รายงานวิจัยฉบับสมบูรณ์

โครงการ การพัฒนาเทคนิคเพื่อใช้ Laser Doppler Flowmeter ในการวัด pulpal blood flow ในฟันมนุษย์

Development of the technique for the use of laser Doppler flowmeter for monitoring pulpal blood flow in intact human teeth

โดย รศ. ทพ. ดร. นพคุณ วงษ์สวรรค์

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สัญญาเลขที่ RSA/13/2543

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สนับสนุนโดย **สำนักงานกองทุนสนับสนุนการวิจัย**

กิติกรรมประกาศ

ก่อนอื่นกระผมใคร่กราบขอบพระคุณ ศ. นพ. วิจารณ์ พานิช เป็นอย่างยิ่งในฐานะที่อาจารย์ได้สร้างคุนูปการในด้านวิจัยให้แก่ประเทศเป็นอย่างยิ่งในขณะที่อาจารย์ดำรงตำแหน่งผู้อำนวยการ สกว.

กระผมใคร่กราบขอบพระคุณ ศ. ดร. วิชัย บุญแสง เป็นอย่างยิ่งในที่ให้โอกาสกระผมในการศึกษาด้านการไหลของเลือดผ่านเนื้อเยื่อในโพรงฟันจากฟันมนุษย์ด้วยเครื่องเลเซอร์ดอปเปลอร์โฟลมิเตอร์

กระผมใคร่กราบขอบพระคุณ ศ. ทพ. สมศักดิ์ จักรไพวงศ์ ที่สนับสนุนการวิจัยโดยเฉพาะด้านเครื่องมือต่างๆ

กระผมใคร่ขอขอบคุณ รศ. ทพ. สุรินทร์ สูอำพัน รศ. ทพญ. มาลินี สูอำพัน รศ. ทพญ. วรุณี เกิดวงศ์บัณฑิต ผศ. ดร. สิทธิชัย วนจันทรารักษ์ คุณศิริรัตน์ วัชรอุดมปัญญา และผู้ป่วยที่ร่วมในโครงการวิจัยนี้

บทคัดย่อ

Project Code : RSA/13/2543

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Project Period : 3 ปี

ในการบันทึกการไหลของเลือดผ่านเนื้อเยื่อในโพรงฟันจากฟันมนุษย์ด้วยเครื่องเลเซอร์ดอปเปลอร์โฟลมิเตอร์นั้น ยังมีสัญญาณการไหลของเลือดจากเนื้อเยื่อปริทันต์และเนื้อเยื่ออื่นๆ ที่อยู่รอบนอกเนื้อเยื่อในโพรงฟัน การศึกษาครั้งนี้ เพื่อหาสัดส่วนขอสัญญาณการไหลของเลือดของเนื้อเยื่อในโพรงฟันที่แท้จริงเมื่อใช้เครื่องมือดังกล่าววัดที่ตัวฟันในมนุษย์ โดยใช้เฝือกครอบกระชับฟันที่ทำด้วยอะคริลิก ปิดคลุมเหงือกรอบฟันที่ต้องการวัดด้วยแผ่นยางทึบสีดำ บันทึกจากฟันหน้าบนซี่ใกล้กลางที่มีสภาพปกติ จำนวน 22 ซี่ จากอาสาสมัคร 14 คน อายุระหว่าง 22-40 ปี ด้วยเครื่องเลเซอร์ดอปเปลอร์โฟลมิเตอร์ รุ่น เอ็ม บี เอฟ 3 ดี42 บันทึกในภาวะต่างๆดังนี้ ภาวะใช้เฝือกครอบกระชับฟันที่ทำด้วยอะคริลิกอย่างเดียว ภาวะใช้เฝือกครอบกระชับฟันที่ทำด้วยอะคริลิกและปิดคลุมเหงือกรอบฟันที่ต้องการวัดด้วยแผ่นยางทึบสีดำ ภายหลังฉีดยาชา ภายหลังดึงเอาเนื้อเยื่อในโพรงฟันออกแล้วนำกลับเข้าไว้ในโพรงฟัน และภายหลังดึงเอาเนื้อเยื่อในโพรงฟันออกแล้วล้างด้วยนอมอลซาลีน สัญญาณการไหลของเลือดด้วยเครื่องเลเซอร์ดอปเปลอร์โฟลมิเตอร์ที่ฟันในภาวะต่างๆมีค่าเท่ากับ 6.98 ± 1.30 , 1.92 ± 1.50, 1.45 ± 0.61, 0.35 ± 0.19, 0.98 ± 0.36 หน่วย พี.ยู ตามลำดับ โดยสรุปสัญญาณการไหลของเลือดด้วยเครื่องเลเซอร์ดอปเปลอร์โฟลมิเตอร์ที่ฟัน สัญญาณที่วัดได้ส่วนใหญ่จะมาจากเนื้อเยื่ออื่นๆที่อยู่รอบนอกเนื้อเยื่อในโพรงฟัน สัญญาณการไหลของเลือดด้วยเครื่องเลเซอร์ดอปเปลอร์โฟลมิเตอร์ที่ฟันมนุษย์ร่วมกับการปิดคลุมเหงือกรอบฟันที่ต้องการวัดด้วยแผ่นยางทึบสีดำมีเพียงร้อยละ 43 เท่านั้นที่เป็นสัญญาณการไหลของเลือดของเนื้อเยื่อในโพรงฟันที่แท้จริงเ

คำสำคัญ: เครื่องเลเซอร์ดอปเปลอร์โฟลมิเตอร์, การไหลของเลือดของเนื้อเยื่อในโพรงฟัน, ฟันมนุษย์

Abstract

Project Code : RSA/13/2543

Project Title : การพัฒนาเทคนิคเพื่อใช้ Laser Doppler Flowmeter ใน
การวัด pulpal blood flow ในฟันมนุษย์

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Project Period : 3 ปี

Records of pulpal blood flow obtained from human teeth with a laser Doppler flow meter include a very large component that is derived from periodontal and other tissues outside the pulp but this contamination can be reduced by covering the surrounding tissues with opaque rubber dam. The present experiments were carried out to determine what proportion of the signal obtained with this dam is derived from non-pulpal tissues. Recordings were made from 22 healthy, intact upper incisors in 14 subjects (aged 22-40 yr.) with and without dam under the following conditions: from the intact teeth, after local anesthesia (LA) and the preparation of a deep cavity in the incisal third of the labial surface of the tooth, and after removal of the pulp and arrest of bleeding. In some cases a reading was also made after replacing the pulp in the pulp cavity. The rubber dam reduced significantly ($P<0.05$) the mean blood flow reading from the intact teeth by 73%, from 6.98 arb. perfusion units (P.U.) (± 1.30 S.D., n=22) to 1.92 ± 1.50 P.U. After LA and cavity preparation, the mean signal with dam was 1.45 ± 0.61 P.U. (n=16). This fell significantly by 76% to 0.35 ± 0.19 P.U. (n=16) when the pulp was removed but not replaced, and by 43% to 0.98 ± 0.36 P.U. (n=14) when the pulp was removed and replaced in the pulp cavity. The latter condition is thought to reproduce most closely the scattering of light in an intact tooth. The results indicate that approximately 43% of the blood flow signal recorded from an intact tooth with opaque rubber dam represents pulpal blood flow.

Key Words: Laser Doppler Flowmeter, Pulpal Blood Flow, Human Teeth

Series 1 experiments

The Sources of Laser Doppler Blood Flow Signals Recordedfrom Teeth in Human Subjects

Introduction

Since the original observations of Gazelius *et al*. (1986, 1988), and Olgart *et al.* (1988), laser Doppler flow meters have been used in many studies to record blood flow from the pulps of intact teeth in man. This is potentially a valuable technique both for research on pulpal blood flow and for clinical diagnosis. However, it is not known what proportion of the signal recorded from a tooth under different conditions is derived from the pulp and how much is from periodontal and other tissues outside the tooth. Some recent studies suggest that between 47 and 60%, depending on the recording conditions (Ingólfsson *et al.,* 1994), or even 80% of the signal recorded from an intact human incisor may not be from the pulp (Amess *et al.,* 1993; Hartmann *et al.*, 1996; Andrew *et al.,* 1999).

Vongsavan and Matthews (1996) studied on the sources of the laser Doppler blood flow signal recorded from teeth in pig. They found that about 15% of the signal recorded with the laser probe centreed 2 mm from the gingival margin was of non-pulpal origin. This was established by recording before and after section of the pulp. These results contrast with those of the other studies which suggested that up to 80% of the signal recorded from human teeth may be of non-pulpal origin. It might be that the discrepancy between these results was due to species differences in the optical properties of dentine and enamel which caused a greater proportion of the incident light to be transmitted to the tooth supporting tissues in human teeth.

Amess *et al.,* 1993 and Andrew *et al.,* (1999) studied the effect of opaque, black rubber dam on the blood flow records obtained from human teeth. They

found that the dam reduced the signal by approximately 80%, indicating that most of that recorded without dam was of non-pulpal origin. It is not known what proportion of the signal obtained with dam is derived from the pulp.

In the present study, this was investigated by recording laser Doppler blood flow signals from the crowns of teeth in adult human subjects, with and without dam, before and after removing the pulps from the teeth.

Materials and methods

Subjects

The experiments were carried out on 22 healthy anterior teeth (upper incisors and canines) in 14 subjects (aged 22 - 40 years, mean 28.3). These teeth were to be root-filled prior to the insertion of a fixed prosthesis. The experiments were carried out in the Main Clinic, Faculty of Dentistry, Mahidol University. The study was approved by the University Ethics Committee and informed consent was obtained from each patient prior to the experiment. All the teeth were caries free and were either intact or had only a small restoration. Radiographic examination and electrical tooth pulp stimulation confirmed that were vital and healthy.

Experimental design and procedure

Series 1 experiments

This series of experiments was carried out on 8 teeth in 5 subjects. Blood flow recordings were made from the teeth with a laser Doppler blood flow monitor (Moor Instruments, Axminster, England; Type MBF3D/42) which uses infra-

red light (wavelength 780 - 820 nm). The probe of the instrument (ext. diam. 1.5 mm) contained two, 0.2 mm diam. optical fibers with their centres separated by 0.5 mm. The probe was fixed to each tooth with a clip-on splint that covered the crown of the test tooth. The splint was constructed from self-curing acrylic resin (semi-transparent, ivory color) on a plaster model of the teeth. The probe tip was inserted into a stainless-steel tube (int. diam. 1.5 mm) which was incorporated into the splint over the central long axis of the crown of the test tooth, perpendicular to the enamel surface and with its centre 2 mm from the gingival margin (Figure 1).

Recordings were made from each tooth under three conditions: (1) the intact tooth, (2) after local anaesthetic (LA) (1 ml, Mepivacaine 3%) had been injected near the apex of the root and a cavity had been cut in the incisal third of the labial surface until the pulp was nearly exposed, and (3) after removal of the pulp with a broach and arrest of bleeding with paper points. At each stage, the blood flow signal was allowed to stabilize for several minutes before measurements were made then the effect on the signal of covering the gingiva and surrounding soft tissue with opaque, black rubber dam (Four D Rubber Co. Ltd., Heanor, England) was determined.

Recordings were made with the normalization (exponent = 2) and linearisation facilities of the Moor instrument enabled (see Vongsavan and Matthews, 1993b). An upper bandwidth setting of 14.9 kHz and a time constant of 0.1 s were used throughout. Data representing blood flow, concentration of moving red cells, velocity of moving blood cells and back-

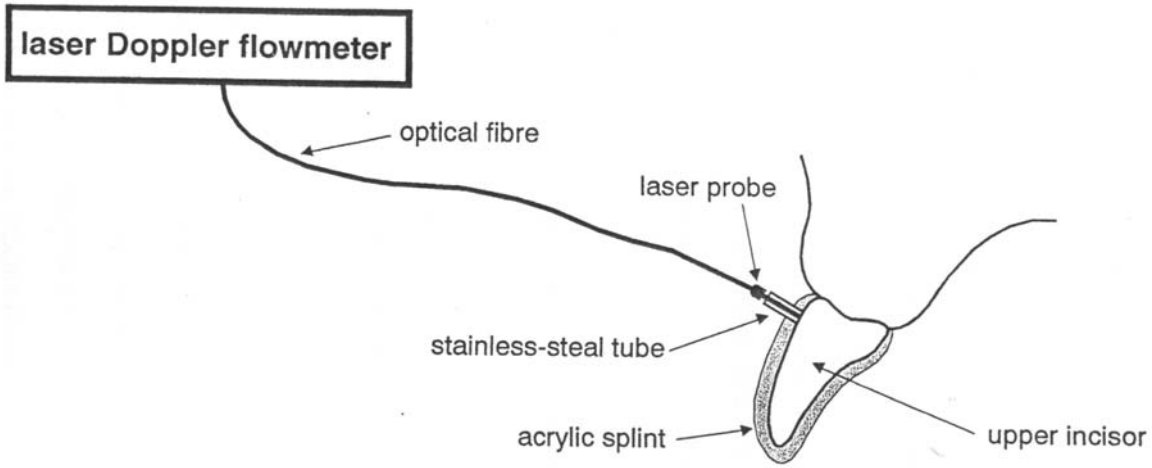

Figure 1.

Diagram of the preparation.

scattered light intensity were collected in digital format from the instrument's serial port with an IBM 486

note book computer. The data were recorded with the Moorsoft (version 4.3) software programme.

After each experiment, records were also made at different light intensities from a stationary reflector. These data were used to calculate the offset of the blood flow signal that would have been present while recorded from the teeth due to noise in the detection system (Vongsavan and Matthews, 1993a). For each set of blood flow values recorded from a tooth during the experiment, the mean and standard deviation were calculated and the offset, determined as described above, was subtracted from the mean.

Series 2 experiments

This series of experiments was carried out on 14 teeth in 9 subjects. The experiments were performed exactly as in Series 1 except that the pulp was replaced in the pulp chamber before the third set of measurements was made. In 8 of the 14 teeth recordings were also made with the pulp chamber empty.

Statistical Analysis

Comparisons between the overall mean blood flow values recorded under each of the different conditions were made using one–way, repeated measures analysis of variance. Where this showed that there were significant differences between the means, the Student-Newman-Keuls test was used to make multiple comparisons between them. P values of less than 0.05 were considered significant. Data are reported as means ± 1 S.D.

Results

Series 1 experiments

Examples of a series of records obtained from one subject are shown in Figure 2.

In eight subjects, the mean blood flow value recorded without rubber dam before any surgical interference was 5.73 ± 1.20 arb. perfusion units (P.U.). When 1 ml of local anaesthetic was injected over the root apex and a cavity cut to almost expose the pulp, this value was significantly reduced to

4.41 ± 1.10 P.U. The mean blood flow value after removal of the pulp and arrest of bleeding, also without dam, was 4.65 ± 1.23 P.U. This value was not significantly different from that obtained immediately before pulp removal, although in 5 of the 8 teeth tested the blood flow signal increased after the pulp had been removed, possibly caused by the trauma of that procedure.

The corresponding values obtained with dam under the same three conditions were 1.49 ± 0.77 P.U., 1.15 ± 0.60 P.U., and 0.31 ± 0.21 P.U. The difference between the mean value from intact teeth and that from the same teeth after local anesthesia and cavity preparation was not significant, but there was a significant difference between the means recorded before and after removal of the pulp. The rubber dam produced a significant reduction in the mean blood flow signal under all three conditions.

These data are summarized in Figure 3.

Series 2 experiments

Examples of records obtained from one subject are shown in Figure 4.

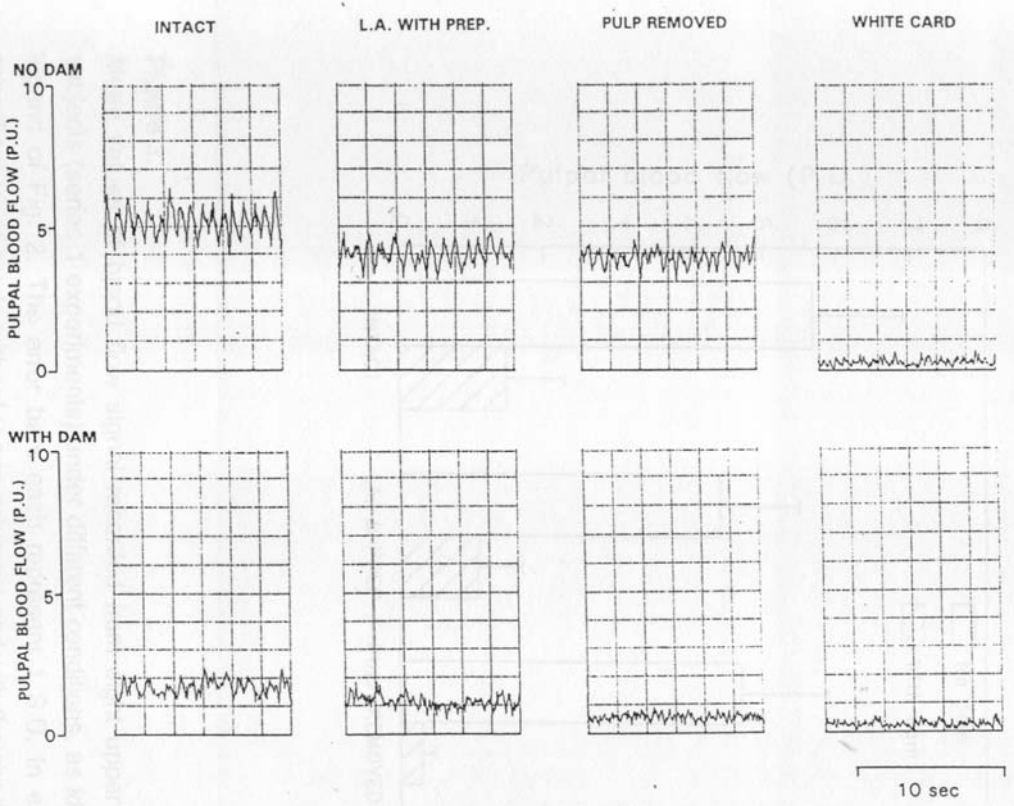

Figure 2.

Laser Doppler records of blood flow obtained from an upper central incisor of a human subject. The lower series of records was obtained with black rubber dam covering the surrounding tissues. Otherwise, the records were obtained under the following conditions: tooth-supporting tissues intact (INTACT), after local anesthesia and cavity preparation (LA. WITH PREP.), and after the pulp had been removed, the bleeding stopped and the pulp chamber left empty (PULP REMOVED). Records were also made at the same light intensities from a stationary reflector (WHITE CARD).

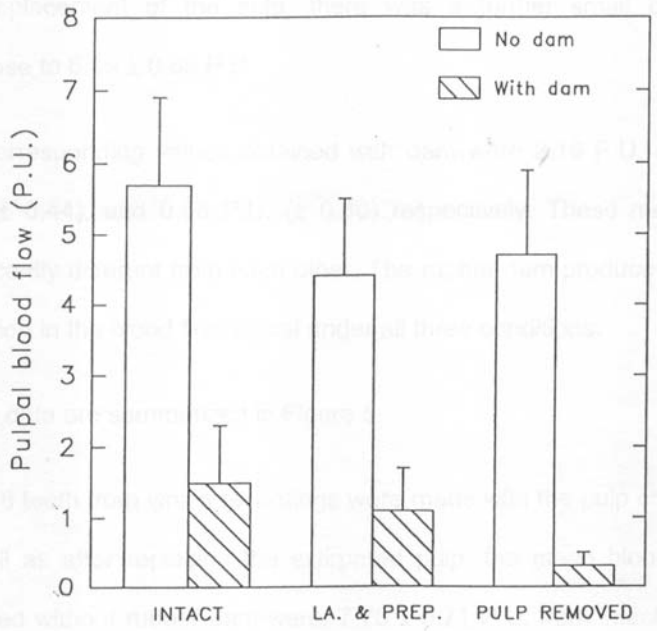

Figure 3.

Mean values of blood flow signal recorded from eight upper incisors in 5 subjects (series 1 experiments) under different conditions, as identified in the legend of Fig. 2. The error bars each represent 1 S.D. In each case the recordings were made without (open columns) and with (hatched columns) the adjacent tissues covered by black rubber dam. In this and all subsequent histograms, the data have been corrected by subtracting the corresponding values recorded at the same light intensity from a stationary reflector.

In 14 subjects, the mean blood flow value recorded initially without rubber dam was 7.71 ± 0.76 P.U. When local anaesthetic was injected over the root apex and a cavity cut to almost expose the pulp, this value decreased significantly to 6.89 ± 0.68 P.U. After removal of the pulp, arrest of bleeding and replacement of the pulp, there was a further small but significant decrease to 6.19 ± 0.65 P.U.

The corresponding values obtained with dam were 2.16 P.U. (± 0.59), 1.73 P.U. (± 0.44), and 0.98 P.U. (± 0.30) respectively. These means were all significantly different from each other. The rubber dam produced a significant reduction in the blood flow signal under all three conditions.

These data are summarized in Figure 5.

In the 8 teeth from which recordings were made with the pulp chamber empty as well as after replacing the extirpated pulp, the mean blood flow values obtained without rubber dam were: 7.78 ± 0.71 P.U. from intact teeth, 6.79 ± 0.42 P.U. after LA and cavity preparation, 6.26 ± 0.55 P.U. after pulp extirpation and arrest of hemorrhage, and 6.26 ± 0.52 P.U. when the pulp was replaced. The mean value recorded after cutting the cavity was significantly greater than both that recorded when the pulp cavity was left empty and that after the pulp had been replaced. After extirpation of the pulp and the arrest of hemorrhage, replacing the pulp in the pulp cavity had no effect on the mean blood flow recording.

With rubber dam, the corresponding values were 2.13 ± 0.66 P.U., 1.75 ± 0.47 P.U., 0.98 ± 0.36 P.U., and 0.39 ± 0.18 P.U. These values are all significantly different from each other. The rubber dam produced a

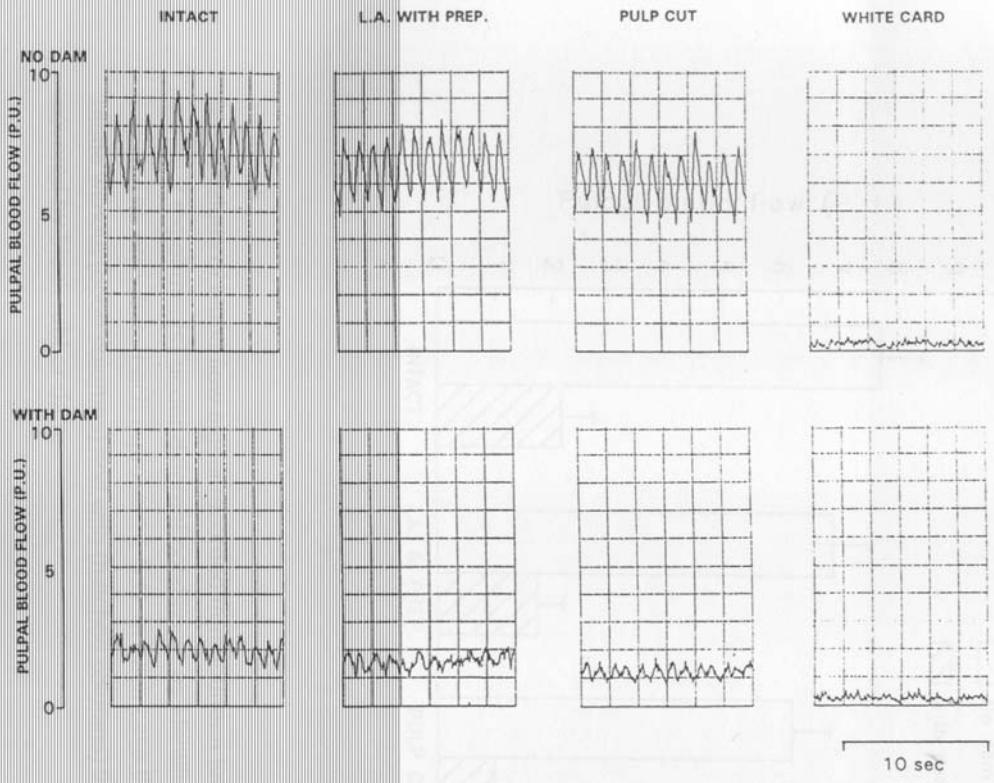

Figure 4.

Laser Doppler records of blood flow obtained from one of the subjects in the second series of experiments. The lower series of records was obtained with black rubber dam covering the surrounding tissues. Otherwise, the records were obtained under the following conditions: tooth-supporting tissues intact (INTACT), after local anesthesia and cavity preparation (LA. WITH PREP.), and after the pulp had been removed, the bleeding stopped and the pulp replaced in the pulp chamber (PULP CUT). Records were also made at the same light intensities from a stationary reflector (WHITE CARD).

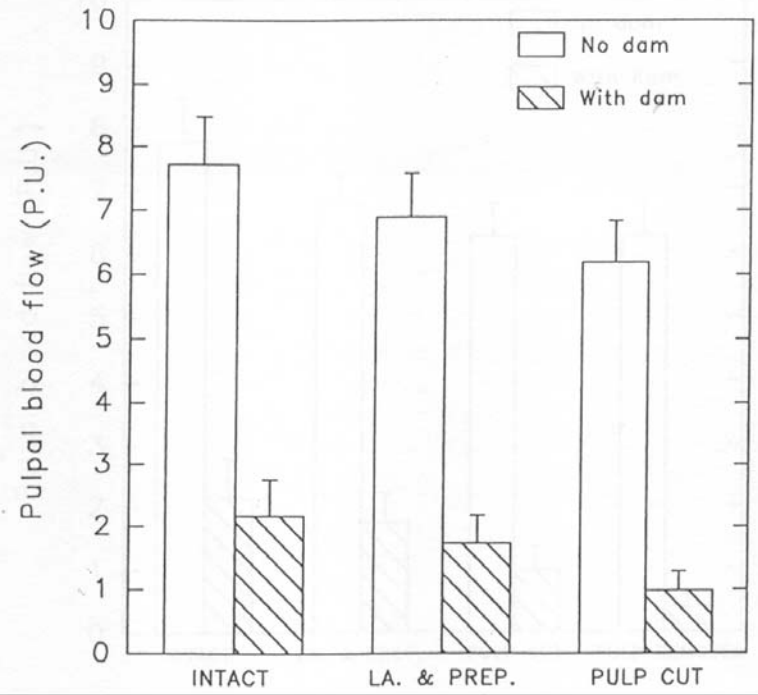

Figure 5.

Mean values of blood flow signal recorded from fourteen teeth in 11 subjects (series 2 experiments) under different conditions, as identified in the legend of Fig. 4. The error bars each represent 1 S.D. In each case the recordings were made without (open columns) and with (hatched columns) the adjacent tissues covered by black rubber dam.

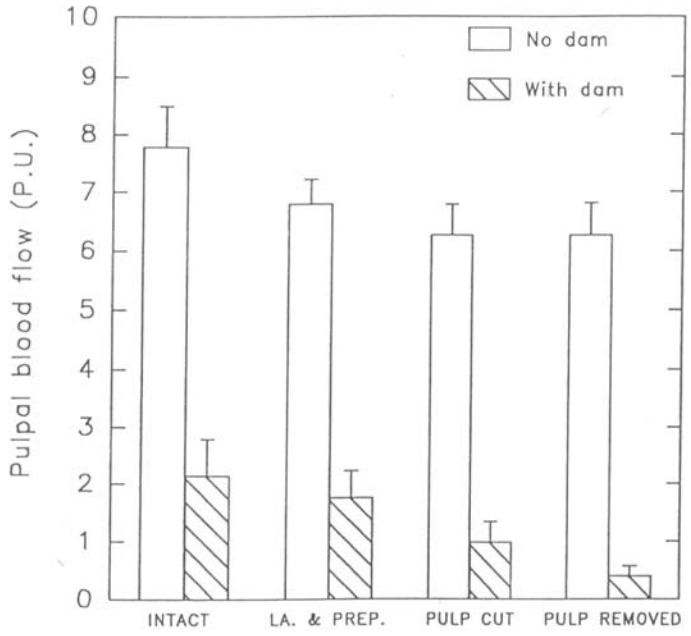

Figure 6.

Mean values of blood flow signal recorded from eight teeth in 5 subjects (series 2 experiments) under different conditions, as identified in the legends of Figs. 2 & 4. The error bars each represent 1 S.D. In each case the recordings were made without (open columns) and with (hatched columns) the adjacent tissues covered by black rubber dam.

significance reduction to the mean blood flow signal under all conditions in this series of experiments.

The data are shown in Figure 6 and examples of records obtained from one subject are shown in Figure 7.

Data from Series 1 and 2 experiments

Combining the data from all 22 teeth, the rubber dam reduced the average blood flow signal recorded from intact teeth by 73%, from 6.98 ± 1.30 P.U. to 1.92 P.U. ± 1.50, which is significant.

When the data from the 16 teeth from both series in which recordings were made with the pulp cavity empty were combined, the mean value after cavity preparation without dam (5.6 ± 1.47 P.U.) was not significantly different from the corresponding figure after removal of the pulp (5.5 ± 1.2 P.U.). With dam however, the change in the mean values was significant, from 1.45 ± 0.61 P.U. to 0.35 ± 0.19 P.U.

Discussion

The results of the present experiments confirm earlier findings (Amess *et al.,* 1993; Hartmann *et al.*, 1996; Andrew *et al.,* 1999) that the signal recorded from an intact tooth in an adult human subject is reduced by up to 80% when the gingiva and surrounding tissues are covered with black rubber dam. This reduction can be accounted for by two effects of the dam: screening of light from periodontal, gingival and other surrounding tissues, and a reduction in gingival blood flow due to compression of the gingival tissue. Both will have reduced the amount of light picked up by the probe that had been scattered

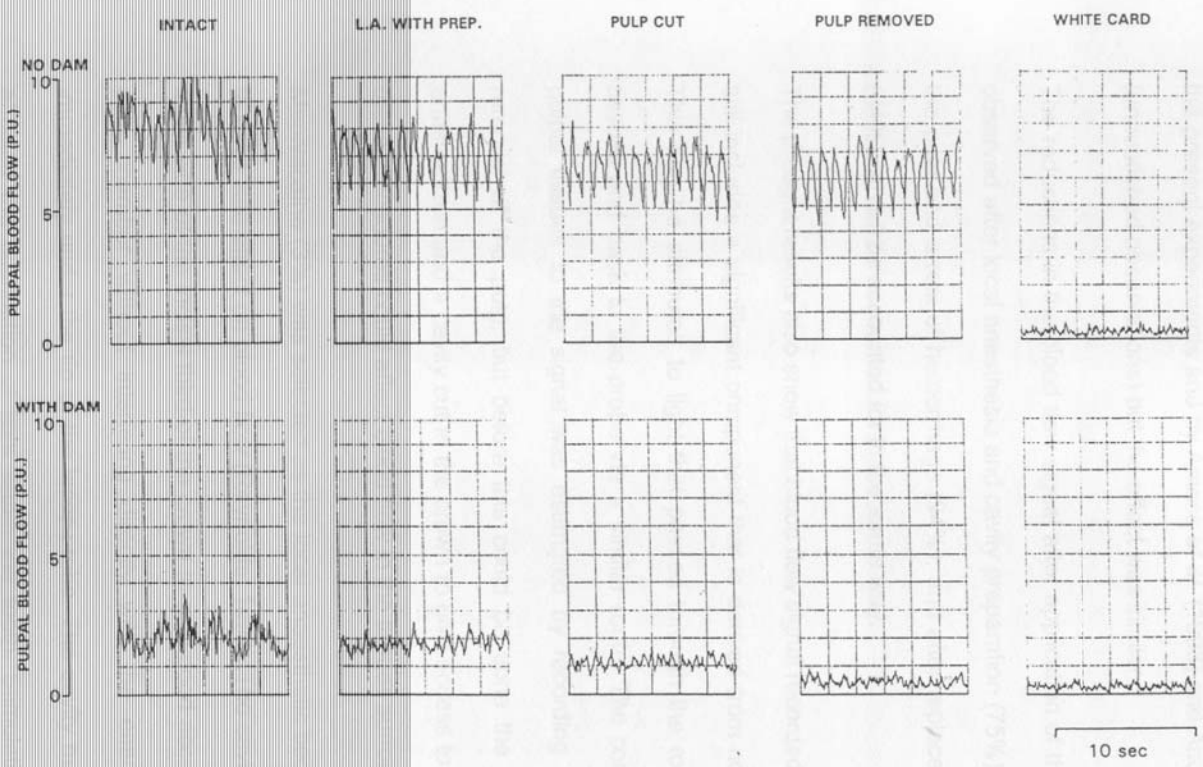

Figure 7.

Laser Doppler records of blood flow obtained from another of the subjects in the second series of experiments. The lower series of records was obtained with black rubber dam covering the surrounding tissues. Otherwise, the records were obtained under the following conditions: tooth-supporting tissues intact (INTACT), after local anesthesia and cavity preparation (LA. WITH PREP.), after the pulp had been removed, the bleeding stopped and the pulp replaced in the pulp chamber (PULP CUT) and with the pulp chamber empty (PULP REMOVED). Records were also made at the same light intensities from a stationary reflector (WHITE CARD).

by blood flowing in non-pulpal tissues. The heavy gauge, Hygenic dam used by Hartmann *et al*. (1996) is more transparent than the Four D dam used in the present experiments and by Amess *et al.* (1993) and Andrew *et al. (*1999) (unpublished observations) but the effect was similar.

The reductions in the blood flow signal after application of the dam that were observed after local anesthesia and cavity preparation (75%), after removal of the pulp and arrest of hemorrhage (94%), and after replacement of the pulp (84%) can all be accounted for in the same way.

The present results also show that blood flow signal recorded with rubber dam still includes a significant component that is derived from non-pulpal tissues. This can be attributed to light that passed through the root to periodontal tissues and back to the probe via a similar route. The contribution of non-pulpal tissues to the signal was estimated by recording before and after removal of the pulp, but before this could be done the tooth had to be anesthetized and a cavity cut in the crown to give access to the pulp. Both of these procedures could affect blood flow in the tissues surrounding the tooth.

Mepivacaine (3%) was selected as the local anaesthetic because it appeared to have little effect on blood flow (Reynolds, 1996). The cavity was cut on the labial side of the incisal third of the crown to give good access to the coronal pulp chamber without interfering directly with the enamel and dentine immediately under the tip of the laser probe. The cavity may however have affected the distribution of light within the tooth. Stimulation of intradental afferent nerves during the cavity preparation may have caused changes in blood flow in tissues around the tooth by an axon reflex mechanism. We

found that the effect of local anesthesia and cavity preparation was to reduce the blood flow signal, both with and without dam. In the pooled data from both series of experiments, the reduction was 14% without dam and 20% with dam. These results indicate that mepivacaine causes vasoconstriction. In other experiments, the injection of a similar volume of normal saline or distilled water over the root apex caused no change in the laser Doppler signal (unpublished observations).

In the first series of experiments, the contribution of pulpal blood flow to the signal recorded from the tooth with dam was estimated by recording before and after removing the pulp, leaving the pulp chamber empty. The results indicated that, on average, 73% of the signal recorded after cavity preparation was from the pulp. It was thought that there might be an error in this estimate because the empty pulp chamber may have affected the amount of light transmitted to tissues outside the tooth. For this reason, in the second series of experiments recordings were made with the pulp replaced in the pulp chamber to reproduce as closely as possible the optical properties of the root of the intact tooth. These data indicate that 43% of the signal recorded after cavity preparation was from the pulp.

In the eight teeth of the second series in which recordings were made both with the pulp replaced and with the pulp chamber empty, the average results were similar to those discussed above. The blood flow signal was significantly greater with the pulp replaced than with the pulp chamber empty. The fact that the signal was greater with the pulp was replaced was probably due mainly to the pulp causing more light to be scattered to tissues outside the tooth. This