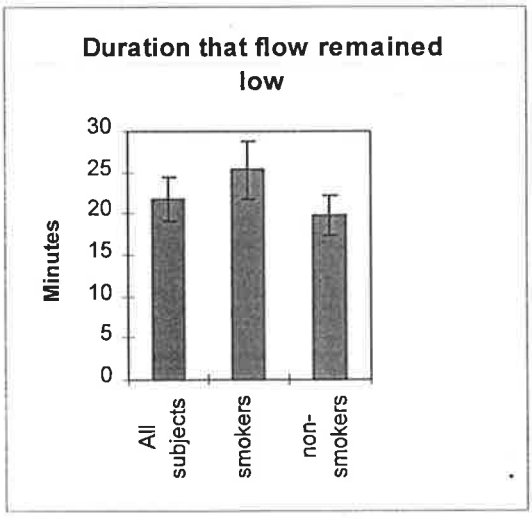


Figure 24

Table 11 and Figure 24 show the period of maximum depression of LDF signals. The bars are SE's.

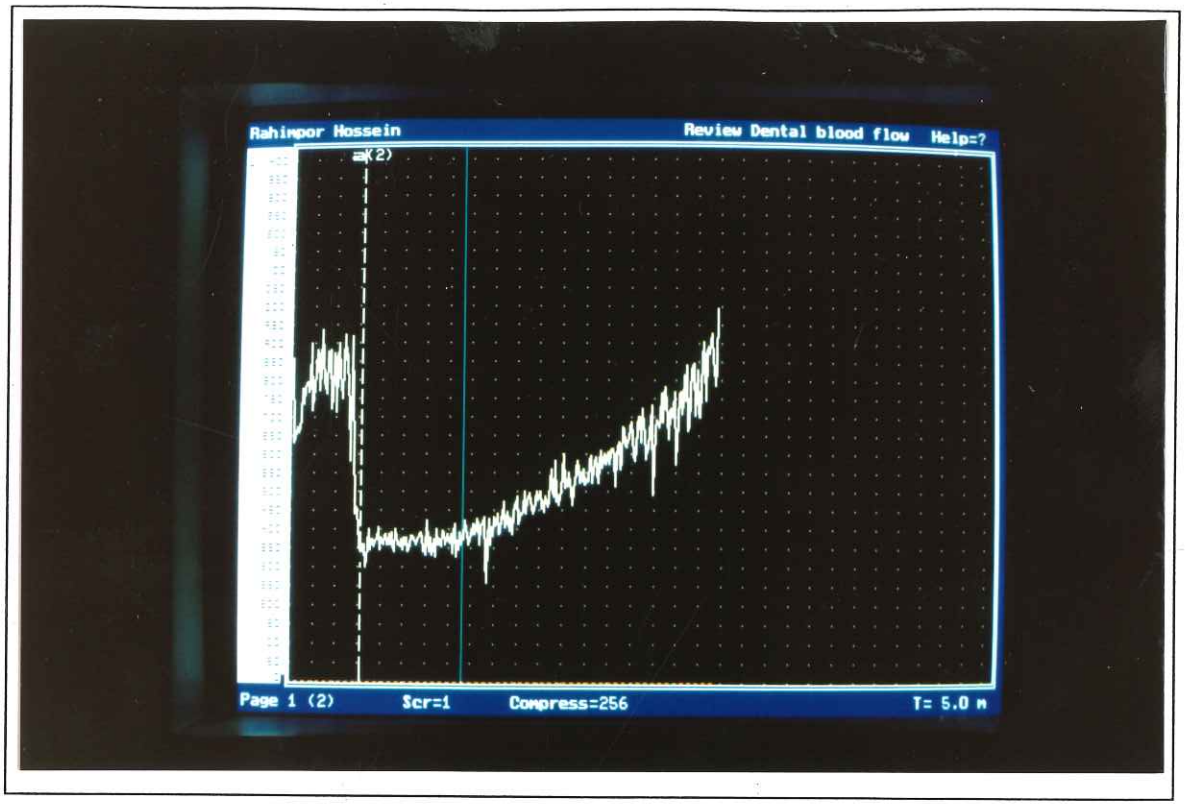


Figure 25 is a photograph showing period of maximum depression of LDF readings (area between 2 vertical cursors). Time between grid dots is 5 minutes. The maximum depression of LDF signals in this subject was 23 minutes.

In four of the smokers, LDF signals remained low and did not return to baseline value even after 70 minutes (average). The procedure was not continued because the subjects found it uncomfortable to keep the stent in the mouth for such a long time. This unusual pattern did not occur in any of the non-smokers.

Recovery of LDF signals to baseline

On average, the recovery of LDF signals to baseline took 67.9 minutes (sd:24.3). This corresponds to sections D and E of the schematic representation in Figure 20 page 50. This period was considerably longer in smokers compared with non-smokers. Using Student's t-test, the difference was statistically significant (t value 3.44, $p<0.05$). The data are given in Table 12 and shown in Figure 26.

Recovery of LDF signals to baseline			
	Recovery duration of LDF signals in all subjects	Recovery duration of LDF signals in smoker	Recovery duration of LDF signals in non-smoker
minimum	34	50	34
maximum	137	137	95
average	67.9	80.2	56.6
SD	24.3	24.2	18.8

Table 12

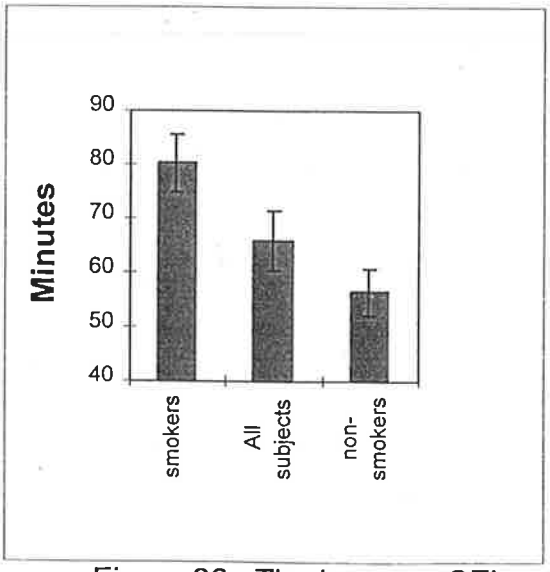


Figure 26: The bars are SE's

Table 12 and Figure 26 that the recovery of LDF signals took considerably longer in smokers than non-smokers.

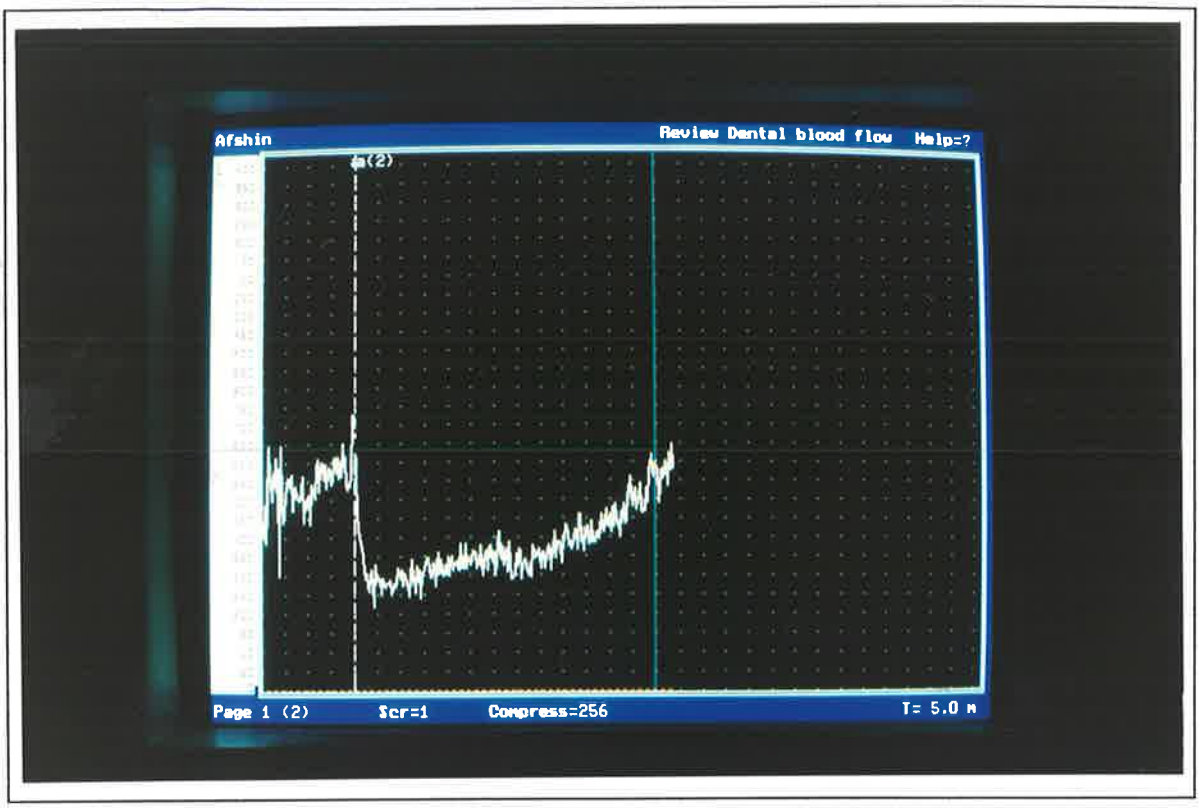


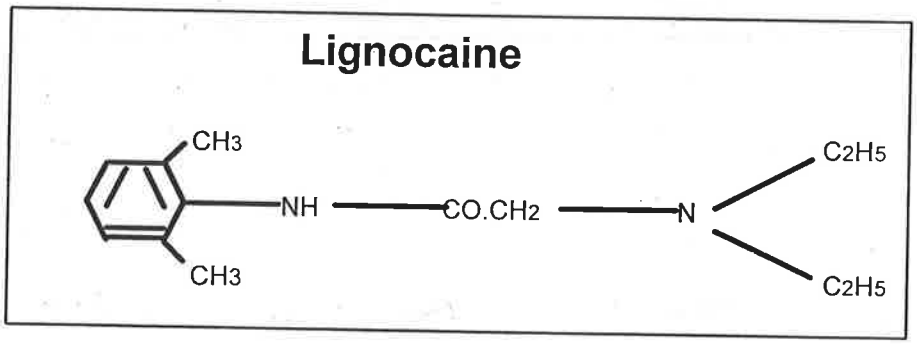
Figure 27 is a photograph showing the complete recovery of LDF signals (area between 2 vertical cursors). ie from time of injection to return to baseline. Time between grid dots is 5 minutes. In this subject, the complete recovery of LDF signals took 65 minutes.

Discussion

The purpose of this study was to investigate the effects of local anaesthetics (mainly lignocaine with adrenaline) on gingival blood flow in humans. In particular, the response of smokers was compared to that of non-smokers.

Lignocaine

Local anaesthetic solutions of lignocaine with adrenaline are very widely used in dental practice. Lignocaine is a xylidine derivative, amide type of local anaesthetic. Its formula is shown below:



Although lignocaine can be used without a vasoconstrictor, it is not very successful at producing anaesthesia of adequate duration. Vasoconstrictor agents are included in many local anaesthetics in order to:

A Reduce the risk of unwanted side effects by delaying absorption from the injection site into the general circulation.

B Reduce bleeding at the site, giving increased visibility and produces a relatively bloodless field which facilitates surgery.

C Allow a smaller volume (dose) of local anaesthetic solution to be given.

D Reduce blood flow in the area of injection thereby delaying absorption into the general circulation, retarding removal of the local anaesthetic agent and ensuring a more prolonged analgesia.

Gray et al (1987) demonstrated that surgical anaesthesia was achieved more than twice as frequently with a local anaesthetic solution containing vasoconstrictor as one without, and it has been recommended that local anaesthetic solutions containing vasoconstrictor should be used routinely for patient comfort (Cawson *et al* 1983).

Adrenaline

The vasoconstrictor most commonly added to local anaesthetic solutions is adrenaline. Adrenaline is an active principle of the adrenal medulla which is formed in natural and synthetic forms. Adrenaline is stable in acid solution and is used in local anaesthetics in concentrations ranging from 1:50,000 to 1:300,000. However, the concentrations commonly used for dental anaesthesia are 1:80,000 and 1:100,000. Adrenaline acts on the effector cells of the autonomic nervous system, the alpha and beta receptors. Stimulation of the alpha receptors causes stimulatory effects, producing peripheral vasoconstriction and pupil dilatation, while stimulation of the beta receptors causes inhibitory effects, producing vasodilatation of skeletal muscle vessels and broncho-dilation (Foldes *et al* 1965).

Vascular effects of adrenaline (Craig and Stitzel 1990)

The chief vascular action of adrenaline is exerted on the smaller arterioles and precapillary sphincters, although veins and larger arterioles also respond to adrenaline. Vasoconstriction occurs in vessels of the skin, splanchnic areas and kidney by the action of their alpha-receptors. Adrenaline has a more complex action on vessels in skeletal muscle because of its high affinity for both α and β_2 receptors. Whether adrenaline produces vasodilation or vasoconstriction in skeletal muscle depends on the dose administered. Low doses of adrenaline dilate the blood vessels; larger doses will result in vasoconstriction. The effect of adrenaline on circulation is also varying according to the route and rate of injection.

Measurement of gingival blood flow

In this study, GBF was measured by LDF. One great advantage of LDF was that the rate of blood flow could be read continuously without contact between the probe and gingiva. The main drawback of LDF is that it produces a relative, rather than an absolute value of the blood cell flux, ie it can not be calibrated in general physiological units such as number of red blood cells flowing through $1mm^3$ of the measuring volume per second. The sensitivity of the LDF system can only be standardised. This is achieved by immersing the probe tip in a solution of polystyrene microspheres (0.48 um diameter) at 20°C: the Brownian motion of the microspheres is defined as 250 perfusion units (Vongsavan and Matthews 1993). LDF measures relative gingival blood flow rather than actual blood flow. Another drawback of LDF is the inherently

variable nature of LDF recordings. There is evidence of a high degree of variability of consecutive readings (not continuous) that is not related to actual changes in blood flow. This is why LDF signals were recorded continuously once, rather than on separate occasions for each subject.

Difficulties encountered during the study

The duration of the procedure on each subject was lengthy. Subjects found it uncomfortable to keep the stent in the mouth for nearly 2 hours and towards the end of the session they were moving the head and body in different directions. This interfered with LDF readings, producing movement artefacts and occasionally disturbing signal acquisition. Some of the subjects (particularly smokers) used to cough frequently during the procedure and it was sometimes necessary to take the stent out of the mouth for a short while, interrupting LDF recordings. Difficulties were also encountered when administering the local anaesthetic. Although a notch was prepared in the stent to direct the needle into mucobuccal fold, the nature of the reflection of the buccal mucosa was different in each subject, making it difficult to inject local anaesthetic at the same point in all subjects. The depth of penetration of the needle was also difficult to control.

More subjects took part in this study (40) than in comparable ones (Table 13).

Table 13

Reference	Study of PBF/GBF (effect of local anaesthetics)	Number of subjects
Odor , 1994a	PBF	13
Odor , 1994b	PBF	10
Pitt Ford, 1993	PBF	10

However, because blood flow is influenced by a number of physiological, psychological, chronological, environmental and the inherently variable nature of LDF recordings, a larger number of subjects should ideally have been involved.

Dose response curve

The results of the dose response curve establishment showed that repeated injection at the same site of lignocaine containing adrenaline had no effects on the LDF signals when different or the same doses were injected at daily intervals. The result indicated that tolerance developed to adrenaline in the receptors of gingival blood vessels and it was apparent that the gingival blood vessels were no longer reactive to adrenaline.

This is the first report of this phenomenon in the dental literature.

The exact mechanism for the development of tolerance is not known. However, it is known that adrenaline acts on α and β adreneregic receptors on smooth muscle walls of blood capillaries. Repeated exposure of receptors to adrenaline may modify their response.

Desensitisation to catecholamines (adrenaline, noradrenaline, isoproterenol)

Repeated exposure of catecholamine-sensitive cells and tissues to catecholamines causes a progressive diminution in their capacity to respond to such agents. This phenomenon is variously termed as refractoriness, desensitisation, or tachyphylaxis, and it significantly limits the therapeutic efficacy and duration of action of catecholamine and other agents (Goodman and Gilman 1992). Although the mechanisms of such adaptive changes are incompletely understood, modification of receptors is the most likely explanation given. The pattern of desensitisation varies according to the extent to which different regulatory components of responsiveness are modified. In some cases, especially when the receptors themselves are altered, the desensitisation may be limited to the actions of β-adrenergic agents. This is often termed as homologous desensitisation. In other cases, stimulation by a β-adrenergic agonist can cause diminished responsiveness to a wide variety of receptor-mediated stimulators of cyclic AMP synthesis. This is termed as heterologous desensitisation(Goodman and Gilman1992). Different types of receptor modifications may occur which contribute to decrease sensitivity to catecholamines.

Potential pathways for receptor modification: (Goodman and Gilman1992)

A Number of receptors:

Repeated administration of catecholamines (adrenaline, noradrenalline, isoproterenol) promote a rapid (minutes) and reversible sequestration

(internalisation) of their receptors and a slower "down regulation" of the receptors, in which the actual number of receptors in the cell declines and therefore the response to the drug is diminished. Both processes may contribute to desensitisation to catecholamines, although the exact underlying mechanisms are poorly understood.

B Reduction in release of Cyclic AMP:

Cyclic AMP is an important enzyme (secondary messenger) which in combination with other factors allow the adrenergic receptors (α & β) to respond to any stimuli. Repeated administration of catecholamines markedly reduces the synthesis of cyclic AMP and as a result causes a progressive diminution in the capacity of cells and tissue response to catecholamines.

C Phosphorylation of receptors:

A special protein kinase, termed the β-adrenergic receptor kinase, phosphorylates the receptors only when they are occupied by an agonist (eg, adrenaline). This leads to decrease efficiency of coupling of the receptors with agonist (adrenaline) and lowers receptor function.

Repeated injection of lignocaine containing adrenaline failed to affect LDF signals when different or similar doses were injected at frequent short intervals. It has not been reported clinically that the efficacy/duration of local anaesthetics given at the same location within short time periods is reduced. This may be because patients are usually not seen on a daily basis and that the same site is not usually subjected to repeated local anaesthetic injection.

However the rapid development of tolerance to adrenaline in this study indicates that local anaesthetics containing adrenaline may not be as effective when injected frequently at the same location. This must be particularly considered during dental or surgical procedures while adrenaline is mainly used to control the bleeding. The phenomenon of tolerance to local anaesthetics containing adrenaline was investigated in only 2 subjects. This was because each subject had to receive the injection for five consecutive days. Many subjects were reluctant to participate in this part of the project.

Controls

Plain lignocaine and saline were used as controls and their effects on GBF was compared with lignocaine containing adrenaline in 4 subjects. Plain lignocaine had no significant effect on LDF signals except in 3 subjects where, LDF readings were increased by a small amount. This slight increase is most probably due to the vasodilatory effects of plain lignocaine (Goodman and Gilman 1992). Normal saline had no effects on LDF signals in any of the subjects. It can be therefore concluded that the reduction in LDF signals after injection of lignocaine with adrenaline, observed in most of the subjects was due to the vasoconstrictive action of adrenaline.

Injection of lignocaine with adrenaline

During the recording of LDF signals, an initial increase of 21.8 % (on average) were observed in 80% of the subjects before the signal level stabilised. This pattern was observed in both smokers and non-smokers. There is no clear explanation for this phenomenon but it is likely that pressure exerted from the

stent at the time of insertion has caused vasoconstriction of gingival blood vessels. This might have dropped blood flow initially which gradually returned to normal due to tissue adaptation which was observed as an initial rise before the signal level stabilised.

Average baseline LDF readings were higher in smokers than non-smokers. This difference was not statistically significant. This difference could be due to chronic effects of smoking on gingival vasculature. Smoking has complex and divergent effects on blood flow in different parts of the body. Smoking increases blood flow to skeletal muscle, intestine, uterus (Lehtovirta and Forss 1980) and brain but it decreases flow to forearm skin (Waeber *et al* 1984) and hands (Sarin *et al* 1974). The site specific effect of smoking may be due to different vasoactive agents released by smoking or different localised vascular changes induced by smoking.

Two studies which used LDF for measuring blood flow reported contradictory results regarding the effect of smoking on GBF. Baab and Öberg (1987a) showed that relative GBF immediately rose (25.4%) during smoking, whereas Kyuichi *et al* (1995) showed a decrease (23%) in GBF during smoking. These contradictory results could be due to the difficulties associated with LDF measurements.

The site of injection was in the muco-labial fold between upper left central and lateral incisors while point of measuring LDF signals was in interdental papilla between the same teeth. There is a significant distance from the point of injection to the point of measuring. Considering this distance and the collateral

circulation of interdental gingiva, the effect of adrenaline on gingival blood vessels would have been significantly greater had the injection site been closer to the gingival papilla.

The present study showed that infiltration of 2% lignocaine with adrenaline (1:80,000) caused significant reduction in LDF signals from gingiva. On average there was 46.12 % reduction in all the subjects and of interest, there was a wide range in the extent of reduction in LDF signals (25.3-80.6%). This wide range in the extend of reduction in LDF signals (observed both in smokers and non-smokers) and large variation in other parameters measured could have been due to the following factors:

A Large variation inherent in laser Doppler flowmetry measurements.

B The relatively large distance between the site of injection and point of measuring GBF.

C Variation in thickness of cortical plate of bone and variation in porosity of the cancellous bone.

D Different thickness and nature of the reflection of buccal mucosa along with difficulty to control depth of needle penetration.

The results in term of effect on LDF signals are of the same order of magnitude as reported by others. Pitt Ford *et al* (1993) reported a 31% reduction in pulpal blood flow following injection of 1 ml of 2% lignocaine with 1:80,000 adrenaline. Odor (1994a) found a 42% reduction in pulpal blood flow in mandibular canine and 24 % in mandibular molars following inferior alveolar block anaesthesia using 2 ml of 2% lignocaine with 1:80,000 adrenaline.

Odor (1994b) showed reduction in PBF after inferior alveolar nerve block [48% reduction of PBF in canine and 25% in molars] using 1 ml of 2% lignocaine with 1:100,000 adrenaline. Those studies had smaller numbers of subjects (10, 13 and 10) in comparison to present study in which 40 subjects were involved. Secondly in those studies the LDF data were not continuously down-loaded to the personal computer (blood flow readings were taken at short time intervals). In the present study, the entire procedure was continuously recorded and simultaneously down-loaded to the personal computer making the readings more reliable.

These authors did not establish a dose response curve, but used an ill-defined dose of 1-2 ml. This is the amount which is usually administered to obtain anaesthesia for dental procedures. However, this study showed that comparable results in reduction in output of LDF signals can be obtained even with a dose of 0.5 ml lignocaine with adrenaline.

Although lignocaine with adrenaline caused significant reduction in LDF signals in the majority of the subjects, no drop was observed in 3 volunteers. As a dose response curve was not established for all subjects, it is most likely that 0.5 ml was a non-effective dose in these individuals. A larger dose was not administered in order to maintain similar doses in all subjects. Deviation from the usual injection site could be an alternative explanation for this observation.

As the local anaesthetic solution affected the vessels supplying the gingiva, it is likely that adrenaline acted on the adrenergic receptors in the smooth

muscle wall of the arterioles causing vasoconstriction. It is known that stimulation of α adrenergic receptors causes vasoconstriction whereas stimulation of β adrenergic receptors causes vasodilatation. Therefore, adrenaline most likely acted on α adrenergic receptors to constrict gingival blood vessels. According to Poiseuilla Hagen law, the flow of a liquid through a tube is proportional to the fourth power of the radius of that tube and therefore, a small decrease in the radius of the supplying vessels resulted in significant reduction in blood flow in the gingiva. This study indicated that there was no statistically significant difference between smokers and non-smokers in the extent of the initial and rapid reduction in LDF signals that followed injection of lignocaine with adrenaline.

Another significant finding of this study was that the recovery of LDF signals to baseline took considerably longer in smokers compared with non-smokers.

The following hypotheses may be forwarded to explain this phenomenon.

A Delay in recovery of LDF values in smokers could be due to nicotine-stimulated release of some vasoactive enzymes. For example, nicotine is known to stimulate release of catecholamines, particularly adrenaline.

Plasma concentration of adrenaline increases with smoking (Lindell *et al* 1993) and urinary excretion of adrenaline decreased upon quitting smoking (Terres *et al* 1994). It is therefore possible that endogenous adrenaline released in response to smoking added to the effects of adrenaline administered in local anaesthetics and further prolonged the duration of drop in LDF values.

B Nicotine has been shown to trigger the release of AVP (arginine vasopressin), a potent vasoconstrictor hormone (Waeber *et al* 1984). As described previously, it is possible that nicotine stimulated release of vasopressin further delayed uptake of adrenaline and recovery of LDF values.

Conclusion and direction for further research

In conclusion this study has shown that :

A The baseline stabilised LDF signals from interdental gingiva between upper left central and lateral incisors were higher in smokers than non-smokers. The difference was not statistically significant.

B Injection of 0.5 ml lignocaine with adrenaline in mucolabial fold between upper left central and lateral incisors caused a significant reduction in LDF signals which were recorded from the gingival papilla.

C The reduction in LDF signals due to injection of adrenaline was of longer duration in smokers than non-smokers. The difference was statistically significant.

D This study also has shown repeated injection of lignocaine with adrenaline on frequent short intervals had little effects on LDF signals from gingiva. This shows that some type of tolerance or desensitisation may develop in the receptors of gingival blood vessels to repeated injection of adrenaline.

As blood flow is influenced by physiological, psychological, chronological, environmental and other factors and LDF recording has also an inherently variable nature, further research in this area should attempt to study the long term effect of smoking on GBF in a larger sample of subjects. As the phenomenon of tolerance to adrenaline developed in gingival blood vessels was investigated in only 2 subjects, this observation warrants investigation in more subjects.

Bibliography

Anderson RR, Parish JA. The optics of human skin. *Journal of Investigative Dermatology* 1981;77:13-19.

Avery JK, Cox CF, Corpron RE, et al. Distribution of adrenergic and cholinergic nerve endings in mouse molars utilising morphological and cytochemical analysis. *Journal of Dental Research* 1974(Abstr#79);53:74.

Baab DA, Öberg PA, Holloway GA. Gingival blood flow measured with a laser Doppler flowmeter. *Journal of Periodontal Research* 1986;21:73-85.

Baab DA, Öberg PA. The effect of cigarette smoking on gingival blood flow in humans. *Journal of Clinical Periodontology* 1987a;14:418-424.

Baab DA, Öberg PA. Laser Doppler measurement of gingival blood flow in dogs with increasing and decreasing inflammation. *Archives of Oral Biology* 1987b;32:551-555.

Baab DA, Öberg PA, Lundstrom A. Gingival blood flow and temperature changes in young humans with a history of periodontitis. *Archives of Oral Biology* 1990;35:95-101.

Barnet NJ, Dougherty G, Pettinger SJ. Comparative study of two laser Doppler blood flowmeters. *Journal of Medical Engineering and Technology* 1990;14:243-249.

Barnett ML, Gilman RM, Charles CH, et al. Computer-based thermal imaging of human gingiva. *Journal of Periodontology* 1989;60:628-633.

Cawson R, Curson I, Whittingston D. The hazards of dental local anaesthetics. *British Dental Journal* 1983;154:253-258.

Clarke NG, Shephard BC, Hirsch RS. The effects of intra-arterial epinephrine and nicotine on gingival circulation. *Oral Surgery Oral Medicine Oral Pathology* 1981;52:577-582.

Clarke NG, Shephard BC. The effects of epinephrine and nicotine on gingival blood flow in the rabbit. *Archives of Oral Biology* 1984;29:789-793.

Clarke NG, Carey SE. Aetiology of chronic periodontal disease: an alternative perspective. *Journal of the American Dental Association* 1985;110:689-691.

Craig CR, Stitzel RE. Modern pharmacology. (3 ed.). Boston, Little Brown, 1990:143-146.

De Rink WG, Bowen PD, Boner RF. Preliminary results with laser Doppler Velocimetry (LDV) in gingival tissues. *Journal of Dental Research* 1980;59(Abstr. #232):325.

Dorman HL, Bishop JG. In vivo oxygen tension measurements in gingival tissue. *Journal of Dental Research* 1964;43:708-711.

Foldes F, Davidson GM, Duncalf D. The intravenous toxicity of local anaesthetic agents in man. *Journal of Clinical Pharmacology and Therapeutics* 1965;6:328-335.

Forsslund G. Stereoscopic capillaroscopy. Method for photogrammetric investigation and registration of peripheral blood-vessel system, with special regard to gingiva and oral mucosa. *Acta Odontologica Scandinavica* 1953;11:1-4.

Forsslund G. The structure and function of capillary system in the gingiva in man. *Acta Odontologica Scandinavica(Supl.#26)* 1959;17:1-144.

Gazelius B, Olgart L, Edwall B, et al. Non-invasive recording of blood flow in human dental pulp. *Endodontics and Dental Traumatology* 1986;2:219-221.

Genco RJ, Goldman HM, Cohen DW. Contemporary Periodontics. Mosby, St.Louis, 1990:18-19.

Giddon DB, Goldhaber P, Kushnir H, et al. Photoelectric monitoring of gingival vascular reaction. *Journal of Periodontology* 1963;34:366-370.

Goodman LS, Gilman A. The pharmacological basis of therapeutics. (8th ed.) McGraw-Hill, Inc, New York, 1992:68-73.

Grant DA, Stern IB, Listgarten MA. Periodontics. (6th ed.) Mosby, St.Louis, 1988:p:46.

Gray R, Lomax A, Rood J. Periodontal ligament injection with or without a vasoconstrictor. *British Dental Journal* 1987;162:263-265.

Haber J, Wattles J, Crowley M, et al. Evidence for cigarette smoking as a major risk factor for periodontitis. *Journal of Periodontology* 1993;64:16-23.

Heithersay GS, Hirsch RS. Tooth discolouration and resolution following a luxation injury: significance of blood pigment in dentin to laser Doppler flowmetry readings. *Quintessence International* 1993;24:669-676.

Hinrichs JE, Jarzembinski C, Hardie N, et al. Intrasulcular laser Doppler readings before and after root planing. *Journal of Clinical Periodontology* 1995a;22:817-823.

Hinrichs JE, Labelle LL, Aeppli D. An evaluation of laser Doppler readings obtained from human gingival sulci. *Journal of Periodontology* 1995b;66:171-176.

Hock J, Nuki K. Erythrocyte velocity in vascular networks of young noninflammed dog gingiva. *Journal of Dental Research* 1976;55:1058-1060.

Hock JM, Nuki K, Schlenker R, et al. Clearance rates of Xenon-133 in non-inflammed and inflamed gingiva of dogs. *Archives of Oral Biology* 1980;25:445-449.

Hock J, Kim S. Periodontal and pulpal blood flow in dogs with periodontitis. *Journal of Dental Research (Special issue)* 1983;62(Abstr. #269):679.

Kaplan ML, Davis MA, Goldhaber P. Blood flow measurements in selected oral tissues in dogs using radiolabelled microspheres and rubidium-86. *Archives of Oral Biology* 1973;23:281-284.

Kaplan ML, Jeffcoat MK, Goldhaber P. Blood flow in gingiva and alveolar bone in beagles with periodontal disease. *Journal of Periodontal Research* 1982;17:384-389.

Karring T, Löe H. A quantitative analysis of the epithelium-connective tissue interface in relation to assessments of the mitotic index. *Journal of Dental Research* 1969;48:634-640.

Kim S, Edwall L, Trowbridge H, et al. Effects of local anaesthetics on pulpal blood flow in dogs. *Journal of Dental Research* 1984;63:650-652.

Kim S. Regulation of pulpal blood flow. *Journal of Dental Research* 1985;64:590-596.

Kim S, Dorscher-Kim JE, Liu M. Microcirculation of the dental pulp and its autonomic control. *Proceeding of the Finnish Dental Society* 1989;85:279-287.

Kindlova M, Matena V. Blood vessels of the rat molar. *Journal of Dental Research* 1962;41:650-660.

King JD. Experimental investigations of periodontal disease. Vital capillary microscopy as a clinical aid to assessment of gingival health and disease. *Dental Record* 1947;57:3-7.

Kinnen E, Goldberg HJV. The application of electrical impedance plethysmography to the study of gingival circulation. *Journal of Periodontology* 1978;49:528-533.

Kvietys P, Shepherd A, Granger D. Laser Doppler, H2 clearance, and microsphere estimates of mucosal blood flow. *American Journal of Physiology* 1986;250:249-253.

Kyuichi K, Yukihiro N, Soh S, et al. Association between periodontal disease and cigarette smoking. In: Bartold PM, ed. Proceedings of the Asian Pacific Society of Periodontology Meeting Australia 1995. Australia: Asian Pacific Society of Periodontology, 1995: 116-126.

Laser Doppler Flowmeter for Microvascular Perfusion. PeriFlux PF 2 and PF 2B User's Handbook 1986;1st Edition.

Lehtovirta P, Forss M. The acute effect of smoking on uteraplacental blood flow in normotensive and hypertensive pregnancy. *International Journal of Gynaecology and Obstetrics* 1980;18:208-211.

Levine AK. Lasers. *American Science* 1963;51:29-34.

Lindell G, Farnebo L, Chen D, et al. Acute effects of smoking during modified sham feeding in a duodenal ulcer patient. *Scandinavian Journal of Gastroenterology* 1993;28:487-494.

Lundberg JM, Brodin E, Hua XY, et al. Vascular permeability changes and smooth muscle contraction in relation to capsaicin sensitive substance P afferents in the guinea-pig. *Acta Physiologica Scandinavica* 1984;120:217-227.

Manson J, Eley BM. Outline of periodontics. Oxford, Boston, Wright: 1995.

Matheny JL, Abrams H, Johnson DT, et al. Microcirculatory dynamics in experimental human gingivitis. *Journal of Clinical Periodontology* 1993a;20:578-583.

Matheny JL, Johnson DT, Roth GI. Aging and microcirculatory dynamics in human gingiva. *Journal of Clinical Periodontology* 1993b;20:471-475.

Neidle EA, Liebman FM. Effects of vasoactive drugs and nerve stimulation on blood flow in the tooth pulp and applied structures of the cat. *Journal of Dental Research* 1964;43:412-422.

Nilsson GE. Signal processor for laser Doppler tissue flowmeters. *Medical and Biological Engineering and computing* 1984;22:343-348.

Nuki K, Hock J. The organisation of the gingival vasculature. *Journal of Periodontal Research* 1974;9:305-313.

Odor T, Pitt Ford T, McDonald F. Adrenaline in local anaesthesia: the effect of concentration on dental pulp circulation and anaesthesia. *Endodontics and Dental Traumatology* 1994a;10:167-173.

Odor TM, Pit Ford TR, McDonald F. Effect of inferior alveolar nerve block anaesthesia on the lower teeth. *Endodontics and Dental Traumatology* 1994b;10:144-148.

Olgart L, Gazelius B. Effects of adrenaline and felypressin (octapressin) on blood flow and sensory nerve activity in the tooth. *Acta Odontologica Scandinavica* 1977;35:69-75.

Olgart L, Gazelius B, Lindh-Stromberg U. Laser Doppler flowmetry in assessing vitality in luxated permanent teeth. *International Endodontic Journal* 1988;21:300-306.

Page RC, Schroeder HE. Pathogenesis of inflammatory periodontal disease. A summary of current work. *Laboratory Investigation* 1976;33:235-237.

Parker I, Parker DAS, De La Lande IS. Catecholamine contents of rabbit gingiva and dental pulp. *Australian Journal of Experimental Biology and Medical Science. Supplements* 1986;64:465-469.

Pitt Ford TR, Seare MA, McDonald F. Action of adrenaline on the effect of dental local anaesthetic solutions. *Endodontics and Dental Traumatology* 1993;9:31-35.

Ramsay DS, Artun J, Bloomquist D. Orthognathic surgery and pulpal blood flow. *Journal of Oral and Maxillofacial Surgery* 1991a;49:564-570.

Ramsay DS, Artun J, Martinen SS. Reliability of pulpal blood-flow measurements utilising laser Doppler flowmetry. *Journal of Dental Research* 1991b;70:1427-1430.

Riva CE, Ross B, Benedek GB. Laser Doppler measurements of blood flow in capillary tubes and retinal arteries. *Investigative Ophthalmology* 1972;11:936-944.

Rowe JW, Kilgore A, Roberston GL. Evidence in man that cigarette smoking induces vasopressin release via airway-specific mechanism. *Journal of Clinical Endocrinology and Metabolism* 1980;51:170-172.

Sarin CL, Austin JC, Nickel WO. Effects of smoking on digital blood flow velocity. *Journal of the American Medical Association* 1974;229:1327-1328.

Staple PH, Copely AL. Observation on the microcirculation in the gingiva of hamsters and other laboratory animals. *Circulation Research (supplement)* 1959;7:243-246.

Stern MD. In vivo evaluation of microcirculation by coherent light scattering. *Nature* 1975;254:56-58.

Tanaka T, Riva C, Ben-Sira I. Blood velocity measurements in human retinal vessels. *Science* 1974;186:830-831.

Terres W, Becker P, Rosenberg A. Changes in cardiovascular risk profile during the cessation of smoking. *American Journal of Medicine* 1994;97(3):242-249.

Uvnas B. Sympathetic vasodilator system and blood flow. *Physiological review* 1960;40:69-71.

Vandersall DC, Zander HA. Experimental obstruction of the periodontal blood circulation. *Helvetica Odontologica Acta* 1967;11:74-79.

Vongsavan N, Matthews B. Some aspects of the use of laser Doppler flowmeters for recording tissue blood flow. *Experimental Physiology* 1993;78:1-14.

Waeber B, Schaller M-D, Nussberger J, et al. Skin blood flow reduction induced by cigarette smoking: role of vasopressin. *American Journal of Physiology* 1984;247h:895-901.

Yamaguchi K, Nanda RS, Kawata T. Effect of orthodontic forces on blood flow in human gingiva. *The Angle Orthodontist* 1991a;61:193-201.

Yamaguchi K, Nanda RS, Kawata T. Effects of orthodontic forces on blood flow in human gingiva. *The Angle Orthodontist* 1991b;61:193-202.

Yeh Y, Cummins HZ. Localised fluid flow measurements with a helium-neon laser spectrometer. *Applied Physics Letters* 1964;4:176-178.

Appendix

Table of abbreviation

%	Percentage
α	Alpha
β	Beta
μg	Microgram
AMP	Amino Mono phosphate
CNS	Central nervous system
DRC	Dose response curve
ED_{50}	Effective therapeutic dose
g	Gram
GBF	Gingival blood flow
LD	Lethal dose
LDF	Laser Doppler flowmetry
mg	Milligram
min	Minute
ml	Millilitre
mm	Millimetre
mm^2	Square millimetre
mm^3	Cubic millimetre
P	Probability
PF	Periflux flowmeter
S	Second
SBF	Skin blood flow
SE's	Standard error mean
SD	Standard deviation

Recording sheets

This form was used for recording the readings from the computer and for further statistical analysis

Name	
Age	
Sex	
Local Anaesthesia used:	
Dosage	
Area of gums	
Level of flow before injection	
Duration after injection till flow reaches the lowest level.	
Percentage (amount)of drop in blood flow.	
Lowest record of flow after injection.	
Duration that flow remains lowest.	
Duration from lowest flow till it levels like before injection.	
Total duration after injection till it reaches the level before injection.	

Consent forms

Informed consent was obtained from all subjects involved in the study.

CONSENT FORM

See also Information Sheet attached.

1. I ..... (please print) hereby consent to take part in the research project entitled "The effect of local anaesthetics on gingival blood flow in humans ....."

2. I acknowledge that I have read the Information Sheet entitled "Blood flow in the gums ....."

   I have had the project, so far as it affects me, fully explained to my satisfaction by the research worker. My consent is given freely.

3. Although I understand that the purpose of this research project is to improve the quality of medical care, it has also been explained that my involvement may not be of any benefit to me.

4. I have been given the opportunity to have a member of my family or a friend present while the project was explained to me.

5. I have been informed that, while information gained during the study may be published, I will not be identified and my personal results will not be divulged.

6. I understand that I am free to withdraw from the project at any time and that this will not affect medical advice in the management of my health, now or in the future.

7. I am aware that I should retain a copy of this Consent Form, when completed, and the relevant Information Sheet.

SIGNED ..... DATE .....

NAME OF WITNESS ..... SIGNED .....
(Please print)
DATE .....

I, ... have described to .....
(Please print)
the nature of the procedures to be carried out. In my opinion she/he understood the explanation.

SIGNED ..... DATE .....

Subject's recruitment

This page was displayed on notice boards around the University to recruit some of the subjects for this study.

Smokers!!

If you are a male smoker (18-30 years) and smoke at least 5 cigarettes a day, how about taking part in a paid research project in the Department of Dentistry.

Interested??

Please call and leave your name and phone number.