รายงานวิจัยฉบับสมบูรณ์

โครงการวิจัยเรื่องการศึกษาคุณสมบัติของใยประสาทในฟันมนุษย์และการศึกษาทางคลินิกผลการใช้วิธีไอออนโตโฟรีซีสให้เนื้อเยื่อในโพรงฟันชา

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คณะทันตแพทยศาสตร์ มหาวิทยาลัยมหิดล

มิถุนายน 2563

สัญญาเลขที่ BRG5980007

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โครงการวิจัยเรื่องการศึกษาคุณสมบัติของใยประสาทในฟันมนุษย์และการศึกษาทางคลินิกผลการใช้วิธีไอออนโตโฟรีซีสให้เนื้อเยื่อในโพรงฟันชา

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รายงานการเงิน

โครงการวิจัยเรื่องการศึกษาคุณสมบัติของใยประสาทในฟันมนุษย์และการศึกษาทางคลินิกผลการใช้วิธีไอออนโตโฟรีซีสให้เนื้อเยื่อในโพรงฟันชา

สัญญาเลขที่ BRG5980007

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สิงหาคม 2563

รายงานโครงการวิจัยฉบับสมบูรณ์

ทุนองความรู้ใหม่ที่เป็นพื้นฐานต่อการพัฒนา

วันที่ 15 มิถุนายน 2559 ถึงวันที่ 14 กันยายน 2562

โครงการวิจัยเรื่องการศึกษาคุณสมบัติของใยประสาทในฟันมนุษย์และการศึกษาทางคลินิกผลการใช้วิธีไอออนโตโฟรีซีสให้เนื้อเยื่อในโพรงฟันชา

BRG5980007

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บทคัดย่อ

รหัสโครงการ: BRG5980007

ชื่อโครงการ:การศึกษาคุณสมบัติของใยประสาทในฟันมนุษย์และการศึกษาทางคลินิก ผลการใช้วิธีไอออนโตโฟรีซีสให้เนื้อเยื่อในโพรงฟันชา

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การศึกษาวิจัยในการศึกษาที่หนึ่งคณะผู้วิจัยประสบผลสำเร็จในการการพัฒนาเทคนิดเพื่อวัดสัญญานประสาทจากเนื้อฟันในมนุษย์ได้เป็นครั้งแรก การศึกษาวิจัยนี้คณะผู้วิจัยได้ศึกษาสัญญานประสาทจากเนื้อฟันในมนุษย์จากตัวกระตุ้นเช่น การเขี่ยไปบนผิวเนื้อฟันที่เผยผึ่ง หรือใช้แรงดันน้ำที่มากกว่าหรือน้อยกว่าบรรยากาศ พบว่าสัญญานประสาทจากเนื้อฟันในมนุษย์จะตอบสนองมากในกรณีของเนื้อฟันที่เผยผึ่งที่ได้กรดกัด และแรงดันน้ำที่น้อยกว่าบรรยากาศ การศึกษาวิจัยในการศึกษาที่สอง คณะผู้วิจัยประสบผลสำเร็จในการหาสัดส่วน ของสัญญานเลเซอร์ ดอปเปลอร์เมื่อนำส่วนของโพรบมาบนพื้นผิงของเนื้อฟันที่เผยผึ่ง โดยพบว่า 68 % ของสัญญานดังกล่าวเป็นสัญญานของส่วนปริมาณเลือดที่มาเลี้ยงเนื้อเยื่อในโพรงฟัน การศึกษาวิจัยในการศึกษาที่สาม คณะผู้วิจัยประสบผลสำเร็จในการการค้นพบปรากฏการณ์ อีเลคโตรออสโมซีสในฟันมนุษย์ได้เป็นครั้งแรก โดยพบว่าระหว่างการไอออนโตโฟรีซีส ยาชาลิโดเคน 2 เปอร์เซ็นต์ ถ้าใช้ขั้วบวกบนเนื้อฟันที่เผยผึ่งจะทำให้เกิดการไหลของของเหลวสู่ภายในโพรงฟัน แต่ถ้าใช้ขั้วลบจะทำให้ของเหลวไหลออกจากโพรงฟัน การศึกษาวิจัยในการศึกษาที่สี่ หา และหก คณะผู้วิจัยประสบผลสำเร็จในการใช้วิธีไอออนโตโฟรีซีส ยาชา 10%อาติเคน ผสมกับ อิพินิฟฟรีน 1:,1,000 แทนยาชาลิกโนเคน 20% ผสมกับอิพินิฟฟรีน 1:,1,000 เนื่องจากอาติเคนจะสามารถบลอกหรือสกัดกั้นเตตราโดทอกซิน รีซีสแตนโซเดียมชันแนลได้ดีกว่า ซึ่งประสบผลสำเร็จในการเนื้อเยื่อในโพรงฟันชาในฟันกรามที่ผุถึง 100 เปอร์เซ็นต์ ในการศึกษาที่สี่ ทดลองในในฟันกรามน้อยที่จะถอนเพื่อจัดฟัน จำนวน 22 ซี่ ใช้วิธีไอออนโตโฟรีซีส ยาชา 10%อาติเคน ผสมกับ อิพินิฟฟรีน 1:,1,000 ใช้กระแส 120 ไมโครแอมป์ เวลา 90 วินาที พบว่าทำให้เนื้อเยื่อในโพรงฟันชากว่า 30 นาทีในฟันทุกซี่ที่ทดลอง ในการศึกษาที่ห้า ทดลองในในฟันกรามที่ผุ จำนวน 28 ซี่ วิธีไอออนโตโฟรีซีสใช้กระแส 200 ไมโครแอมป์ เวลา 2-3 นาที พบว่าทำให้เนื้อเยื่อในโพรงฟันชาทุกซี่สามารถกรอฟันและอุดฟันได้สำเร็จโดยผู้ป่วยไม่มีความรู้สึกเจ็บหรือเสียวฟันเลย วัดปริมาณเลือดที่มาเลี้ยงฟันใช้วิธีวัดด้วยเครื่องเลเซอร์ ดอปเปลอร์โฟลมีเตอร์ ก่อนและหลังจากอุดฟันไป 6 เดือน พบว่ามีปริมาณเลือดที่มาเลี้ยงไกล้เคียงกัน ในการศึกษาที่หก ทดลองในในฟันกรามที่ผุ จำนวน 18 ซี่ % วิธีไอออนโตโฟรีซีสใช้กระแส 200 ไมโครแอมป์ เวลา 2-3 นาที พบว่าทำให้เนื้อเยื่อในโพรงฟันชาทุกซี่สามารถกรอฟันและอุดฟันได้สำเร็จโดยผู้ป่วยไม่มีความรู้สึกเจ็บหรือเสียวฟันเลย ประเมินการมีชีวิตของเนื้อเยื่อในโพรงฟันด้วยเครื่องระตุ้นไฟฟ้า อาการทางคลินิก และภาพรังสี ก่อนและหลังจากอุดฟันไป 6 เดือน 12 เดือนและ 18 เดือนพบว่าเนื้อเยื่อในโพรงฟันมีชีวิตและเป็นปกติ วิธีไอออนโตโฟรีซีสใช้ขั้วบวกบนผิวของเนื้อฟันที่เผยผึ่งจึงไม่น่าที่จะผลักเชื้อแบคทีเรีย

ลงไปสู่เนื้อเยื่อในโพรงฟัน เนื่องจากเชื้อแบคทีเรียมีประจุเป็นลบ โดยสรุปวิธีไอออนโตโฟรีซีสยาชา 10%อาติเคน ผสมกับ อิพินิฟฟรีน 1:,1,000 สามารถทำให้เนื้อเยื่อในโพรงฟันทีผุชาได้และสามารถกรอฟันและอุดฟันได้สำเร็จ โดยไม่ต้องฉีดยาชา เทคนิคนี้มีความปลอดภัยต่อเนื้อเยื่อในโพรงฟันจากการประเมินทางคลินิกถึง 18 เดือน

คำสำคัญ: อาการเจ็บปวด ภาวะเสียวฟัน สัญญานประสาท วิธีไอออนโตโฟรีซีส อีเลคโตรออสโมซีส ยาชา ฟันมนุษย์ที่มีโรคฟันผุ

Abstract

Project Code: BRG5980007

Project Title: Investigation on the properties of intra-dental nerves and clinical evaluation on the use of iontophoretic delivery of local anesthetic drugs through dentine to obtain pulpal anesthesia in man

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Project Peroid: 15th June 2016- 14th September 2019

The first series of experiments, the technique for recording nerve discharges from human dentine was successfully developed (8 teeth in 5 subjects). The action potentials were recorded from various type of stimuli egs. mechanical, positive and negative hydrostatic pressure. The responses were greater from etched than unetched dentine, and negative pressures evoked greater responses than the corresponding positive pressures. The secondt series of experiments, these experiments we validated the used of laser Doppler flow meter for pulpal blood flow recording when the probe was placed on the exposed dentine surface. It was demonstrated that when recording blood flow from exposed coronal dentine with either infrared or red light in a tooth isolated with opaque rubber dam, about 68% to the signal was contributed by the pulp. the series III experiments, we could demonstrate the physical phenomena called “Electroosmosis in human dentine” for the first time. Electroosmosis was an fluid flow through the dentine when iontophoresis or a D.C. current was passed between the dentine surface and the pulp. During iontophoresis of 2% lignocaine with epinephrine through dentine with a smear layer, currents of 0.2, 0.4, and 0.6mA applied with the cavity electrode as anode produced inward flow rates of 2.25±0.87, 5.00±1.62, 8.60±1.97 (mean ± s.d.) nL/s/mm² respectively, and applying the currents in the opposite direction caused outward flows of 0.76±0.72, 1.00±1.01, 1.12±1.18nL/s/mm² respectively. In series IV, V and VI experiments, the solution of 10% articaine with epinephrine 1:1,000 was used instead of lignocaine with epinephrine 1:1,000 as previous works because it superior to block tetrodotoxin resistant sodium channel. In series IV experiments, The experiments were carried out on 22 non-carious premolars in 6 subjects. These teeth were scheduled to be extracted for orthodontic purposes. It was shown in all teeth, iontophoresis of 10% articaine with 1:1000 epinephrine through exposed etched dentine on pulpal anesthesia lasts up to 30 minutes. In series V experiments, the experiments were carried out on 28 carious molar and premolar teeth in 17 healthy subjects. The objective of this study was to compare the vitality of pulp in teeth that anesthetized with iontophoresis of 10% articaine with 1:1,000 epinephrine and standard injection technique with 4% articaine with 1:100,000 epinephrine in 6 months. The vitality of pulp was assessed electric pulp test.and pulpal blood flow recording with a laser Doppler flowmeter. It was shown that both teeth anesthetized with iontophoresis of 10% articaine with 1:1000 epinephrine through carious dentine and standard local anesthesia technique of 4% articaine with 1:100,000 epinephrine presented the vital and healthy pulp. In series VI experiments, The experiments were carried out on 18 carious premolars or molars in 9 subjects. The objective of this study was to evaluate the use of iontophoretic delivery the solution of 10% articaine with epinephrine 1: 1,000 through exposed carious dentine to anaesthetized dentine before cavity preparation and filling. Pulpal blood flow was recorded before and after iontophoresis. Radiographic examination, electrical pulp test and clinical assessment were performed up to 18 months. It was shown that iontophoretic delivery of 10% articaine with 1:1000 epinephrine through carious

dentine could be used to anesthetize dentine prior to cavity preparation and filling and caused no adverse effects during 18 months follow up.

Key Words: Pain, Dentine Sensitivity, Nerve discharges, Iontophoresis, Electroosmosis, Local anesthetic, Carious human teeth

Research project

Investigation on the properties of intra-dental nerves and clinical evaluation on the use of iontophoretic delivery of local anesthetic drugs through dentine to obtain pulpal anesthesia in man
Project no. BRG5980007

This project was composed of six series of experiments. In series I experiments entitled “Action potentials recorded from dentine in man” These series of experiments obtained from the grant proposal section 1: Investigation on the properties of intra-dental nerves in man. This was the first that the nerve discharges could be recorded from dentine in man. These series of experiments were very complicated and had to use very sensitive equipments that kindly help by Professor Bruce Matthews, Emeritus professor, University of Bristol United Kingdom. He was the reconized distinguish scientist from the International Association for Dental Research.

In series II experiments entitled “Pulpal blood flow recorded from exposed dentine with a laser Doppler flow meter using red or infrared light”. These experiments we validated the used of laser Doppler flow meter for pulpal blood flow recording when the probe was placed on the exposed dentine surface. The paper was published in *Archives of Oral Biology* (Impact factor = 1.663, From Journal Citation Reports®, 2018). Then in the series III experiments, we could demonstrate the physical phenomena called “Electroosmosis in human dentine for the first time. The paper was submitted to *Archives of Oral Biology*. This paper was primarily accepted but need some revision. In series IV experiments entitled “Effect of iontophoresis of articaine with epinephrine into exposed dentine on pulpal blood flow and the sensitivity of dentine in man”. Series V experiments entitled “A comparison on the pulp vitality of the teeth anaesthetized with iontophoresis of 10% articaine with epinephrine 1:1,000through carious dentine versus standard local anesthesia technique”. Series VI experiments entitled “Evaluation of teeth that use the iontophoretic delivery of 10% articaine with 1:1,000 epinephrine through carious dentine for pain control in operative dentistry in man”. These three series of experiments obtained from the grant proposal section 2: “Clinical evaluation on the use of iontophoretic delivery of local anesthetic drugs through dentine to obtain pulpal anesthesia in man”. We demonstrated that pulpal anesthesia in man could be obtained by using iontophoretic delivery of local anesthetic drugs through dentine. This was the first that the pulpal nerve was anesthetized without needle. The technique was proofed that could be used in carious

teeth and the follow up period was up to 18 months without any harmful effect to the dental pulp. It was safe and do not push the bacteria into the dental pulp because most of bacteria have the negative net surface charge. The electrode that used on the exposed carious dentine surface was anode.

Series I experiments

Action potentials recorded from dentine in man

Abstract

In experimental animals, action potentials can be recorded from the terminals of intradental nerves by simply placing a relatively gross electrode in contact with the overlying dentine. Objectives: To obtain such records from human teeth. Methods: The experiments were carried out on premolar teeth scheduled for extraction in young adults. A 3.5mm deep, 0.76mm diam. cavity was cut, without anaesthesia, towards the pulp horn at the tip of each of 2 cusps. A slow-speed, pin drill was used with water coolant to cut dentine. Each cavity was etched with acid and dried then filled with fissure sealant. A pointed, 0.3 mm diameter wire was pushed against the cavity floor while the resin cured, then the wire was removed and the void filled with saline. A silver wire was inserted into the saline in each cavity and simultaneous recordings made between each wire and a reference electrode in the gingival crevice. A class I cavity was cut just into dentine for the application of test stimuli. Results: Successful recordings were made from 4 teeth in 3 subjects. No spontaneous discharge was recorded in the absence of stimulation or during mechanical stimulation of the class I cavity prior to etching the exposed dentine. After etching, responses were recorded during mechanical stimulation and during drying. The action potentials had amplitudes of up to 50µV. Responses of some sensory units were recorded at both electrodes, but with different waveforms, and others were present at only one. No responses could be recorded when larger areas of dentine were exposed for recording. Alternative methods of bonding the resin to the exposed dentine are under investigation, and of measuring the effective area of electrode contact. Conclusion: Single unit action potentials can be recorded from dentine in man if the area of dentine exposed for recording is small.

Introduction

In experimental animals, action potentials can be recorded from the terminals of intra-dental nerves by simply placing a relatively gross electrode in contact with the overlying dentine. In the cats, dentine was exposed by cutting off the tip of the cusp. Stimuli were applied to the same area of dentine as that from which the recordings were made. The success of this technique appears to depend upon the dentinal tubules acting as biological microelectrodes, detecting the action potentials in nerve terminals located in, or close to, the pulpal ends of the tubules (Scott DJr, and Tempel,1965; Horiuchi and Matthews, 1974; Vongsavan and Matthews, 2007). It is not possible to record in a similar way from nerve terminals in any other tissue in vertebrates, apart from cornea (Carr et al., 2009). Similar recordings could be obtained from dentine in dog (Horiuchi and Matthews) but despite several attempts, it has not been able to do this in human teeth.

The present experiments were carried out to to develop the technique for recording the action potentials from dentine in human subjects.

Materials and methods

Subjects

The experiments were carried out on 8 healthy, premolar teeth in 5 subjects (ages: 20-25 years). All the teeth were scheduled to be extracted for orthodontic reasons. Radiographic and clinical examinations confirmed that the teeth were fully erupted, vital, and free of caries. One tooth had a very small, class II restoration.

The experiments were carried out in the Endodontics Department of the Faculty of Dentistry, Mahidol University in the Maha Chakri Sirithon Dental Hospital at the Salaya Campus of the University. The study was approved by the Ethics Committee on the Use of Human Rights Related to Human Experimentation of the Mahidol University and complied with the principles of the Declaration of Helsinki. The experiment procedures were clearly explained to each subject and their written consent to these procedures being carried out was obtained. The subject could terminate the experiment at any stage. The privacy rights of the subjects were observed at all times.

Dentine recording

3.5 mm deep cavity was cut, without anaesthesia, towards the pulp horn at the tip of each of 2 cusps (Fig. 1). The enamel was penetrated with a diamond bur in an air-rotor

hand-piece, then a slow-speed, 0.76 mm diam. pin drill was used, with water coolant, to extend the cavity into dentine. Each cavity was etched with acid, dried with paper points, then filled with self-curing fissure sealant. A pointed, 0.3 mm diameter wire was pushed against the cavity floor while the resin cured, then the wire was removed and the void filled with saline. A silver wire was inserted into the saline and the opening of the cavity was sealed with silicone rubber. A class I cavity was cut just into dentine for the application of test stimuli (Fig. 1). Recordings were made in one of two ways: either differentially between the two recording cavities, or between each cavity and a common reference electrode in the gingival crevice

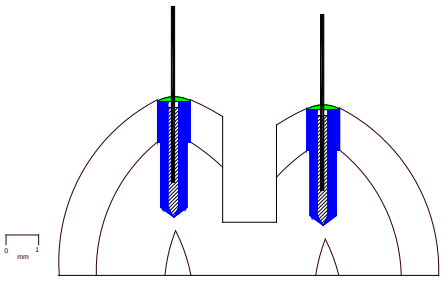

Fig. 1.. Diagram of the dentine recording electrodes and a class I cavity for stimulation.

Dentine stimulation and dentine sensitivity assessment

Each cavity was tested with 5 s stimuli of ±400 mm Hg. If either the positive or negative pressure stimulus caused pain, stimuli of ±100, ±200, and ±300 mm Hg were applied in random order, with 2 min. between the stimulus sequences. This procedure was repeated after etching the cavity floor with 35% ortho-phosphoric acid for 30 s. The acid was applied with a fine cannula and removed by rinsing the cavity with Ringer's solution applied similarly. If at any level, stimuli of ±400 mm Hg produced no pain, it was assumed that the thresholds to both positive and negative pressure stimuli were greater than this and that less intense stimuli would have produced no pain.

After each stimulus, the subject indicated the maximum intensity of any pain produced by placing a mark on a simple visual analogue scale (VAS) calibrated from 0 (no sensation) to 100 mm (the most severe pain one can imagine) (Holland et al., 1997). As well as recoding the maximum intensity of any pain felt in the form of a VAS score in this way, the subject provided a continuous record of changes in the intensity of any pain during the stimulus by squeezing appropriately a wrist-exerciser that was equipped with strain-gauges.

Results

Successful recordings were made from 7 teeth in 5 subjects. No spontaneous discharge was recorded in the absence of stimulation or during mechanical stimulation of the class I cavity prior to etching the exposed dentine. After etching, responses were recorded during mechanical stimulation and during drying. The action potentials had amplitudes of up to 50µV.

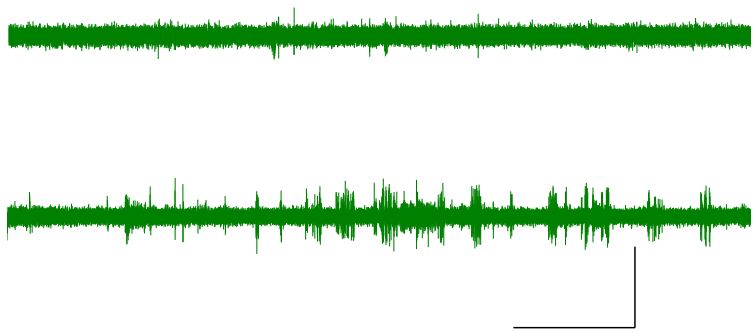

Fig. 2. shows the first successful recording. A differential recording was made between the buccal and palatal cavities during mechanical stimulation of exposed dentine in a separate class I cavity, before and after etching the cavity.

Responses of some sensory units were recorded at both electrodes but with different waveforms, and others were present at only one (Figs. 3 & 4). These responses were recorded during mechanical stimulation of etched dentine. Simultaneous, independent records were obtained from the buccal and palatal cavities. The subject reported pain only during the first part of the records in Fig. 3.

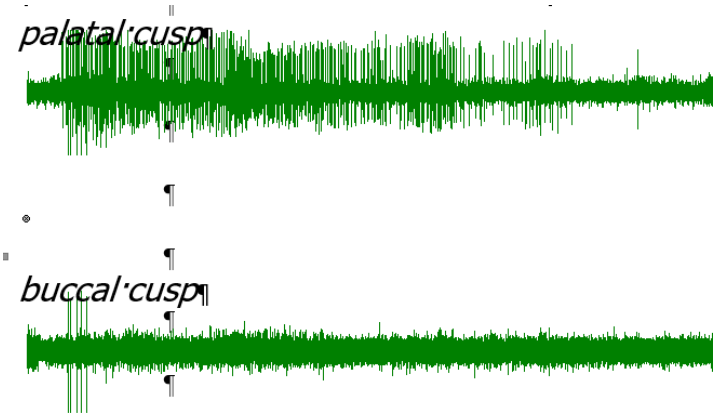

Fig. 3. A differential recording was made between the palatal cavities and buccal during mechanical stimulation of exposed dentine in a separate class I cavity, before and after etching the cavity. The stimulus caused pain after etching.

Unetched Dentine and Etched Dentine

Stimulation of unetched dentine produced very few responses. Four of the 8 test cavities did not respond to any of the stimuli. After etching the dentine, more cavities responded to stimulation The responses to the +400 mm Hg stimulus also tended to increase as the cavity was deepened, The example of the record was shown in Fig. 4.

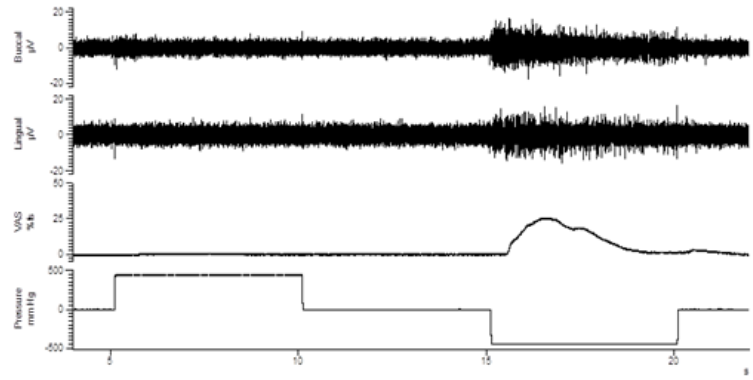

Fig. 4. Recordings of responses to 5 s, +400 and -400 mm Hg stimuli. In each panel, the top record is from the electrode in the test (buccal) cavity, the second record is that in the control (distal) cavity, the third trace is the output of a device by which the subject indicated the intensity of any pain felt (VAS), and the fourth trace is a record of the stimulus. In the upper 2 records, the bandpass of the amplifier was 100 Hz – 1 kHz in both cases.

Discussion

We demonstrated that positive pressure stimuli, and etching the dentine increased its sensitivity to both forms of stimulus and the sensitivity increased progressively as the cavity was deepened. Also, as was found in another study (Charoenlarp *et al.*, 2007) the dentine was more sensitive to negative than to positive pressure stimuli, and etching the dentine increased its sensitivity to both forms of stimulus. These trends were present at all levels of the dentine. The recordings from dentine indicate that the properties of the sensory receptors that respond to hydrostatic pressure stimuli in human teeth are similar to those in cat teeth (Vongsavan and Matthews, 2007).

Our results are consistent with the hydrodynamic mechanism of dentine sensitivity, in which action potentials are generated in nerve terminals in the inner dentine or superficial pulp as a result of movement of the contents of the dentinal tubules. The effects of cavity depth and of etching, on the sensitivity of the dentine to hydrostatic pressure stimulation can be explained by the effects that these procedures would have had on the hydraulic conductance of the dentine.

It would be possible to investigate the effect of cavity depth on the sensitivity of dentine to mechanical stimuli in man by repeating the present experiments but with probing or drilling as the test stimulus. Drilling has been used to investigate topical anaesthesia of dentine under caries (Smitayothin *et al*., 2015).

It is not known why impulses were not recorded from the test cavity in two of the teeth in the present study: it may have been that the cavity did not communicate with tubules that either contained nerve terminals or terminated close to nerve terminals in the pulp horn. When we have deliberately placed a recording cavity in the cervical region of a tooth, where there are likely to be fewer nerve terminals (Byers and Dong, 1983; Holland *et al*., 1987), we have never recorded action potentials.

In three teeth, action potentials were recorded from the control cavity during stimulation of the test cavity. This pattern of response can be explained by axons branching and having terminals in the pulp horns of both cusp, so that some action potentials generated under the test cavity would produce secondary action potentials in other branches that would be propagated antidromically into the pulp horn under the control cavity. In other experiments (Ajcharanukul *et al*., 2006), we did not record from the area of exposed dentine to which stimuli were applied, but used simple recording electrodes that did not incorporate a method of applying stimuli, and we relied upon branching of the nerve fibres to enable action potentials to be recorded from one area of dentine that had been generated in another. A disadvantage of this method compared with that used in the present experiments is that a smaller proportion of the action potentials generated are likely to be recorded.

The electrodes we used were selected after preliminary experiments in which we investigated many different designs. The best recordings were obtained from cavities that were as small as possible in diameter, extended deep into the dentine, and exposed tubules that terminated over the pulp horn, where a high proportion contain nerve terminals (Byers and Dong, 1983; Holland *et al*., 1987). Also, as noted above, the probability of recording

action potentials was greatest with electrodes that incorporated a method of applying stimuli to the tubules from which the recordings were made. These findings, together with evidence obtained in cats by recording orthodromic and antidromic action potentials from single pulpal nerve fibres (Horiuchi and Matthews, 1974) and the effects on the antidromic responses of sectioning either the pulp horn (Horiuchi and Matthews, 1974) or the pulpal nerves outside the tooth (Holland *et al*., 1987), indicate that the action potentials that can be recorded from dentine originate from nerve terminals that are located in the exposed tubules and the superficial pulp close to the ends of those tubules.

In other trials to find a better way of recording from human dentine, we recorded from an array of 5, 0.2 mm diameter electrodes that were formed in the floor of a 2 mm diameter cavity. The aim was to improve the identification of single units by making multiple, closely-spaced recordings for analysis with spike-sorting software (Buzsaki, 2004). We also thought the array would increase the chances of at least one electrode making contact with tubules that terminated over the pulp horn. But the method was unsuccessful: no impulses were recorded. It seems that exposing the larger area of dentine resulted in impulses being blocked for some reason; although the subjects still felt pain when the dentine was stimulated.

It is not possible to record nerve action potentials from the surface of any other tissue with relatively large electrodes such as were employed in the present experiments. They can be recorded from the surface of the cornea (Carr, *et al*., 2009) but this requires micro-electrodes. In dentine, the tubules exposed in the floor of a cavity appear to function as a bundle of parallel, biological microelectrodes in series with the much larger electrode in the cavity. The potentials recorded from the cavity floor, 2 mm or more from the nerve terminals, must be produced by extracellular currents flowing through the tubules during the propagation of impulses in the nerves.

The action potentials we recorded from human premolars were much smaller than those recorded with similar techniques from cat canines (Horiuchi and Matthews, 1974; Vongsavan and Matthews, 2007). The reason for this is probably that the pulp horn is much more slender in a cat canine than in a human premolar. As a result, the resistance of the tissues around the nerve terminals is higher in the cat teeth and the extracellular current densities associated with the propagation of action potentials in the terminals, correspondingly larger. Even larger action potentials, up to 400 µV, have been recorded

from the cusps of rat molars (Matthews et al., 2008), and in these teeth the pulp horns are even more slender than that in a cat canine.

To detect the action potentials in the present study, the noise in the recordings had to be kept to a minimum. Surprisingly, despite working in a clinical environment with no Faraday cage, mains electrical interference was not a problem and was avoided by using batteries to power all the equipment, turning off all mains-powered equipment in the immediate vicinity of the subject, and by using a simple 50 Hz notch filter in the amplifiers. The mains supply in the building in which the experiments were carried out is channelled through earthed, metal ducting.

The first recordings of action potentials from human dentine were made by Ewall and Olgart, (1977) using methods similar to those developed by (Scott and Tempel, 1965) in the cat. They recorded from cavities that were cut on the buccal side of the teeth. A similar method was used later by Ahlquist and others (Ahlquist et al., 1984; Ahlquist et al., 1994).

The only alternative to dentine recording for monitoring the discharge evoked in peripheral sensory nerve fibres by pain-producing stimuli in man, is micro-neurography (Hagbarth, 2002). This technique involves inserting a microelectrode through the skin or mucous membrane into a peripheral nerve trunk. It has been used to record from pulpal nerve fibres in the inferior alveolar nerve (Iwata et al., 1991; Ikeda and Suda, 1998).

Acknowledgements

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Series II experiments

Pulpal blood flow recorded from exposed dentine with a laser Doppler flow meter using red or infrared light

Abstract

Objective: To determine the percentage of the blood flow signal that is derived from dental pulp when recording from exposed dentine in a human premolar.

Design: Recordings were made from 7 healthy teeth in 5 subjects (aged 22-33 yr.) with a laser Doppler flow meter (Periflux 4001) using either a red (635 nm) or an infrared (780 nm) laser. After exposing dentine above the buccal pulpal horn (cavity diam. 1.6 mm, depth 3 mm) and isolating the crown with opaque rubber dam, blood flow was recorded alternately with infrared or red light from the exposed dentine under four conditions: before and after injecting local anaesthetic (3% Mepivacaine without vasoconstrictor) (LA) over the apex of the root of the tooth; after exposing the pulp by cutting a buccal, class V cavity in the tooth; and after sectioning the coronal pulp transversely through the exposure.

Results: There was no significant change in mean blood flow recorded with either light source when the tooth was anaesthetized or when the pulp was exposed. After the pulp had been sectioned, the blood flow recorded with infrared light fell by 67.8% and with red light, by 68.4%. The difference between these effects was not significant.

Conclusions: When recording blood flow from exposed coronal dentine with either infrared or red light in a tooth isolated with opaque rubber dam, about 68% to the signal was contributed by the pulp. The signal:noise ratio was better with infrared than red light, and when recording from dentine than enamel.

Introduction

Laser Doppler flow meters have been used to record blood flow from dental pulp for thirty years, since the original studies of Gazelius, Olgart, Edwall & Edwall (1986) and Olgart, Gazelius & Lindh-Strömberg (1988). This technique has the principal advantage over alternative methods that it is non-invasive. Also, continuous recordings can be obtained. Disadvantages are that the records cannot be calibrated in absolute units (Vongsavan & Matthews, 1993), and records from the crown of a tooth always include a component that is derived from tissues outside the tooth, such as the gingiva and periodontal ligament, as well as the pulp (Soo-ampon, Vongsavan, Soo-ampon, Chuckpaiwong & Matthews, 2003).

Most of the observation in humans have been made by placing the laser Doppler probe on enamel in the bucco-cervical region of a tooth. Under these conditions, the contamination of the records from non-pulpal tissues can be reduced by covering the adjacent gingiva with opaque dam or some other suitable material. For example, Soo-ampon et al. (2003) found that the use of opaque rubber dam reduced the blood flow signal recorded from an intact, anterior tooth in man by an average of 73%. Akpinar, Er, Polat & Polat (2004) used an opaque periodontal paste to cover the gingiva with similar results. Soo-ampon et al. (2003) also showed that the signal remaining after applying opaque rubber dam was further reduced by 57% when the pulp was removed and replaced in the root canal. Thus only approximately 10% of the signal recorded from a tooth without dam, and 43% of that recorded with dam could be attributed to blood flow in the pulp.

Laser Doppler blood flow records can be obtained with different wavelengths of light, which do not all penetrate tissues to the same extent. For example, infrared light (780 nm) penetrates deeper than red light (635 nm) (Bonner & Nossal, 1990), thus a record from the cervical region of a tooth that was obtained with infrared light might be expected to include a greater contribution from tissues outside the pulp than one obtained with red light. However, Kijsamanmith, Timpawat, Vongsavan & Matthews, (2011a, 2011b) found no such difference. With both wavelengths, and using opaque rubber dam, the proportion of the signal due to dental pulp was around 46 % for anterior teeth and 60 % for premolars.

Another limitation often encountered when trying to record pulpal blood flow through enamel with a laser-Doppler flow meter is that the signal obtained is very weak and close to the limit of resolution of the instrument (Soo-ampon et al., 2003). Banthitkhunanon, Chintakanan, Wanachantararak, Vongsavan & Matthews (2013) showed that it might be possible to avoid this problem by recording from exposed dentine. Blood

flow signals obtained from exposed dentine in deep cavities were more than 10X those obtained from the corresponding area of enamel. This result was obtained in an *in vitro* study in which diluted blood was pumped through the pulp cavity. More recently, Sukapattee, Wanachantararak, Sirimaharaj, Vongsavan & Matthews (2016) found that pulpal blood flow values recorded from exposed dentine in premolars and molars during and after cutting minimum depth, full-crown preparations were about double those recorded from the corresponding area of enamel prior to the crown preparation

The aim of the present experiments was to determine what proportion of the blood flow signal recorded from dentine near the tip of a cusp of a premolar was derived from the pulp. Both red (635 nm) and infrared (780 nm) light were tested to determine if, in this location, the wavelength of the light would affect the result.

2 Materials and Methods

The experiments were carried out on 7 healthy premolar teeth in 5 human subjects (aged 22-33 yr., mean 27.3). These teeth were scheduled to be extracted for orthodontic purposes. All the teeth were intact. Radiographic examination and electrical pulp stimulation confirmed that they were vital and healthy.

The experiments were carried out in the Endodontics Clinic of the Maha Chakri Sirithon Dental Hospital at the Salaya Campus of the Faculty of Dentistry, Mahidol University. The study was approved by the Ethics Committee of Faculty of Dentistry/Faculty of Pharmacy, Mahidol University, Institutional Review Board (Certificate no. COA.No.MU.DT/PY-IRB 2015/049.0610) and complied with the principles of the Declaration of Helsinki. The experimental procedures were clearly explained to each subject and their written consent to these procedures being carried out was obtained. The subject could terminate the experiment at any stage. The privacy rights of the subjects were observed at all times.

2.1 Cavity preparation

A 1.6 mm diameter, 3.0 mm deep cavity directed towards the pulp horn was cut at the tip of the buccal cusp. The enamel was first removed with a 1.0 mm diam. round, diamond bur in an air-rotor drill under a constant stream of water. The cavity was then deepen and the floor flattened with a 1.6 mm diameter, tungsten carbide, flat fissure bur in a very slow hand-piece and with copious water spray. The enamel surface around the cavity was etched with 35% phosphoric acid (3M ESPE; St. Pual, MN, USA) and a

cannula made from a 4 mm length of 16G stainless steel needle (ext. diam. 1.59 mm, int. diam. 1.19 mm) was sealed into the cavity with flowable composite resin (3M ESPE; St. Pual, MN, USA), care being taken to avoid the resin flowing onto the cavity floor.

2.2 Pulpal blood flow recording

Blood flow recordings were made with a Periflux System 4001 two-channel, laser Doppler flow meter (Perimed AB, Järfälla, Sweden). One channel was equipped with an infrared (780 nm) laser and the other, a red (635 nm) laser. Time constants of 0.03 s were used for the infra-red channel, which showed any rapid fluctuations in blood flow; and 0.2 s for the red, which showed slower changes with a better signal:noise ratio. Measurements of mean blood flow over several seconds were not affected by these settings. The blood flow data were recorded from the digital output of the flow meter with a computer running the PeriSoft (version 1.13) software program.

The flow meter probe (type 415-159) had an ext. diam. of 1.0 mm and contained 2 optical fibres (diam. 0.125 mm, separation 0.25 mm). The probe was inserted into the cannula in the cavity until it touched the exposed dentine (Fig. 1). Light was transmitted between the dentine and the probe through air; no attempt was made to improve the optical contact between them by introducing an alternative contact medium. Recordings were made with the probe connected alternately to each of the two channels of the flowmeter. The probe was zeroed separately with infrared and red light and calibrated according the manufacturer's instructions so that the Brownian motion of a standard suspension of latex particles (Vongsavan & Matthews, 1993) gave a reading of 250 arbitrary perfusion units (P.U.).

After the cavity at the tip of the tooth had been prepared and the cannula inserted, the crown was isolated by the application of opaque black rubber dam (Four D Rubber Co. Ltd., Heanor, England). Blood flow recordings were made under four conditions: (1) before and (2) after the tooth had been anaesthetised by a submucosal injection of local anaesthetic (1.7 ml Mepivacaine 3% without vaso-constrictor, Septodont; Saint-Maur-des-Fossés, France) administered buccally over the apex of the test tooth (3) after exposing the pulp by cutting a buccal, class V cavity in the tooth; and (4) after sectioning the coronal pulp transversely with the tip of a fine scalpel blade (No.11) through the exposure. ~~At each stage, the blood flow signal was allowed to stabilize for several minutes~~ After each intervention, the blood flow signal was allowed to stabilize before further measurements

were made. The time required for this, and in the case of the local anaesthetic injection also for the anaesthetic to take effect, was typically between 3 and 5 minutes. ~~before then~~ Measurements were made from a recording of approximately 15 seconds duration. The total backscattered light intensity was also noted.

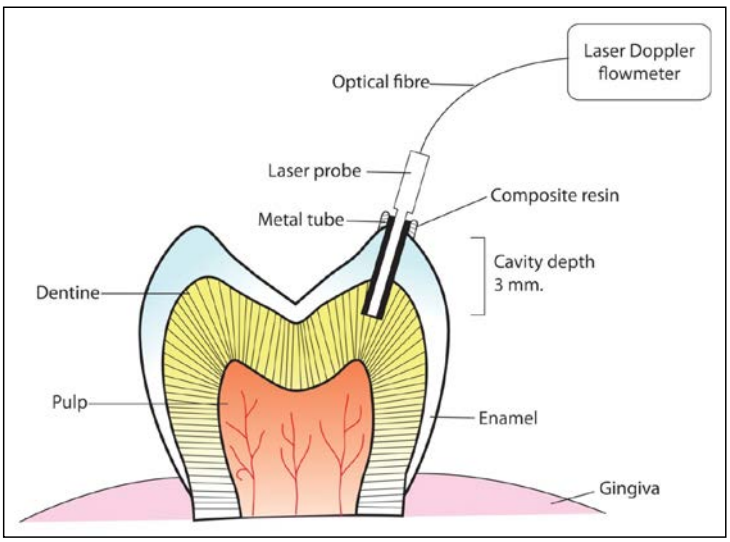

Fig. 1. Diagram of the experimental set up (not to scale).

After each experiment, recordings were also made at different light intensities from a stationary reflector (white card). These data were used to calculate the offset of the blood flow signal that would have been present due to noise in the detection system while recording from the teeth (Vongsavan & Matthews, 1993). For each blood flow recording obtained during an experiment, the mean and standard deviation values of blood flow were calculated in P.U. and the offset appropriate for the intensity of backscattered light present, determined as described above, was subtracted from the mean.

After the experiments, the tooth was extracted with forceps and sectioned bucco-lingually through the cusps with a diamond disc and water coolant. The remaining dentine

thickness was measured between the floor of the tip cavity and the closest point of the pulp chamber.

2.3 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 (ANOVA). Where this showed that there were significant differences between the means, the Tukey test was used to make multiple comparisons between them. The mean blood flow values that were obtained under corresponding conditions with the two light sources were compared with Student's paired t-test. The statistical analyses were carried out with Sigmaplot® software (version 12, Systat Software Inc., CA, USA).

P values of less than 0.05 were considered significant.

Results

A full set of data was obtained with both infrared and red light from every tooth. Examples of the records obtained from one tooth under the different conditions of the experiment are shown in Fig. 2.

With infrared light, ANOVA indicated that there was a significant difference between the mean blood flow values recorded under different conditions. After cutting the tip cavity and application of rubber dam, the mean value was 7.05 (S.D. 3.77, n=7) P.U. The administration of the local anaesthetic resulted in a fall in the mean to 5.31 (S.D. 3.16) P.U. but this change was not significant. After cavity preparation to expose the pulp, the mean value (5.68 , S.D. 3.67 P.U.) also did not change significantly. After sectioning the coronal pulp however, the mean blood flow signal decreased significantly ($P<0.05$, Tukey test) by 67.8% to 1.63 (S.D. 1.30) P.U.

A similar result was obtained with red light. The mean after preparing the tip cavity and applying the rubber dam was 3.44 (S.D.2.13, n=7) P.U. This fell insignificantly to 3.12 (S.D. 1.60) P.U. after the administration of local anaesthetic. Cavity preparation to expose the pulp produced no significant change in the mean blood flow value (3.26, S.D. 2.84P.U.) whereas sectioning the coronal pulp significantly($P<0.05$, Tukey test) reduced this value by 68.4% to 0.94 (S.D. 0.77) P.U.

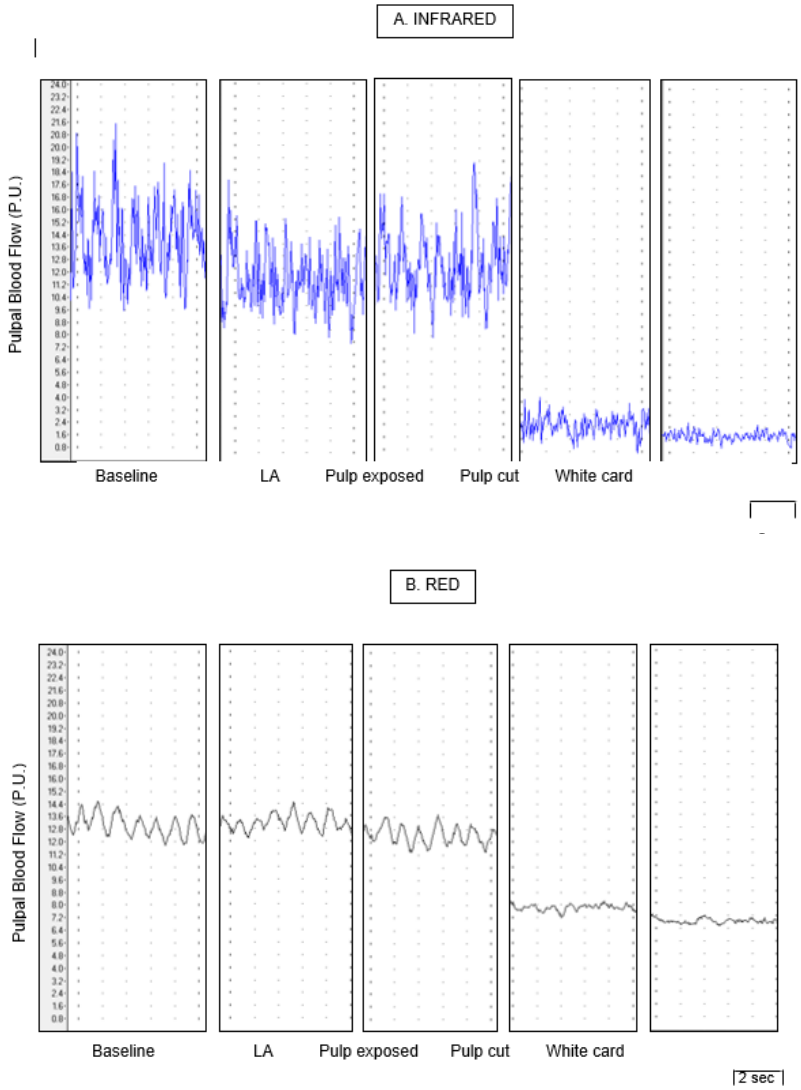

Fig. 2. Laser Doppler records of blood flow recorded with infrared (A) and red (B) light from exposed dentine in one tooth after the preparation of the tip cavity and applying opaque black rubber dam (Baseline), after the administration of the local anaesthetic (LA), after exposing the pulp through a cervical cavity (Pulp exposed), and after sectioning the pulp (Pulp cut). The final record in each case is a control obtained with the same intensity of back-scattered light from a stationary reflector (White card). For the records obtained with infrared light, a time-constant of 0.03 s was used; whereas for red light, it was 0.2 s.

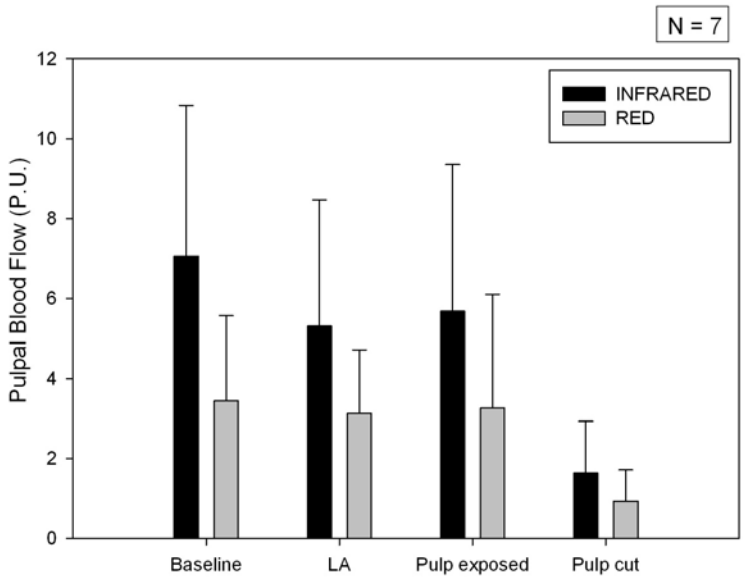

Fig. 3. Mean values of the pulpal blood flow signal recorded from 7 teeth with infrared (black columns) and red (grey columns) light after the preparation of the tip cavity and applying opaque black rubber dam (Baseline), after the administration of the local anaesthetic (LA), after exposing the pulp through a cervical cavity (Pulp exposed), and after sectioning the pulp (Pulp cut). In each case, the value was corrected by subtraction of the reading from the white card. The error bars indicate one S.D.

A summary of the data is shown in Fig.3. The mean blood flow values obtained with infrared light were higher than the corresponding values for red light under all the experimental conditions. The differences between the mean values obtained with infrared and red light after cutting the tip cavity and application of rubber dam, after the administration of local anaesthetic, and after cavity preparation to expose the pulp were all significant (P = 0.008, 0.018, and 0.007 respectively, *Student's paired t-test*); but the difference between the means after sectioning the pulp was not significant.

The mean % reduction in the blood flow signal following sectioning of the pulp (67.8% with infrared and 68.4% with red light), provides an estimate of the contribution of pulpal blood flow to the signal obtained before this was done. The difference between these values was not significant (*P*=0.920, *Student's paired t-test*).

The infra-red records did not reveal any high frequency fluctuations in blood-flow such as a dicrotic notch in the pulsations synchronised to the heart-beat.

The average remaining dentine thickness under the tip cavity was 1.87 (S.D. 0.19) mm.

Discusson

This study confirmed the earlier finding (Banthitkhunanon et al., 2013) that a much larger laser-Doppler blood flow signal can be recorded from exposed dentine than from enamel. Thus, by recording from dentine, the method does not have the disadvantage often encountered when recording from enamel in human teeth, that the signal amplitude is close to the limit of resolution of the flow meter (Soo-ampon et al., 2003). This would apply particularly in elderly individuals since pulpal blood flow tends to decrease with age (Ikawa, Komatsu, Ikawa, Mayanagi & Shimauchi, 2003). Another method of improving the signal:noise ratio in recordings of pulpal blood flow would be to reduce the upper limit of the bandwidth of the system that measures the Doppler shift in frequency of the light when it is scattered by moving blood cells, from the usual 24 kHz or 20 kHz (as in the present experiments) to 5 kHz (Qu, Ikawa & Shimauchi, 2014). This optimises the detection system to match the relatively slow peak blood velocities in a tooth.

It was also demonstrated that when recording from exposed dentine at the tip of the cusp of a premolar, with the crown isolated with opaque rubber dam, approximately 70% of the signal was derived from pulp as opposed to tissues outside the tooth . This is higher than has been found in studies in which recordings were made from enamel in human teeth, where the proportion was between 43 and 60% (Kijsamanmith et al., 2011a, 2011b; Soo-ampon et al., 2003). Kijsamanmith, Timpawat, Vongsavan & Matthews (2011b) recorded from the cervical buccal enamel of premolars using methods that were otherwise very similar to those of the present study and found that 60% of the signal was from pulp. Although the proportion of the signal derived from pulp was increased by recording from

dentine, there was still a considerable contribution from other tissues. When recording from the enamel of deciduous teeth in pigs, Vongsavan & Matthews (1996) demonstrated that up to 85-93% of the signal was from pulp. Although these teeth are of similar size to human teeth, the difference between the results of that and the present study may be due to differences in the size of the pulp, which is larger (Vongsavan & Matthews, 1996), and in the thickness of the enamel and dentine which is smaller, in pig incisors . Although the recordings were made from exposed dentine in the present study, on average 1.9 mm remained over the pulp.

Commented [BM1]: «

Isolating the crown of a tooth with opaque rubber dam is known to decrease the contribution of tissues outside the pulp to the blood flow signal recorded. Without black rubber dam, only approximately 10% of the signal recorded from human enamel was from pulp (Soo-ampon et al., 2003). Polat, Er, Akpinar & Polat (2004) also found that the contribution was between 3 and 14% when recording without covering the gingiva. The effect of dam may be to compress the gingival margin and thus reduce its blood flow (Ikawa, M., Personal Communication), as well as to screen the tissues from the light of the flow meter. The dams in common use clinically are not as opaque as the material used in the present study. The use of an opaque dam, or some other similar procedure to cover the gingiva, is therefore essential to ensure that the majority of the laser Doppler signal recorded from the crown of a tooth is derived from the pulp.

The proportion of the signal recorded with dam that was derived from the pulp in the present study was the same for both red and infrared light, as found in a previous study under similar conditions but recording from the buccal enamel surface of premolars (Kijsamanmith et al., 2011b). It therefore appears that the greater penetration of tissues by infrared light (Bonner & Nossal, 1990) results in a deeper penetration into the pulp as well as the surrounding structures. The signals obtained from the pulp with infrared were consistently larger than those with red light.

The performance of a laser Doppler flow meter in recording from dentine would be expected to be improved by introducing a suitable fluid with a high refractive fluid as a coupling medium between the laser Doppler probe and exposed dentine surface. This interface was occupied by air in the present experiments. Preliminary experiments carried out while recording both blood flow and nerve action potentials (Wanachantararak, Ajcharanukul, Vongsavan & Matthews, 2016) from exposed dentine at the tip of a cusp of a human premolar indicate that liquid paraffin may be a suitable medium for this purpose.

The higher resolution of the records from dentine compared with those from enamel, did not reveal any additional details of fluctuations in blood flow synchronised to the heart- beat, such as a dicrotic notch, which might have been present due to the very low compliance of the pulp (Matthews & Andrew, 1995). In recordings from enamel, Qu et al. (2014) found that blood flow in the pulpal circulation was characterized by a lack of high velocity components.

It is surprising that exposure of the pulp did not produce an increase in its blood flow. This may have been because the local anaesthetic blocked a neurogenic inflammatory response.

In conclusion, when recording pulpal blood flow with a laser Doppler flow meter a larger signal is obtained, and a greater proportion of that signal is derived from pulp, when the probe is placed on exposed dentine rather than enamel. When recording from dentine, infrared light gives a larger signal than red light but the proportion of that signal that is derived from the pulp is unaffected. Dentine may become exposed and accessible for recording as a result of attrition, trauma or cavity preparation. The method could be particularly useful for making repeated measurements of blood flow from a tooth to monitor changes in the severity of pulpitis, the effects of medication, or the progress of pulpal revascularization.

Acknowledgements

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Series III experiments

Electroosmosis in human dentine *in vitro*

Abstract

Objective: To determine the rate of fluid flow through human dentine due to electroosmosis during iontophoresis of either 2% lignocaine with epinephrine, Ringer's solution, epinephrine, or distilled water.

Design: Experiments were carried out on 24 intact extracted human premolars. Dentine was exposed at the tip of the buccal cusp. The pulp cavity was filled with Ringer's solution at a pressure of 11 mm Hg and fluid flow through the dentine was measured using a capillary connected to the pulp cavity. Current was passed between a stainless-steel electrode in the cavity and one in the pulp cavity. The effect of removing the smear layer from the exposed dentine was also investigated.

Results: During iontophoresis of 2% lignocaine with epinephrine through dentine with a smear layer, currents of 0.2, 0.4, and 0.6mA applied with the cavity electrode as anode produced inward flow rates of 2.25±0.87, 5.00±1.62, 8.60±1.97 (mean ±s.d.) nL/s/mm² respectively, and applying the currents in the opposite direction caused outward flows of 0.76±0.72, 1.00±1.01, 1.12±1.18nL/s/mm² respectively. The flow rates recorded during iontophoresis of Ringer's solution, epinephrine or distilled water did not differ significantly from those produced during iontophoresis of lignocaine with epinephrine. Removing the smear layer had little effect on the flow rates produced.

Conclusions: It is concluded that electroosmosis can be produced in human dentine, it can enhance the effect of iontophoresis in transporting charged molecules through dentine, particularly large molecules, and it could also enable uncharged molecules to be carried through dentine into the pulp.

Introduction

In an incidental observation made while investigating the relationship between the rate of fluid flow through dentine and the discharge of pulpal nerves in the cat, it was noticed that the resting flow rate changed when current was passed through the dentine. An inward flow through the dentine was produced when a D.C. current was passed between the dentine surface and the pulp, and an equal outward flow when the current was reversed. The rate of fluid flow depended on the intensity of the current, with 0.3 mA generating a flow rate equal to that produced by an hydrostatic pressure gradient of about 100 mm Hg. This flow was attributed to electroosmosis (Vongsavan & Matthews, 1995).

Electroosmosis is a phenomenon by which flow of a fluid is produced when an electric current is passed through electrolyte-filled channels with charged walls (Pikal, 2001; Ou, Wu, Sandberg & Weber, 2014). The current produces a concentration gradient of ions and hence an osmotic gradient along the channels. In the present study, we have measured the fluid flow rates through dentine due to electroosmosis that were produced during the iontophoresis of either lignocaine with epinephrine, epinephrine, Ringer's solution, or distilled water in extracted human teeth. The effect of etching to remove the smear layer from exposed dentine was also investigated.

Materials & methods

The experiments were carried out on 24 intact human premolars *in vitro*. All teeth were free of caries and extracted for orthodontics purposes. After extraction, the teeth were stored in 0.9% normal saline solution with amoxycillin (500 mg/L) at 4°C and used within 24 hours. The experimental protocol was approved by the Institutional Review Board, Faculty of Dentistry/Faculty of Pharmacy, Mahidol University (COE. No. MU-DT/PY-IRB 2016/017.0508).

Brief outline of experiments

The crown of each tooth was fixed to an acrylic block and the pulp cavity filled with Ringer's solution at a pressure of 11 mm Hg above atmospheric. A cavity was cut at the tip of the buccal cusp to expose dentine, and filled with one of 4 test solutions: 2% lignocaine with epinephrine 1:100,000, epinephrine 1:1,000, Ringer's solution, or distilled water. Current was passed through the dentine between a stainless-steel electrode in the cavity and one in the pulp cavity. All the solutions were tested in each tooth, both before and after etching the exposed dentine. Currents of 0.2, 0.4, and 0.6 mA were applied, for 2 min. each, with the cavity electrode as anode and with the current reversed. Fluid flow through the

dentine was measured while the currents were passed.

Tooth preparation

Each tooth was sectioned transversely 2mm below the cemento-enamel junction with a diamond disc under water. Any remaining coronal pulpal tissue was removed with fine tweezers. The pulp chamber was then irrigated with water from a triple syringe for 10 min. to remove any remaining tissue. Dentine was exposed at the tip of the buccal cusp by cutting a cavity (diameter approximately 2mm, depth approximately 3mm) with an air-rotor and diamond burs (Nos. 201 and 204, Intensive1, Viganello-Lugano, Switzerland) under water. The cut cervical surface of the crown was sealed with cyanoacrylate cement (Alteco Inc., Osaka, Japan) to an acrylic block (Figure 1) into which had been sealed a stainless-steel tube (18G, o.d. 1.27mm, i.d. 0.84mm). This tube was connected to a glass capillary with a uniform internal diameter of 300µm (DADE®, Miami, Florida, U.S.A.). The pulp chamber, stainless steel tube and capillary were filled with Ringer's solution and kept in the same horizontal plane. The free end of the capillary was connected to a manometer set at 11mm Hg above atmospheric pressure to create an intra-pulpal pressure and hydrostatic pressure gradient across the dentine similar to that present *in vivo*. This is the pupal pressure recorded in cat teeth (Vongsavan & Matthews, 1992) and is close to 14mm Hg reported by Ciucchi, Bouillaguet, Holz & Pashley (1995). After finishing the experiment, the surface area of exposed dentine was measured in a scanning electron microscope. Each tooth was sectioned bucco-lingually through the cusps with a diamond disc and water coolant. The remaining dentine thickness was measured between the floor of the cavity and the closest point of the pulp chamber along the dentinal tubules with Vernier callipers.

Flow measurement

The volume flow of fluid through the exposed dentine was measured (Kijsamanmith, Surarit & Vongsavan, 2016) by recording the movement of a small air bubble introduced into the capillary (Figure 1). Outward flows through the dentine (i.e. away from the pulp cavity) are represented by positive values, and inward flows by negative values.

The flow that occurred during anodal or cathodal-current iontophoresis with each solution was determined before and after etching the exposed dentine with 35% phosphoric acid for 30 seconds to remove the smear layer formed during drilling.

To check that the flow in the capillary that occurred when current was passed was not due to electrolysis and gas bubble formation in the stainless-steel needle, some

duplicate recordings of flow were made while passing the current between the electrode in the cavity and a stainless-steel wire inserted into the cannula at the end of the capillary beyond the bubble.

Iontophoresis and Solutions

An iontophoresis machine (DENTAPHOR™-II MODEL 6111D, Life-Tech Inc®, USA) was used to pass a D.C. current of 0.2, 0.4, or 0.6mA, of either polarity, through the dentine. The current was passed between a stainless-steel electrode that was inserted into the cavity and either the stainless-steel needle (Figure 1) or a stainless-steel wire inserted into the cannula beyond the glass capillary. Currents passed with the cavity electrode as the cathode will be referred to as cathodal currents and those with the cavity electrode as the anode, as anodal currents.

The test solutions were: 2% lignocaine hydrochloride with epinephrine hydrochloride 1:100,000 (Octocaine® 100, Novocol Pharmaceutical of Canada Inc.) pH 3.4; epinephrine hydrochloride 1:1,000 (GPO, The Government Pharmaceutical Organization, Thailand) pH 3.2; Ringer's solution; and distilled water. A lignocaine solution with epinephrine has been used in experiments in which iontophoresis was used to anesthetize dentine (Smitayothin, et al., 2015; Thongkukiatkun et al., 2015), and epinephrine alone has been iontophoresed through dentine to produce vasoconstriction in the pulp (Thongkukiatkun et al., 2015). The saline was included as a control, and the distilled water was used to determine if it was essential to include ions in the solution for electroosmosis to occur through dentine.

After drying the cavity with cotton pellets, 50µL of one of the solutions was inserted into the cavity. The solutions were tested in random order. Between solutions, the electrodes were disconnected; the cavity, pulp chamber and connecting tubes rinsed with Ringer's solution; and the hydrostatic pressure in the pulp chamber was raised to 300mm Hg above atmospheric for 5 minutes to wash out any remaining test solution from the dentinal tubules.

Statistical Analysis

The flow rates are represented as means ±1 S.D. With each test solution, the flow rates observed at different currents and with each polarity were compared using one-way, repeat measures, analysis of variance (RM ANOVA). Where this showed a significant effect, Student-Newman-Keuls test was used to determine the significance of differences between the means. P values <0.05 were considered significant. The flow rates recorded

with the same current but with different test solutions were compared in the same way. The flow rates recorded with each set of variables before and after etching were compared using Student's paired-t-test.

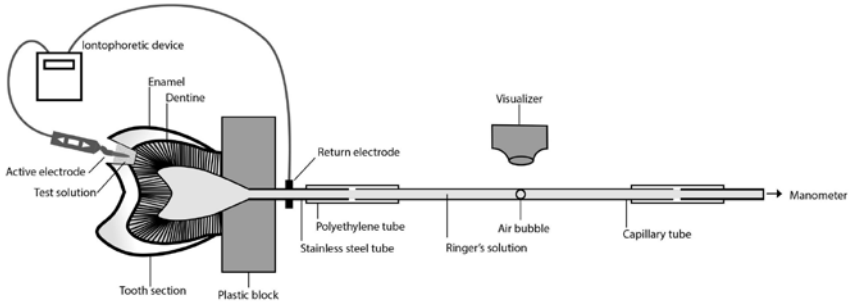

Figure 1. Diagram of the experimental set up.

Results

The mean flow rates recorded with each test solution, before and after removing the smear layer, and with different current strengths are shown in Figures 2a, 2b, 2c and 2d; and the means and standard deviations of the flow rates obtained with different current intensities, in Figures 3a, 3b, and 3c.

Ringer's solution

With Ringer's solution in the test cavity, before removing the smear layer and before applying current, an outward flow 0.68±0.83nL/s/mm² was recorded. This was due to the pulpal pressure of 11mmHg. When cathodal currents were applied an outward flow was also recorded in all cases, and the mean flow rate increased slightly as the current intensity was increased (0.85±1.02, 0.95±1.04, 1.10±1.20nL/s/mm² with 0.2, 0.4, and 0.6mA respectively). With anodal currents, the flow reversed, and the mean inward flow rates increased to 2.27±0.87, 5.00±1.61, 8.14±1.93nL/s/mm² respectively. The overall effect of the intensity of the current on the flow rate was significant for both current polarities (P<0.05, RM ANOVA); and the individual means with anodal currents were all

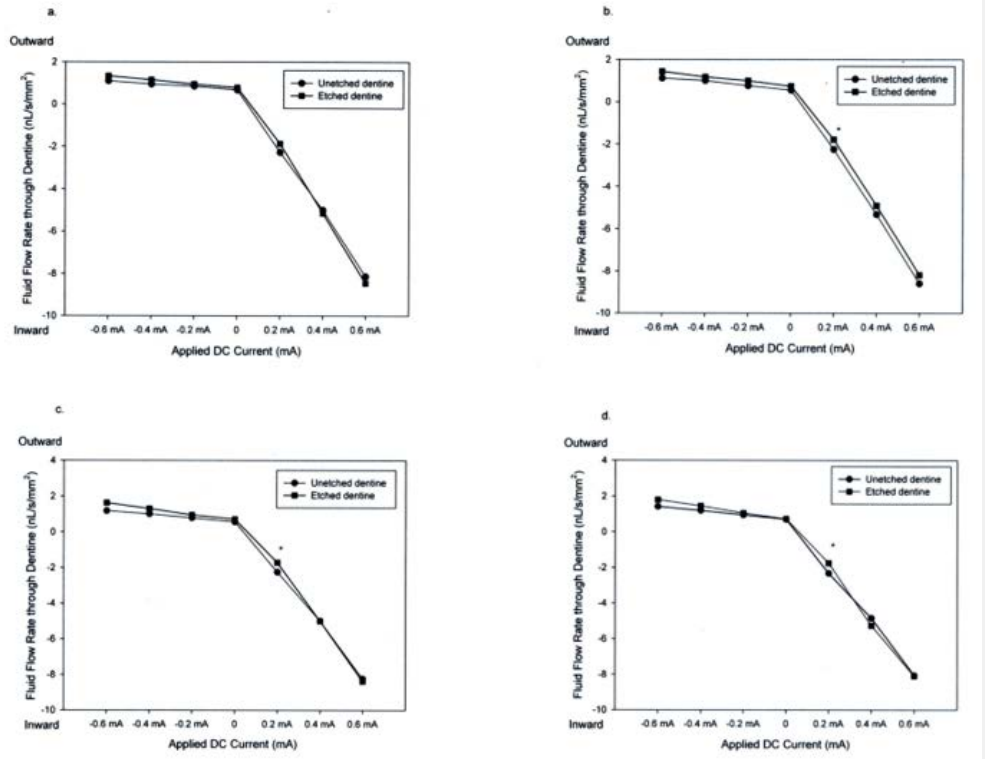

Figure 2. The relationship between the intensity of current applied to the dentin and the flow rate of fluid through the dentin that was produced. 'Outward' refers to flow from the pulp cavity to the exposed dentin surface, and 'Inward' to flow in the opposite direction. Positive currents were delivered with the electrode in the cavity as the anode and negative currents, with the cavity electrode as the cathode. Circular symbols represent mean flow rates recorded before the exposed dentine surface was etched to remove the smear layer and the square symbols, after etching. Each graph represents measurements made with a different test solution in the cavity: **a.** Ringer's solution, **b.** 2% lignocaine with epinephrine 1:100,000, **c.** epinephrine 1:1,000 and **d.** distilled water.

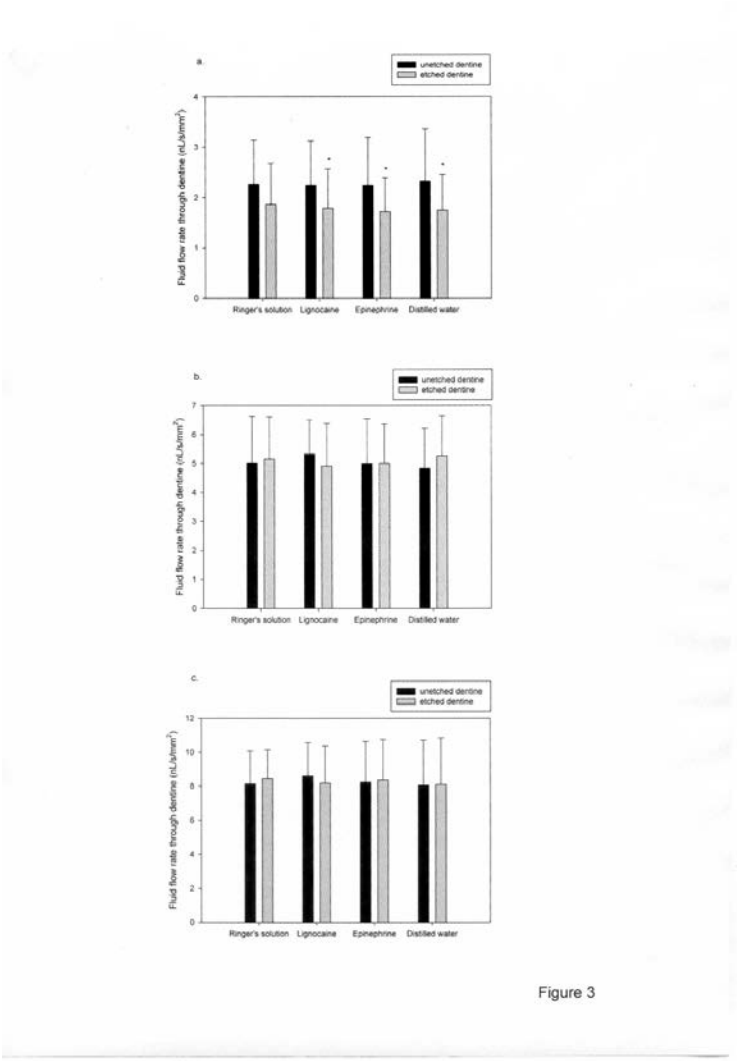

Figure 3

Figure 3. Barcharts showing the means and standard deviations of the flow through the dentine recorded with different test solutions in the cavity, before (solid columns) and after (hatched columns), during the application of D.C. anodal currents with intensities of:**a.** 0.2 mA, **b.** 0.4 mA, **c.** 0.6 mA Asterisks indicate those cases in which the difference between the mean values recorded before and after etching were significant ($P<0.05$, Student's paired t-test).

significantly different from each other (P<0.001, Student-Newman-Keuls test). With cathodal currents, the mean flows were all significantly different from each other (P ranged from 0.006 to <0.001, Student-Newman-Keuls test) except those produced by 0.2 and 0.4 mA (P=0.066).

The corresponding values after removing the smear layer were: with no current, an outward flow of 0.79±0.73nL/s/mm²; with cathodal currents of 0.2, 0.4 and 0.6mA there were outward flows of 0.95±0.79, 1.15±0.89, and 1.33±0.94nL/s/mm² respectively; and with anodal currents, inward flows of 1.86±0.82, 5.15±1.46, and 8.46±1.68nL/s/mm² respectively. The overall effect of the intensity of the current on the flow rate was significant for both polarities (P<0.05, RM ANOVA); and the individual means were all significantly different from each other (Student-Newman-Keuls test), both with cathodal (P ranged from 0.007 to <0.001) and anodal (P ranged from 0.003 to <0.001) currents.

The difference in the flow rate recorded in individual teeth before and after etching was not significant (P>0.05, Student's paired t-test) under any of the conditions tested.

Lignocaine with epinephrine

With 2% lignocaine with epinephrine 1:100,000 in the cavity, no current being passed and before removal of the smear layer, the average outward flow rate was 0.58±0.63nL/s/mm². When cathodal currents were applied, the mean outward flow rates were 0.76±0.72, 1.00±1.09, 1.12±1.18nL/s/mm² with currents of 0.2, 0.4, and 0.6mA respectively. The corresponding results obtained with anodal currents were inward flows of 2.25±0.87, 5.00±1.62, and 8.60±1.97nL/s/mm². The overall effect of the intensity of the current on the flow rate was significant for both polarities (P<0.05, RM ANOVA); and the individual means with anodal currents were all significantly different from each other (P<0.001, Student-Newman-Keuls test). With cathodal currents, the differences between the mean flows obtained with no current and 0.6mA, no current and 0.4mA, and 0.2 and 0.6mA were significantly different (P ranged from 0.03 to 0.001, Student-Newman-Keuls test), and the other differences were insignificant.

The corresponding values after removing the smear layer were: with no current, an outward flow of 0.74±0.79nL/s/mm²; with cathodal currents of 0.2, 0.4 and 0.6mA, outward flows of 1,00±0.97, 1.17±1.20, and 1.44±1.26nL/s/mm² respectively; and with anodal currents, inward flows of 1.79±0.78, 4.91±1.48, and 8.19±2.20nL/s/mm² respectively. The overall effect of the intensity of the current on the flow rate was significant for both polarities (P<0.05, RM ANOVA); and the individual means were all

significantly different from each other (Student-Newman-Keuls test), with the cavity electrode both as the cathode (*P* ranged from 0.039 to <0.001) and as the anode (*P* ranged from 0.007 to <0.001).

The difference in the flow rate recorded in individual teeth before and after etching was not significant (*P*>0.05, Student's paired t-test) under any of the conditions tested except with an anodal current of 0.2mA (*P*=0.019).

Epinephrine

The results with epinephrine 1:1,000 were very similar to those obtained with Ringers and with 2% lignocaine with epinephrine 1:100,000. The mean flow before etching with no applied current was an outward rate of 0.57±0.61nL/s/mm². Cathodal currents of 0.2, 0.4, and 0.6mA, produced outward flows of 0.79±0.69, 1.01±0.83, 1.18±0.91nL/s/mm² respectively, and with currents of the opposite polarity, the corresponding flows were 2.25±0.94, 4.98±1.55, 8.24±2.40nL/s/mm² inward. The overall effect of the intensity of the current on the flow rate was significant for both anodal and cathodal currents (*P*<0.05, RM ANOVA); and the individual means with both cathodal and anodal currents were all significantly different from each other (*P* ranged from 0.017 to <0.001, Student-Newman-Keuls test).

The corresponding values after removing the smear layer were: with no current, an outward flow of 0.72±0.60nL/s/mm²; with cathodal currents of 0.2, 0.4 and 0.6mA, outward flows of 0.95±0.70, 1.30±0.91, and 1.63±1.25nL/s/mm² respectively; and with anodal currents, inward flows of 1.72±0.67, 4.99±1.37, and 8.38±2.37nL/s/mm² respectively. The overall effect of the intensity of the current on the flow rate was significant for both anodal and cathodal currents (*P*<0.05, RM ANOVA); and the individual mean flows obtained with cathodal currents were all significantly different from each other (*P* ranged from 0.011 to <0.001, Student-Newman-Keuls test), except for those obtained with 0 and 0.2mA. With anodal currents, the flow rates were all significantly different from each other (*P* ranged from 0.017 to <0.001, Student-Newman-Keuls test).

The difference in the flow rate recorded in individual teeth before and after etching was not significant (*P*>0.05, Student's paired t-test) under any of the conditions tested except for an anodal current of 0.2mA (*P*=0.008).

Distilled water

With distilled water in the cavity and no current being passed, and before etching the exposed dentine, the average outward flow rate was 0.70±0.93nL/s/mm². Despite the lack of charged ions in the test solution, passing a current between the cavity and the pulp chamber induced flows similar to those recorded with other solutions. With cathodal currents, outward flow rates at rates of 0.95±1.19, 1.19±1.70, and 1.42±1.88nL/s/mm² were produced by currents of 0.2, 0.4, and 0.6mA respectively. The corresponding values obtained with anodal currents were inward flows of 2.32±1.04, 4.84±1.37, and 8.06±2.65nL/s/mm². The overall effect of the intensity of the current on the flow rate was significant for both anodal and cathodal currents ($P<0.05$, RM ANOVA). With cathodal currents, the differences between the flows recorded with 0 and 0.6mA, with 0 and 0.4mA, and with 0.2 and 0.6mA were significant (P ranged from 0.012 to <0.001, Student-Newman-Keuls test). With anodal currents, all the flow rates were significantly different from each other (P ranged from 0.008 to <0.001, Student-Newman-Keuls test).

The corresponding values after removing the smear layer were: with no current, an outward flow of 0.73±0.69nL/s/mm²; with cathodal currents of 0.2, 0.4 and 0.6mA, outward flows of 1.05±0.78, 1.45±0.89, 1.80±1.17nL/s/mm² respectively; and with anodal currents, inward flows of 1.75±0.71, 5.26±1.38, 8.10±2.74nL/s/mm². The overall effect of the intensity of the current on the flow rate was significant for both anodal and cathodal currents ($P<0.05$, RM ANOVA); and the individual mean flows were all significantly different from each other, both with cathodal (P ranged from 0.004 to <0.001, Student-Newman-Keuls test), and anodal (0.021 to <0.001) currents.

The difference in the flow rate recorded in individual teeth before and after etching was not significant ($P>0.05$, Student's paired t-test) under any of the conditions tested except for an anodal current of 0.2mA ($P=0.012$).

The average remaining dentine thickness was 1.06 ± 0.31mm.

Duplicate recordings

When duplicate flow recordings were made while passing anodal and cathodal currents either between the electrode in the cavity and the stainless-steel needle or between the electrode in the cavity and a stainless-steel wire inserted into the cannula beyond the bubble in the capillary, the results were the same. This control showed that the flow was not due to electrolysis of the saline and bubble formation at the electrode on the pulpal side of the dentine.

Discussion

Our study is the first to demonstrate electroosmosis in human dentine. The flow created in human dentine *in vitro was* similar to that demonstrated in the cat *in vivo* Vongsavan & Matthews (1995): the direction of the flow in relation to the direction of the current was the same but the relationship of current intensity to flow rate was different. With electroosmosis of cat dentine *in vivo*, the slopes of the lines relating anodal and cathodal current intensity to flow rate were similar, whereas in human dentine *in vitro* they were very different, the slopes for anodal currents were much sleeper than those for cathodal currents.

The direction of fluid flow with both polarities of applied current was from anode to cathode, which indicates that there was a negative surface charge on the inside of the dentinal tubules. Cations in the tubular fluid would have been attracted to the walls of the tubules, leaving an excess of anions away from walls that would have been carried towards the cathode by applied currents, dragging water with them. The electroosmotic flow would have summed with the slow outward flow that would have been created by the hydrostatic pressure gradient of 11 mm Hg across the dentine.

The fluid flow rates produced by electroosmosis increased as the current intensity was increased but the rate was much faster with anodal than cathodal currents. Theoretically, the two rates should have been the same but in opposite directions; the reason that the rates were so different in the present study is not known.

The fact that the relationship between the intensity of the applied current and the flow rate produced was so similar with all the test solutions investigated, would have been due to the composition of the fluid in the tubules being the same (normal saline) in all cases. The distilled water would have been made electro-conductive by ions from the saline that would have flowed into the cavity with the tubular fluid.

Etching the dentine to remove the smear layer had little effect on the flow rates recorded, although this was significant in some cases with an anodal current of 0.2mA (Figure 3a). The small effect of etching is as expected for electroosmosis, with which pore size does not affect flow rate. However, the flow produced by the hydrostatic pressure gradient would have been affected by the resistance in the tubules. The fact that no significant difference was found between the flow rates recorded before and after etching with no current flowing can be attributed to the relatively low sensitivity of the flow recording method used, compared with the more complex technique employed in other studies (Vongsavan & Matthews, 1992).

Iontophoresis has been used to deliver local anesthetics through dentine into pulp (Thongkukiatkun et al., 2015; Smitayothin et al., 2015), and electroosmosis would have enhanced the inward transport of the anesthetic. Evidence that that the fluid flow produced by electroosmosis can augment the delivery of drugs and peptides during iontophoresis has been obtained in studies on mouse, pig and human skin (Marro, Kalia, Delgado-Charro & Guy, 2001; Pikal, 2001; Kim & Oh, 2018). Electroosmosis has also been demonstrated in brain tissue (Ou, Wu, Sandberg & Weber, 2014).

In conclusion, the results of the present experiments indicate that electroosmosis can be used to deliver drugs through dentine to the pulp, and can enhance the transport of drugs carried in by iontophoresis. It would be particularly effective with large ions such as proteins (Pikal, 2001). Uncharged molecules could also be transported into the pulp by the induced fluid flow.

Acknowledgments

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Series IV experiments

Effect of iontophoresis of articaine with epinephrine into exposed dentine on pulpal blood flow and the sensitivity of dentine in man

Abstract

Objective: To determine the effect of iontophoresis of 10% articaine with 1:1000 epinephrine through exposed etched dentine on pulpal blood flow and the sensitivity of dentine in human subjects.

Design: The experiments were carried out on 22 non-carious premolars in 6 subjects. These teeth were scheduled to be extracted for orthodontic purposes. Each tooth was isolated with an opaque black rubber dam and dentine was exposed at the tip of the buccal cusp by preparing a cavity. Then it was etched with 35% phosphoric acid for 20 seconds. The baseline pain sensation caused by probing and air blast was assessed on 100 mm Visual Analogue Scale (VAS).In group I, the cavity was filled with 25 µl of 10% articaine with 1:1,000 epinephrine. Next, the drug was delivered with the iontophoretic device (Dentaphore-II model 611 D Life-tech, Inc. USA) with current of 0.12 mA for 90 sec. Pain assessment to both stimuli was tested again every 2 min for 6 min, then every 10 minutes for 30 minutes. Pulpal blood flow (PBF) was recorded in each condition with a laser Doppler flow meter (Periflux 4001, Perimed®, Sweden). In group II, the experiments were performed exactly the same as for group I, except that distilled water was used prior to articaine and epinephrine.

Results: In group I, the baseline pain and sensation caused by airblast and probing were 7.36 ± 1.27 and 5.14 ± 1.29 cm. respectively. Immediately after iontophoresis of articaine with epinephrine, the pulpal anesthesia was obtained (VAS = 0) in all cases ($p<0.05$, one-way RM ANOVA on rank and Dunett's test). The corresponding pulpal blood flow values were 0.88 ± 0.05,0.28 ± 0.01, 0.28 ± 0.01, 0.26 ± 0.01, 0.24 ± 0.02, 0.21 ± 0.01, 0.19 ± 0.01, 0.20 ± 0.02 PU ($p<0.05$, one-way RM ANOVA and Tukey's test) consequently, at baseline, 0, 2, 4, 6, 10, 20 and 30 min after application. In group II, distilled water application had no significant effect on both VAS and pulpal blood flow values. It is concluded thationtophoresis of 10% articaine with 1:1000 epinephrine through exposed etched dentineon pulpal anesthesia lasts up to 30 minutes. It is suggested that this technique will have potential benefit for pain control in operative dentistry.

Introduction

It has been shown recently that dentine can be anaesthetised rapidly by the topical application of a solution containing 20% w/v lignocaine HCl and 0.1% w/v epinephrine HCl if an iontophoretic, anodal current of 120 µA is passed for 90 s between the solution and the dentine (Thongkukiatkun et al., 2015). These experiments were carried out on freshly exposed, healthy dentine; if the technique could be used for cavity preparation in carious teeth, it would avoid the need for the administration of local anaesthetics by injection, which causes patients pain and anxiety. Injections are one of the most anxiety-provoking procedures in dental treatment for both children and adults (Corah, 1985; Giangrego, 1986; Milgram et al 1988; Weine et al., 1982). Later, Smitayothin et al. (2015) studied the exposed dentin was etched and topical application of 20% lidocaine and 0.1% epinephrine, with an iontophoretic current of 120 µA for 90 s, the dentin was anaesthetise in all cases. In contrast to the experiments that performed by Smitayothin et al. (2015) that studied in the exposed unetched dentin in the patients, it was found that iontophoretic delivery of lidocaine with epinephrine anesthetized dentin that the cavity preparation was performed without pain up to 87.5% of carious molars. Although not all of the teeth were anesthetized, the most likely explanation for the greater resistance to anaesthesia of carious dentine compared with normal dentine is that, as a result of inflammatory changes in the pulp associated with the caries, Na+ channels of a type that are not sensitive to lignocaine were expressed in the nerve terminals and that these continued to support the propagation of action potentials despite the presence of the anaesthetic (Renton et al, 2005; Wells et al. 2007; Warren et al. 2008; Kistner et al., 2010; Suwanchai et al., 2012).

Articaine hydrochloride is an effective local anesthetic in amide group. Molecular structure of articaine containing a thiophene ring and ester side chain which rapid onset, intermediate duration and safety in local anesthesia and potency 1.5 times of lidocaine. Articaine were reported that they were superior than lidocaine to block to TTX-resistant Na^+ channel in the peripheral nerve. Articaine hydrochloride seems to have potential for iontophoetic application because it has rapid onset, intermediate duration and safe local anesthetic drug (Oertel et al. 1997). In the present experiments, we aimed to determine the effect of iontophoresis of 10% articaine HCl with 1:1000 epinephrine through exposed etched dentine on pulpal blood flow and the sensitivity of dentine in human subjects.

Materials and methods

Subjects

The experiments were carried out on 22 non-carious premolar teeth in 6 healthy subjects (age: 16-30 years) that were scheduled for extraction due to orthodontic treatment. All teeth were intact, non-carious and completely formed root which were assessed by dental history, clinical and radiographic. The study was approved by the Ethics Committee on Human rights Related to Human Experimentation of Faculty of dentistry of Mahidol University. The experiment procedures were clearly explained to each subjects and parents or guardians before the inform consent was signed.

Tooth preparation

The teeth were isolated with opaque black rubber dam sheet (Four D Rubber Co.,Ltd., Heanor, England) which was stabilized by the splint. The cylindrical shaped cavity on the buccal cusp of the premolar tooth was prepared with a round diamond bur number 201 high-speed handpiece with water coolant. The cavity was finished by using cylinder diamond bur number 204. The finished cavity has approximately 3 mm. in diameter and 3 mm. depth from the tip of the cusp (Figure 1). After that, the composite resin wall (Filtex-Flow, 3M Dental product, USA) was built up around the cavity approximately 1 mm. to made a chamber for filling the drug (Figure 1). The whole cavity was etched with 35% phosphoric acid (Scottbond multipurpose® 3M Dental Product, USA) for 20 seconds to remove smear layer.The cavity was then rinsed with water and dried by cotton pellet**.**

Pain assessment

The baseline pain sensation of the exposed etched dentine was performed with two forms of stimuli. First, a 3 seconds air blast at room temperature was directed onto the exposed dentine via triple syringe (pressure = 60 pound per $inch^2$). Later, gently stroking the exposed dentine in the middle floor of the cavity with explorer (tip diameter 0.15 mm., force approximately 20 g.). After the applications of each stimulus, the patient will be asked to rank the pain sensation using a horizontal 10 cm. visual analogue scale (VAS)

Recording of pulpal blood flow

The baseline blood flow from the teeth was recorded with a laser Doppler blood flow monitor (Periflux 4001, Perimed). Which emitted infrared light.The probe of the instrument was fixed to each tooth with a clip-on splint (Figure 2). Recording of pulpal blood flow was made for approximately 1 minute for each measurement period.

Iontophoresis

An iontophoretic device (Dentaphore-II model 611 D, Life-tech, Inc. USA) is battery-operated device, which generates a precise dose of direct current. The two electrodes were connected to a direct current source. The drugs were placed under anodal electrode (oral electrode) and the cathodal electrode was placed in the hand of the patients. (Figure 1)

Experimental design and procedure

Group 1. Iontophoresis 25 µl 10% articaine with epinephrine (1:1,000) [pH 3.4]

16 sound teeth

The teeth were isolated with opaque black rubber dam sheet, prepared the cavity approximately 3 mm. in diameter and 3 mm. depth. Clip-on splint was placed and sealed with the tooth with composite resin. The teeth were etched 20 seconds with 35% phosphoric acid (Scottbond multipurpose® 3M Dental Product, USA), rinsed and both stimuli were applied to the dentine and baseline pain perceptions were assessed with the VAS, the baseline blood flow was assessed by Laser Doppler Flow Meter. After that, the 10% articaine with 1:1,000 epinephrine 25 µl were placed in the cavity and the current was applied to the tooth 0.12 mA for 90 sec. After that, switched-off the current, rinsed the cavity with water, pain perception at T0 and blood flow at T0 were assessed again. The cavity was kept moist by placing a distilled water. Both pain and blood flow were assessed in the first 6 minutes perform every 2 minutes (T2, T4, T6) and then perform every 10 minutes until 30 minutes (T10, T20, T30).But two premolar teeth cannot measure pulpal blood flow by laser Doppler flow meter due to the position of the teeth so we assessed only VAS.

Group 2. Iontophoresis 25 µl distilled water before 25 µl 10% articaine with epinephrine (1:1,000) [pH 3.4] 6 sound teeth

The experiments were carried out on six teeth in 6 patients. Exactly the same procedures as group 1 were performed but the cavity was filled with distilled water first. Both forms of stimuli and pain assessment were performed at the time after application and every 10 minutes for 20 minutes. Then, 10% articaine with epinephrine was applied to the cavity. The pain stimuli and blood flow assessment were done again, in the first 6 minutes perform every 2 minutes (T2, T4, T6) and then perform every 10 minutes until 30 minutes (T10, T20, T30).

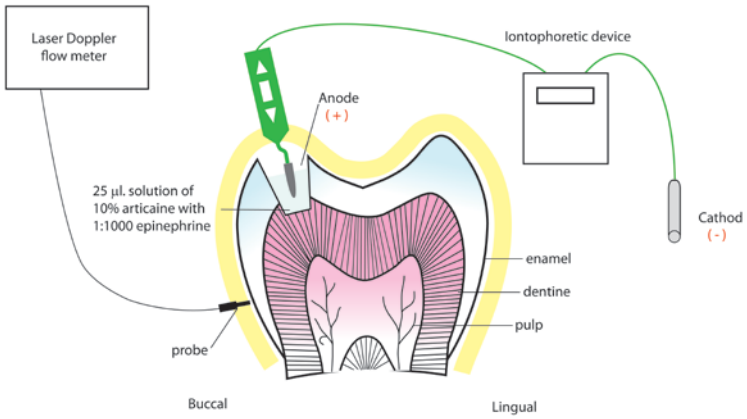

Figure 1. Schematic diagram of the prepared cavity on buccal cusp in the premolar

Statistical analyses

The differences of pulpal blood flow and VAS pain score to both forms of stimuli under different conditions were tested using one way repeated measured analysis of variance (1 Way RM ANOVA) in parametric data and 1 Way RM ANOVA on ranks in non-parametric data. At P <0.05. All pairwises multiple comparison were made using Tukey's method in parametric data and Dunnett's method in non-parametric data at P <0.05.And remaining dentine thickness were measured by T-test in parametric data at P <0.05

Results

The examples VAS and pulpal blood flow records obtained from subject in group 1 shown in Figure 3. The means baseline VAS score caused by probing and air blast obtained from group 1 experiments were 5.14 ± 1.29 cm and 7.36 ± 1.27 cm, respectively. The corresponding value significant decreased to 0 ($p<0.05$, one- way RM ANOVA on rank, Dunett's test) respectively immediately after iontophoresis. The subjects did not feel any pain to both stimuli Immediately after iontophoresis and last for 30 minutes. The corresponding pulpal blood flow values were 0.88 ± 0.05,0.28 ± 0.01, 0.28 ± 0.01, 0.26 ± 0.01, 0.24 ± 0.02, 0.21 ± 0.01, 0.19 ± 0.01, 0.20 ± 0.02 PU ($p<0.05$, one-way RM ANOVA and Tukey's test) consequently, at baseline, 0, 2, 4, 6, 10, 20 and 30 min after application. The data are shown in Figure.2

In group II is a control group,Figure 3 show the examples of pulpal blood flow records and VAS pain scores to both stimuli. The distilled water application had no significant effect on the mean VAS caused by both form of stimuli and pulpal blood flow value.The data are shown in Figure 3.

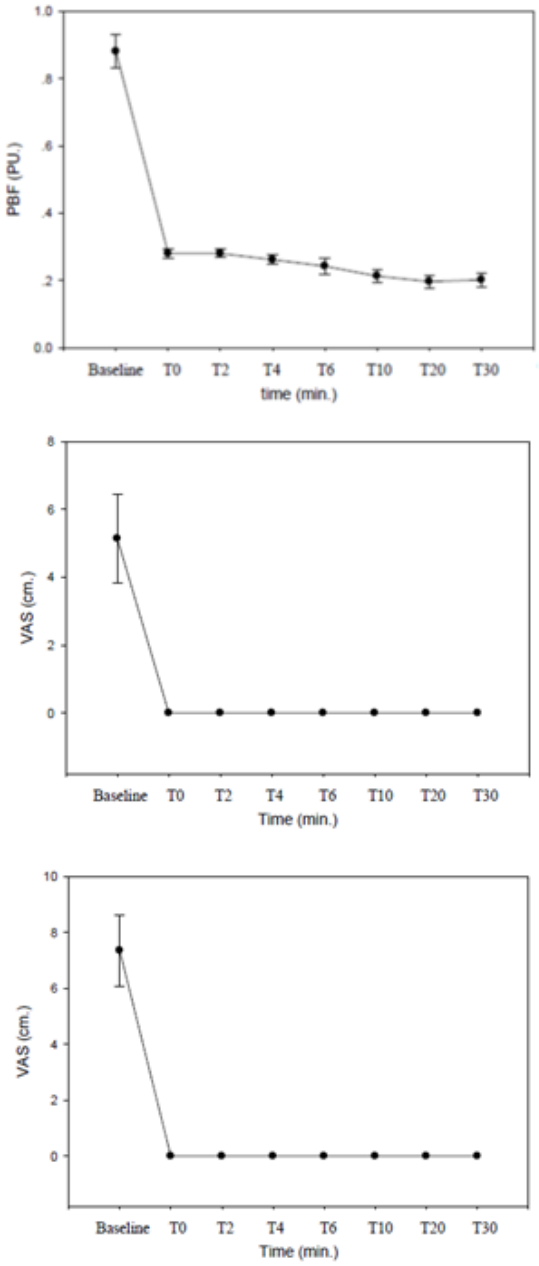

Figure 2. The mean of pulpal blood flow, VAS scores recorded with air blasting and probing stimulation of dentine obtained from subjects in group 1 (N=16)

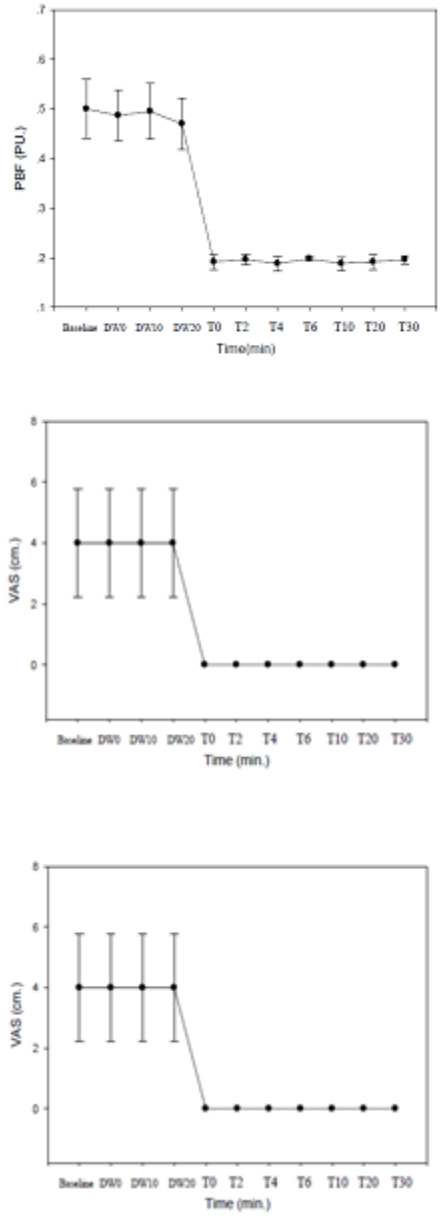

Figure 3. The mean of pulpal blood flow, VAS scores recorded with air blasting and probing stimulation of dentine obtained from subjects in group 2 (N=6)

Discussion

This study was the first to demonstrate that iontophoresis of 10 % articaine with epinephrine through the exposed dentine produces pulpal anesthesia in the premolar teeth in human subjects. The pulpal anesthesia was found immediately after iontophoretic technique. The quick onset of pulpal anesthesia from these experiments were similar to iontophoretic delivery of 20% lidocaine with epinephrine (Thongkukiatkun et al., 2015). The quick onset of pulpal anesthesia was thought that due to the properties of articaine and lidocaine,[6] because when similar methods were used with 2.5% bupivacaine with epinephrine, the mean onset was 0.67 and when mixed 1.25% bupivacaine and 10% lidocaine with epinephrine, the mean onset was quicker to 0.14 minute (Akkarachaneeyakorn, 2009). The result from the present experiment that uses articaine were more than 30 minutes that enough the time require for operative procedure such as filling the tooth with composite filling. Similar result was obtained when lidocaine and bupivacaine were used in the normal pulp.

Thongkukiatkun *et al*. reported that when the use of iontophoretic technique of 20% lidocaine with epinephrine through exposed dentine for only 90 seconds could cause pulpal anesthesia in normal premolar teeth in every case (Smitayothin et al. 2015). However, when similar method was used in carious teeth and the time used were up to 10 minutes, the pulpal anesthesia were obtained only 87.5% of cases Smitayothin et al. (2015). The unsuccessful group was thought that the human dentine was very permeable (Pashley et al., 1991; Elgalaid et al., 2007) and these caused the bacterial toxin diffused into the pulp caused pulpal inflammation Ajcharanukul et al., 2011). The inflammatory mediators such as prostaglandin E2, adenosine, and serotonin have potential to sensitize intradental fiber to develop Tetrodotoxin resistant Na^+ channels (TTX) (Renton et al, 2005; Wells et al. 2007; Warren et al. 2008; Kistner et al., 2010; Suwanchai et al., 2012). Recently, Tetrodotoxin resistant voltage gate sodium channel were reported in irreversible pulpitis in man which were NaV 1.8 andNaV 1.9 (Wells et al. 2007; Warren et al. 2008). Niu et al. (2008) demonstrated that tooth with pulpal inflammation associated with decrease in the efficacy of lidocaine and these may contribute to anesthetic failure.

Articaine was chosen in this study because there were evidences shown that it could block tetrodotoxin resistant voltage gate sodium channel than lidocaine. Bupivacaine was another local anesthetic drug that superior than lidocaine to block Tetrodotoxin resistant Na^+ channels (TTX)(Scholz et al., 1988). However, the disadvantage of bupivacaine was

that had longer onset which might not appropriate to use clinically.Therefore, the mixture of bupivacaine-lidocaine may reduce the onset of action to delivery bupivacaine through exposed dentine but it still need longer time to produce pulpal anesthesia. Articaine was the potential local anesthetic drug to use because its rapid onset and could block Tetrodotoxin resistant NaV 1.8. (Wang, et al., 2009). However, further experiment in carious teeth should be investigated in human subjects.

Articaine is one of the safer local anaesthetics due to its rapid metabolism into an inactive metabolite, decreasing the risk of systemic toxicity and overdose, even after repeated injection (Oertel et al., 1997). At present, few of the adverse reactions to articaine have been reported, including hypersensitivity, ophthalmologic complications, ischaemic skin necrosisand fever, chills and arthralgia. Controversy exists regarding articaine safety following non-surgical dental procedures with an IANB, which suggests articaine having a higher incidence of paresthesia (persistent anaesthesia or an abnormal or unprovoked sensation).

Nevertheless, direct damage to the nerve caused by 4% drugs has never been scientifically proven. Some research points to needle trauma of drugs that from dosed dependent. And a meta-analysis that compared efficacy and safety of articaine and lignocaine shown no difference in post-injection adverse events between lidocaine and articaine (Katyal, 2010). In this study did not have paresthesia in all volunteers because of the little amount of anesthetic agent of articaine were used. Which was only 2.5 mg that anesthetic agent was much less than 4% articaine in 1 catridge (68 mg). And the articaine in this study has pH 3.4 that had aciduric properties but in normal pulp had buffer capacity so the small amount of articaine wouldn’t damage pulpal tissue However, further clinical studies to evaluate adverse events of this technique in histology.

In children, the manufacturer did not recommend articaine use in children younger than four years of age, but an early retrospective report on 211 children under four years of age gave initial evidence reports no adverse systemic reactions (Wright et al., 1989). The other literature on articaine use in children shows that it is safe and effective for clinical procedures in children of all ages but should be aware that the common adverse articaine reactions in children have been reported to be numbness and soft tissue injuries, with prolonged numbness being the most common, mainly occurring in children younger than seven (Brickhouse et al., 2008; Yapp et al., 2011). But in this technique make pulpal anesthesia without soft tissue engaged so this adverse reaction didn’t be aware. Further

study should be investigate in adverse reaction to the pulpal tissue from iontophoretic technique of 10% articaine with epinephrine.

In our experiments, pain assessment was performed by probing and air blast. These stimuli caused pain when they were applied to the dentine. The transduction mechanism of dentine is caused by the movement of fluid in the dentinal tubules and disrupts the intradental nerves ending which known as “Hydrodynamic hypothesis of dentine sensitivity” (Brännström et al., 1967). It was found that intradental nerves were excited more readily by stimuli that produced fluid flow outwards from the pulp, a given amount of fluid flow generated more action potentials when the flow was directed outwards rather than inwards (Vongsavan and Matthews, 1992). They demonstrated that probing causes inward flow of fluid through dentine whereas air blast produced outward flow. The results from the present experiment confirmed the previous studies that VAS pain scores produced from air blasting stimulation were significantly greater than probing.

Iontophoresis was an alternative noninvasive technique to deliver ionized drugs into the pulp. Constant current (0.12 for 90 second) was chosen in the present study on the ground that application of direct electric current to the cervical dentine, up to 2 mA for 2 minutes caused neither histological nor ultrastructure alterations in the dog tooth pulp[44]. In vitro studies have used current of 0.1-2 mA to demonstrate current increased permeation of ions through dentinal tubules. In human 1 mA has been used in trials to increase diffusion of fluoride ion into dentine (Gangarosa, 1983). Such current intensities are likely to excite pulpal nerves and cause pain when it was applied to exposed dentin in humans. In our studies, we have used lower current intensities (0.12 mA) which made the patients felt comfortable, only 1 volunteer that felt dull pain during iontophoresis which rated in VAS about 1 and others felt comfortable without pain.

The results from this study showed that iontophoresis of distill water did not alter the pain sensation to both forms of stimuli. This indicated that electrical current could not make pulpal anesthesia which similar results reported earlier (Thongkukiatkun et al., 2015; Smitayothin et al. 2015). Blood flow significant reduction occurs after application of 10% articaine with epinephrine 1 : 1,000 under iontophoretic technique but not significant reduction when distilled water was used. Thongkukiatkun*et al.* 2015also reported that iontophoresis of epinephrine 1 : 1,000 caused similar pulpal vasoconstriction did not produce any anesthesia indicated that in this condition the pulpal vasoconstriction was not

in the stage of ischemia this vasoconstriction (Thongkukiatkun et al., 2015; Smitayothin et al. 2015).

This procedure could be a benefit tool in clinical use especially in children because its noninvasiveness and shortening the time for drug application. It might also be alternatively use to make pulpal anesthesia. such as in the hot tooth, drilling the cavity in hypersensitive teeth and pulp treatment in children. That may useful in needle phobia patient and reduce complications after needle injection such lip biting, hematoma.

However, further clinical studies to evaluate the effect of iontophoresis of 10% articaine with epinephrine (1:1,000; 1 mg/ml) to exposed carious dentine on pulpal anesthesia are needed

In conclusion, Iontophoresis of 10% articaine with epinephrine 1:1,000 (1 mg/ml) through exposed etched dentine produced pulpal anesthesia immediately (onset = 0 minute). The subjects did not report dentinal pain evoked by probing and airblast stimuli and sustained at least 30 minutes.The results from this experiments suggested that iontophoresis 10% articaine with epinephrine have potential to use for pain control in carious teeth for filling procedure especially for children or one that have needle phobia.

Acknowledgements

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Series V experiments

A comparison on the pulp vitality of the teeth anaesthetized with iontophoresis of 10% articaine with epinephrine 1:1,000through carious dentine versus standard local anesthesia technique

Abstract

Objective: The objective of this study was to compare the vitality of pulp in teeth that anesthetized with iontophoresis of 10% articaine with 1:1,000 epinephrine and standard injection technique with 4% articaine with 1:100,000 epinephrine in 6 months. The vitality of pulp was assessed electric pulp test.and pulpal blood flow recording with a laser Doppler flowmeter.

Design: The experiments were carried out on 28 carious class I molar and premolar teeth in 17 healthy subjects. In the experimental group (14 teeth), the teeth were anesthetized using iontophoresis 25 µl of 10% articaine with epinephrine 1:1,000. First, the teeth were isolated with composite resin wall and soft caries was removed with spoon. The cavity was etch with 35% phosphoric acid for 30 seconds and rinse for 10 seconds to remove smear layer. Then filled the cavity with 0.12% chlorhexidine for 1 minute to reduce bacteria the iontophoresis was performed with current of 0.12 mA for 2 or 3 minutes. In the control group (14 teeth), the teeth were anesthetized using standard inferior alveolar nerve block (IANB) or local infiltration of 1.7 ml. 4% aritcaine with epinephrine 1:100,000. The vitality of the pulp of the tooth was assessed electric pulp test (SybronEndo, USA) and pulpal blood flow recording (Periflux 4001, Sweden) at baseline , one month and six months.

Results: In both group, all teeth were positive to electric pulp test from baseline, one month and six months peroid. The EPT readings were similar from the baseline and up to 6 months. In iontophoresis group, in 8 teeth pulpal blood flow were recorded, the baseline were 1.47 ± 1.06 P.U., one month 1.31 ± 1.13 P.U. and six months 1.45 ± 1.11 P.U. repectively. There were not significantly difference (P>0.05, One Way Repeated Measures Analysis of Variance). In the standard local anesthesia technique group, the corresponding value were 1.35 ± 0.97 P.U., one month 1.39 ± 0.90 P.U. and six months 1.42 ±1.12 P.U. respectively which no significantly difference.

Conclusions: It was concluded that both teeth anesthetized with iontophoresis of 10% articaine with 1:1000 epinephrine through carious dentine and standard local anesthesia

technique of 4% articaine with 1:100,000 epinephrine present the vital and healthy pulp. It was suggested that teeth that anesthesized using iontophoretic delivery technique could be used and was safe to the pulp.

Introduction

Pain control in dental procedure usually achieved by local infiltration and inferior alveolar nerve block (Malamed, 2004). However, injection was one of the most fearful operations during dental procedure (Corah, 1985; Milgram et al., 1988). Local anesthetic injection also causes complications as hematoma, lip and cheek biting, paresthesia and trismus (Malamed, 2004). Attempted have been made on direct application of the local anesthetic agent on dentine, which, recently, Thongkukiatkun et al. (2015) demonstrated that dentine can be anaesthetised by the topical application of a solution containing 20% w/v lidocaine HCl and 0.1% w/v epinephrine HCl with iontophoresis of anodal current of 120 µA is passed for 90 s. Subsequent experiments, it was also shown that iontophoresis of a mixture of lidocaine and epinephrine into the dentine possible to anesthetise a high proportion of carious teeth, to permit cavity preparation to be carried out without pain (Smitayothin et al. 2015). During iontophoresis, there is another physical phenomenon called electroosmosis occures. Electroosmosis results when an electric field is applied to a charged membrane such as the skin and causes a solvent flow across this membrane (Sieg et al, 2004). Vongsavan and Matthews (1995) demonstrated electroosmosis in cat dentin in vivo. It was shown that there was an inward fluid flow through dentin when anodal currents were passed between the solution and the dentine. It was suggested that this inward flow of fluid might have disadvantage effects that push bacteria which present in in the carious cavity (Michelich et al., 1980) down into the pulp and cause pulp necrosis. The study was designed to evaluate the outcome of this effect, therefore the objective of this study was to compare the pulp vitality in teeth that anesthetized with iontophoresis of 10% articaine with 1:1,000 epinephrine versus conventional injection inferior alveolar nerve block 4% articaine with 1:100,000 epinephrine after 6 months. The vitality of pulp was assessed by electric pulp test and pulpal blood flow recording with a laser Doppler flowmeter.

Materials & methods

Subjects

The experiments were carried out on 28 carious molar and premolar teeth in 17 healthy subjects (age: 16 - 30 years, mean 21.7 year) with class I lesion and were needed for restoration. All teeth were vital which confirmed by clinical signs and symptoms, tooth vitality test and did not have any pathology which were assessed by dental history, clinical

sign, electric pulp test and radiographic examination. The teeth with pulpitis, pulp necrosis, partial eruption and the other classes of lesion were excluded. The experiment was approved by Ethic's Committee of Mahidol University (MU-DT/PY-IRB 2012/033.2906), and complied with the principles of the declaration of Helsinki. The experiment procedures were clearly explained to each subjects and parents or guardians before the informed consent was signed.

Assessment of pulp vitality of the teeth

Two methods were used to test the vitality of pulp. First method was electrical tooth pulp stimulation or electric pulp test. The second method was monitoring the pulpal blood flow with a laser Doppler flowmeter.

Electric pulp test

The electric pulp test was performed by using electric pulp test machine (Vitality scanner model 2006, SybronEndo, USA). After teeth were isolated, the subject was asked to hold the probe to complete the current. The control tooth which was the same type of tooth and adjacent to the tested tooth was tested first to establish the baseline response and confirm normal sensation. The tester electrode, coated with conducting medium, was put on the incisal 1/3 of buccal aspect of tested tooth. And the current was initiated. Subject was asked to remove the finger from the probe when feel warming or tingling sensation. Then the current from control and tested teeth were recorded. If there were any sign or symptom of irreversible pulpitis or pulp necrosis, that tooth would be excluded from the study.

Pulpal blood flow recording

The impression was taken to build up the working model for acrylic cap. During first and second visit laboratory work on acrylic cap was done using self-curing acrylic resin (semi-transparent, ivory color). The cap was cover clinical crown of test tooth and a socket for laser Doppler probe was made with custom tube (Ø = 1 mm.) at standard position (the central long axis of the buccal surface of the tested tooth, perpendicular to the buccal surface and its center located 3 mm from the gingival margin) and the wing of cap was extent to cover occlusal surface of adjustment teeth to make the cap stable. On the second visit, the teeth was isolated with opaque black rubber dam (Four D Rubber Co.Ltd., Heanor, England) in order to reduce to a minimum the contribution of blood flow in tissues outside the tooth. The cap was stabilized by wedge jet and acrylic cap. Then pulpal blood flow was recorded with a Laser Doppler blood flow monitor (Periflux 4001, Perimed®, Sweden) via the angle probe (401) to confirm pulpal vitality and collect the information of

baseline pulpal blood flow. Recording of pulpal blood flow were made for approximately 1 minute at each of the following time: at baseline and after filling then during the follow up period at 1 month and 6 months.

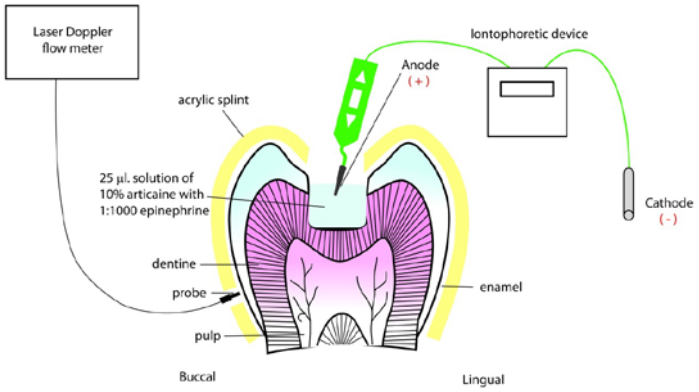

Fig. 1. Diagram of the experimental set up (not to scale).

Iontophoresis technique

The tooth was prepared with airotor until the subject complains tooth sensitivity then soft caries was removed with spoon. The cavity was etch with 35% phosphoric acid (Scottbond multipurpose® 3M Dental Product, USA) for 30 seconds and rinse for 10 seconds to remove smear layer. Then filled the cavity with 0.12% chlorhexidine for 1 minute to reduce bacteria.

An iontophoretic device (Dentaphore-II model 611 D, Life-tech, Inc. USA) is battery-operated device, which generates a precise dose of direct current. The two electrodes are connected to a direct current source. The drugs were placed under anodal electrode (oral electrode) and the cathode electrode was placed in the hand of the patients. (Fig. 1.).

Experimental design and procedure

Experimental group: the 14 carious molar and premolar teeth were iontophoresis 25 µl 10% Articaine with epinephrine (1:1,000)

The teeth were selected randomly and soft caries were removed with spoon. Then, the cavity was prepared with an airotor until the subject complained of pain. Then it was etched with 35% phosphoric acid for 15 seconds, rinsed with water and 0.12% chohexidine for 1 minute. Baseline pain sensation caused by drilling and air blast stimuli were assessed on 100 mm. Visual Analogue Scale (VAS) was recorded. After that, the cavity was filled with 25 µl of 10% articaine with 1:1,000 epinephrine. By using iontophoresis, the anesthetic agent was introduced to dentinal tubule with current of 0.12 mA for 2 minutes. Pain assessment to all stimulation was done again. If the pain still presented after iontophoresis (VAS score > 0). Supplement dose was repeated again with the same technique for another 1 minute.

Control group *(standard technique for local anesthesia)*: the 14 carious molar and premolar teeth were injected 1.7 ml of 4% Articaine with epinephrine (1:100,000)

After pulpal anesthesia was obtained cavity preparation and restoration were performed. Teeth in both groups was restored with resin composite (Z350) with three step bonding. Occlusion was checked and recorded pulpal blood flow with laser Doppler blood flow monitor under a black opaque rubber dam as after operative blood flow. Follow up phase

Each subject was asked to come back for follow up at 1 month and 6 months respectively. The tooth was examined clinically, confirmed tooth vitality by EPT and laser Doppler blood flow monitor in every recall visits. The subjects were asked about sign or symptom. In 6 months radiographic examination was taken. If any teeth has restoration defect such as marginal discoloration, partial or total loss of restoration or secondary caries was exclude from the study.

Statistical analysis

The different of flux on baseline and to period of follow up in each group were tested using one way repeated measured analysis of variance (1 Way RM ANOVA) if they are parametric and Friedman analysis if they are non-parametric**.** All pairwises multiple comparison in parametric data was made using Tukey's method at $P < 0.05$. and Dunnett's method in non-parametric data at $P < 0.05$.

Results

In all 14 teeth in iontophoresis group, 9 teeth required 2 minutes iontophoretic delivery of 10% articaine with epinephrine whereas 5 teeth required minutes to produce

pulpal anesthesia, cavity preparation and filling were carried out without pain. In all teeth were positive to electric pulp test from baseline, one month and six months peroid. The EPT readings were similar from the baseline and up to 6 months. The examples of the records of the radiograph after filling for 6 months, and pulpal blood flow from the baseline and 6 months of the teeth anesthetized using iontophoresis of 10%articaine with 1:1,000 epinephrine or standard technique of local anesthesia of of 4% articaine with 1:100,000 epinephrine were shown in Figs. 2-5.

In 8 teeth that pulpal blood flow was recorded, the baseline were 1.47 ± 1.06 P.U., one month 1.31 ± 1.13 P.U. and six months 1.45 ± 1.11 P.U. repectively. There were not significantly difference ($P>0.05$, One Way Repeated Measures Analysis of Variance). The data were summarized in Fig. 6.

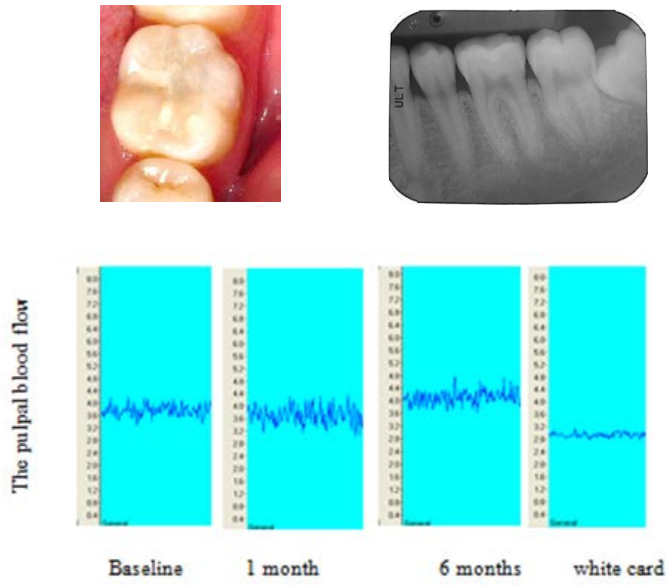

Fig. 2. The records of the photograph of tooth after filling, the radiograph after filling for 6 months, and pulpal blood flow of tooth 36 after anesthetized using iontophoresis of 10% articaine with 1:1,000 epinephrine. Pulpal blood flow was recorded at baseline, 1 month and 6 months.

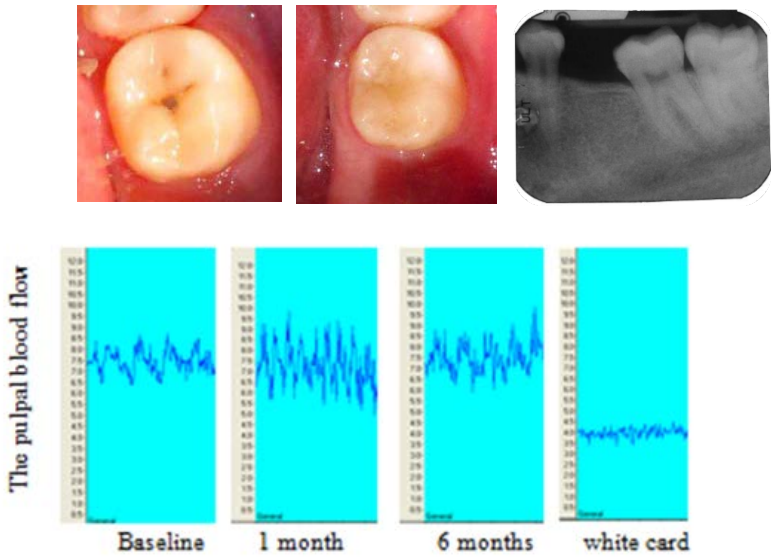

Fig. 3. The records of the photographs of tooth before and after filling, the radiograph after filling for 6 months, and pulpal blood flow of tooth 37 after anesthetized using iontophoresis of 10%articaine with 1:1,000 epinephrine. Pulpal blood flow was recorded at baseline, 1 month and 6 months.

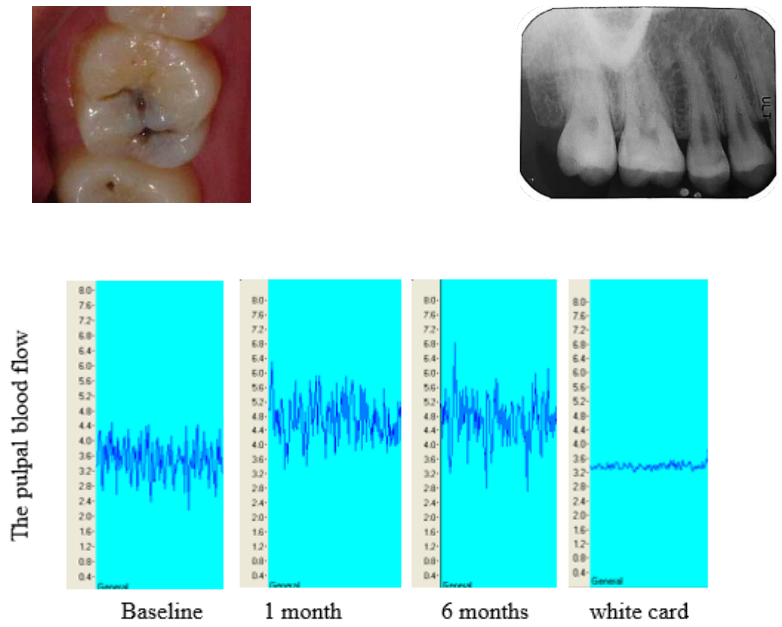

Fig. 4. The records of the photographs of tooth before and after filling, the radiograph after filling for 6 months, and pulpal blood flow of tooth 16 after anesthetized using local infiltration of 4% articaine with 1:100,000 epinephrine. Pulpal blood flow was recorded at baseline, 1 month and 6 months.

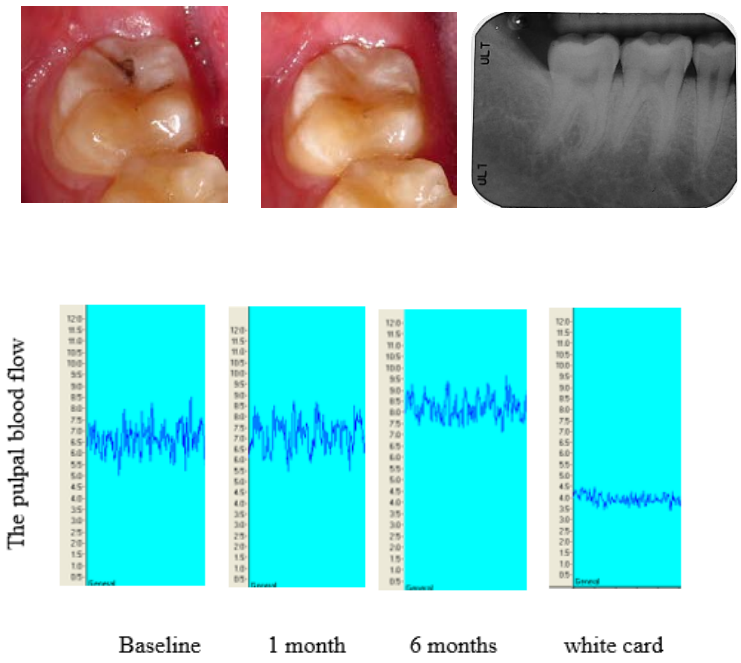

Fig. 5. The records of the photographs of tooth before and after filling, the radiograph after filling for 6 months, and pulpal blood flow of tooth 47 after anesthetized with inferior alveolar nerve block of 4% articaine with 1:100,000 epinephrine. Pulpal blood flow was recorded at baseline, 1 month and 6 months.

In the control group (14 teeth), the teeth were anesthetized with inferior alveolar nerve block or local infiltration technique of 4 % articaine with epinephrine 1:100,1000. All teeth pulpal anesthesia were obtained that cavity preparation and filling were done without pain. In all teeth were positive to electric pulp test the EPT readings were similar from the baseline and up to 6 months. In 8 teeth that pulpal blood flow was recorded, the baseline were 1.35 ± 0.97 P.U., one month 1.39 ± 0.90 P.U. and six months 1.42 ±1.12 P.U. respectively which no significantly difference (P>0.05, One Way Repeated Measures Analysis of Variance). The data were shown in Figure 6.

There were no significant different between baseline pulpal blood flow between the iontophoresis group and the injection group (P>0.05, Mann-Whitney Rank Sum Test).

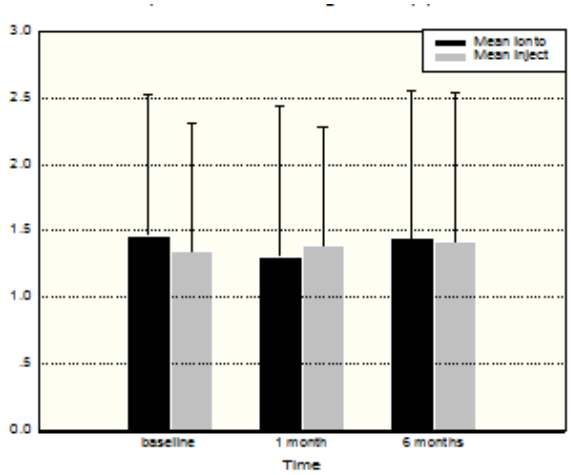

Fig. 6. Comparison of mean pulpal blood flow value from 8 teeth in each group at baseline , 1 month and six months. Black colum : Teeth anesthesized using iontophoretic delivery technique of 10% articaine with 1:1000 epinephrine through carious dentine. Gray colum : Teeth anesthesized using inferior alveolar nerve block of of 4% articaine with 1:100,000.

Discussion

The results from the present experiments demonstrated that up to six months, both the teeth that anesthezied with iontophoresis of 10% articaine with 1:1,000 epinephrine and conventional injection inferior alveolar nerve block 4% articaine with 1:100,000 epinephrine present the vital pulp that assessed by electrical stimulation and pulpal blood flow recording. During the follow up period all the subjects had no complaint on the treated teeth. None of the restorations failed (no marginal discoloration, no secondary caries, no post-operative sensitivity and normal soft tissue) and all restorations had 100% retention rate during 6 months. In both groups, the tooth vitality were confirmed with EPT and a cold test in patients who already on brace. The radiographic findings had no sign of pulp necrosis. Even none of the resent tooth vitality test represented the histologic health of pulp. In this study combination of clinical signs and symptoms, electric pulp test and radiographs use to confirm the tooth vitality and in 16 teeth (8 teeth in each group) the pulpal blood flow were used.

The results from the present experiments suggested that our method of cleasing the cavity by soft caries removal with spoon acid etcing with 35% phosphoric acid for 30 seconds and rinse for 10 secondsand filled the cavity with 0.12% chlorhexidine for 1 minute was the effective method for the bacterial removal. The results from our laboratory showed that after using this method. No bacteria were found in the dentinal tubule under SEM studied in vitro (Narkatok et al., 2020). It was suggested that it would be safe even during iontophoretic delivery of articaine and epinephrine results the inward electroosmosis flow of fluid through dentine (Vongsavan and Matthews, 1995).

Thongkukiatkun *et al*. were the first who use of this technique of iontophoretic delivery of 20% lidocaine with epinephrine through exposed sound dentine for only 90 seconds could produce pulpal anesthesia in premolar teeth. However, when a similar method was used in carious teeth even the time used were up to 10 minutes, the pulpal anesthesia were obtained only 87-95% of cases (Smitayothin, et al., 2015). The result of unsuccessful group was rethought that the intradental nerves in the pulp beneath carious dentine were sensitized and developed the socalled tetrodotoxin resistant Na^+ channels (TTX) (Renton et al., 2005; Wells et al., 2007; Warren et al., 2008; Kistner et al., 2010). Articaine was used in this study because it was reported that it was superior than lidocaine to block tetrodotoxin resistant voltage gate sodium channel. Articaine could block NaV 1.8 and also had a rapid onset (Malamed2004). Articaine is one of the safe local anesthetics

used in dentistry nowadays due to its rapid metabolism into an inactive metabolite and it decreased the risk of systemic toxicity and prevented overdose (Malamed2004). Articaine is inactivated in the liver and as well by hydrolyzed in the tissue and blood. Articaine is also the only local anesthetic agent which is inactivated in both mechanisms. The hydrolization is very fast process and start immediately after injection. About 85 to 90% of administed articaine is inactivated in this way. The main metabolic product is arti-cainic acid (or more accurately: articainic carboxylic acid), which is untoxic and inactive as local anesthetic. Less than 10% of articaine are metabolized in the liver, only about 5% is excreted unchanged. The elimination half-time of articaine is about 15 to 20 minutes. it is also safe because it is a high clearance drug with clearance of 6,000 ml/min(Becker and Reed, 2006). At present, few of adverse reactions of articaine have been report, including hypersensitivity, ophthalmologic complications, ischemic skin necrosis and fever, chills and arthralgia. The most controversy issue about articaine was the safety following non-surgical dental procedures with inferior alveolar nerve block. Haas and Lennon (1995) reported that articaine having higher incidence of paresthesia (persistant anaesthesia or an abnormal or unprovoked sensation) than lidocaine. However, some of evidences found that no significant difference in post-injection paresthesia incidences between both local anesthetic drugs. Recently, Katyal *et al*., (2010) used a meta-analysis to compare efficacy and safety of articaine and lidocaine also shown that no difference in post-injection adverse events between them.

In this study no incidence of paresthesia or other post-operative complications was found in all volunteers. After 6 months follow up, there was no clinical signs and symptoms in all teeth tested. However, further clinical studies were needed to be evaluated in longer period of time. Constant current (0.12 for 120 second) was chosen in the present study because it was used in earlier study and also did not cause any pain in the subjects. It seemed that the amount of this current did no harm to the dental pulp. Previous reported found that current up to 2 mA for 2 minutes caused neither histological nor ultrastructure alterations in the dog tooth pulp (Walton et al., 1979). In vitro studies have used current of 0.1 – 2 mA to demonstrate current increased permeation of ions through dentinal tubules (Gangarosa, 1983). In human 1 mA has been used in trials to increase diffusion of fluoride ion into dentine. Such current intensities are likely to excite pulpal nerves caused pain when it was applied to exposed dentine in humans. In our studies, we have used lower current intensities (0.12 mA) which made the patients felt comfortable. Though in nine

subjects with both treatments were done as split mouth design. There was no significant different between VAS scores on each technique application.

In our experiments, all the class I teeth were followed up for 6 months to evaluate that there was any adverse effect in this procedure which none of case was found. However, the further 1 year and 2 years followed up and others type of restoration were in the process of evaluation. It was concluded that both teeth anesthetized with 10% articaine with 1:1000 epinephrine and IANB of 4% articaine with 1:100,000 epinephrine present the vital and healthy pulp. It was suggested that teeth that anesthesized using iontophoretic delivery technique of 10% articaine with 1:1000 epinephrine through carious dentine was safe to the pulp.

Acknowledgements

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Series VI experiments

Evaluation of teeth that use the iontophoretic delivery of 10% articaine with 1:1,000 epinephrine through carious dentine for pain control in operative dentistry in man

Abstract

Objectives: The objective of this study was to evaluate the use of iontophoretic delivery the solution of 10% articaine with epinephrine 1: 1,000 through exposed carious dentine to anaesthetized dentine before cavity preparation and filling. Pulpal blood flow was recorded before and after iontophoresis. Radiographic examination, electrical pulp test and clinical assessment were performed up to 18 months.

Methods: The experiments were carried out on 18 carious premolars or molars in 9 subjects. The experiment was approved by Ethic's Committee of Mahidol University (MU-DT/PY-IRB 2012/033.2906). The teeth were isolated with composite resin wall and soft caries was removed with spoon. The cavity was etch with 35% phosphoric acid for 30 seconds and rinse for 10 seconds to remove smear layer. Then filled the cavity with 0.12% chlorhexidine for 1 minute to reduce bacteria. The teeth was isolated with opaque black rubber dam (Four D Rubber Co.Ltd., Heanor, England) in order to reduce to a minimum the contribution of blood flow in tissues outside the tooth. Pulpal blood flow was recorded with a Laser Doppler blood flow monitor (Periflux 4001, Perimed®, Sweden) before and after iontophoretic delivery the solution of 10% articaine. Radiograhic examination, Electrical pulp test and clinical evaluation were assessed 1, 6, 12 and 18 months.

Results:

The mean VAS scores from airblast were significantly decreased from 4.833 ± 1.581 to 0±0 cm. ($p<0.001$, Paired t-test) corresponding to drilling which decrease from 4.056 ± 2.127 to 0±0 ($p<0.001$, Paired t-test) after iontophoresis. In 9 teeth, the pulpal blood flow were monitored, the means pulpal blood flow at baseline were 2.3467 ± 1.6478 P.U. After iontophoresis of 10% articaine with 1:1,000 epinephrine, they were significantly decrease to 1.0722 ± 0.9105 P.U. ($P<0.005$, N=9, *Paired t-test*). The pulpal anesthesia was obtained in all tested teeth. There were 72.22 % of them pulpal anesthesia were obtained with iontophoresis time of 2 minutes while the Other 27.78% needed another 1 minute

suplement. During follow up period of 3 months, 6 months, 1 year and 18 months in all 18 teeth had no clinical sign or symptom. All subject had no signs and symptoms on the teeth. The positive response to EPT and no sign of radiographic failure.

Conclusions: It is concluded that iontophoretic delivery of 10% articaine with 1:1000 epinephrine through carious dentine could be used to anesthetize dentine prior to cavity preparation and filling and caused no adverse effects during 18 months follow up.

Introduction

Local infiltration and inferior alveolar nerve block of local anesthetic drugs are the most commonly technique used for pain control during operative procedure in dentistry (Malamed, 2004). However, needle injection is thought to be one of the most fearful operations during dental procedure (Corah, 1958; Giangrego, 1986; Milgrom et al., 1988; Weine, 1982). Attempts have been made to use alternative methods to control pain in operative procedure for example to topically application of the drugs through exposed dentine. The first success for the use of this technique reported by Amess and Matthews (1995) by using 50 % lidocaine on the third molar in human subjects for 10 minutes abolished the sensation to both airblast and probing stimuli. Rirattanapong *et al*. (2013) also reported that when filled the solution of 50% lidocaine into the cavity of premolar tooth and left for 30 minutes produced pulpal anesthesia. The disadvantage of this technique was that the time up to 30 minutes to allow lidocaine to diffuse into the pulp is still too long for clinical used. Thongkukiatkun *et al*. (2015) was reported that pulpal anesthesia was obtained after iontophoresis of 20% lidocaine with 1:1,000 epinephrine for 90 sec in the non-carious premolar teeth. With this technique, it looked very promising because it could reduce the concentration of lidocaine to only 20% and reduce the time used to only least than 2 minutes. However, when the same technique was used in carious teeth, pulpal anesthesia was obtained 87 % of teeth tested (Smitayothin et al., 2015) even the time used was up to 10 minutes. It was suggested that the pulpal anesthesia in the carious teeth in some cases could not be achieved because there was some degree of pulpal inflammation in those teeth. Inflammatory mediator, prostaglandin E2, adenosine and serotonin, was known to alter tetrodotoxin resistant sodium channels (TTX-resistant Na^+ channel) ((Wood et al., 2004). Na_V 1.8 was upregulated in the inflamed dental pulp (Suwanchai et al., 2012; Renton et al., 2005). These kind of sodium channels need much higher concentration of lidocaine to block (Gold et al., 1996). Articaine were reported that they were superior than lidocaine to block to TTX-resistant Na^+ channel in the peripheral nerve (Wang et al., 2009). The meta-analysis was found articaine advantage over lidocaine in achieving pulpal anesthesia (Brandt et al., 2011). Recently, Kittiyanpanya *et al*. (2012) use iontophoretic delivery of 10% articaine with epinephrine (1: 1,000; 1 mg/ml) for 90 second through exposed dentine in the premolar could abolish pain sensation evoked by probing and air blast stimuli and sustained at least 30 minutes in human subjects. Rukchon (2012) demonstrated that iontophoresis 10% articaine with epinephrine 1:1,000 within 3 minutes could be used to anesthetize the class I carious teeth prior to filling. The propose

of this part was to determine the pulpal vitality of the teeth that anesthetized using the method of iontophoresis of 4% articaine with 1:1,000 epinephrine through class I carious dentine before filling with composite resin. The pulpal vitality of the teeth was evaluated up to 18 months.

Materials & methods

Subjects

The experiments were carried out on 18 carious molar and premolar teeth in 9 healthy subjects (age: 16 - 30 years, mean 20.8 years) that were needed restoration. All teeth were carious dentine class I lesion, vital and did not have any pathology which were assessed by dental history, clinical and radiographic examination. (Figure 1) The teeth with pulpitis, partial eruption and the other class of lesion were excluded. The experiment was approved by Ethic 's Committee of Mahidol University (MU-DT/PY-IRB 2011/033.2906, and complied with the principles of the declaration of Helsinki. The experiment procedures were clearly explained to each subject and parents or guardians before the informed consent was signed.

General preparation

Tooth preparation

The teeth were isolated with an opaque black rubber dam sheet (Four D Rubber Co.,Ltd., Heanor, England) and soft caries was remove with spoon.(Figure 4.2). Then the cavity was prepare with the round airotor bur until the subject complained of hypersensitivity. The whole cavity was etch with 35% phosphoric acid (Scottbond multipurpose® 3M Dental Product, USA) for 15 seconds to remove a smear layer. The cavity was rinsed with water and filled 0.12% chohexidine to 1 minute for reduce the bacteria and dry by a cotton pellet.

Application of pain-producing stimuli and pain assessment

The baseline pain sensation of the exposed dentine was performed with three forms of stimuli. First, a 3-second cavity preparation with a airotor bur and a cylinder diamond bur no.204. Then, a 3-second air blast at room temperature was directed onto the exposed dentine via a triple syringe (pressure = 60 pound per $inch^2$). Last, the exposed dentine was gently stroked in the middle floor of the cavity with explorer (tip diameter 0.15 mm., force approximately 20 g.). After the applications of each stimulus, the subjects were asked to rank the baseline of pain sensation using a horizontal 100 mm. visual analogue scale (VAS). The VAS scores were recorded in the same way as iontophoresis

10% articaine with 1:1,000 epinephrine. When the VAS score was zero (subject report no pain to all stimulation) were the stage of pulpal anesthesia.

Iontophoresis

An iontophoretic device (Dentaphore-II model 611 D, Life-tech, Inc. USA) is battery-operated device, which generates a precise dose of direct current. The two electrodes are connected to a direct current source. The drug was placed under anodal electrode (oral electrode) and the cathode electrode was placed in only one hand of the patients. (Figure 4.4)

Recording of pulpal blood flow

The baseline blood flow from the teeth was recorded with a laser Doppler blood flow monitor (Periflux 4001, Perimed®, Sweden), which emitted infrared light. During recording, opaque black rubber dam (Four D Rubber Co.Ltd., Heanor, England) was applied on the tooth in order to reduce to a minimum the contribution of blood flow in tissues outside the tooth. The probe of the instrument (415-159h) was fixed to each tooth with a composite flow (Figure 4.5). Recording of pulpal blood flow were made for approximately 1 minute at each of the following time: before and after iontophoresis with 10% articaine with 1:1,000 epinephrine.

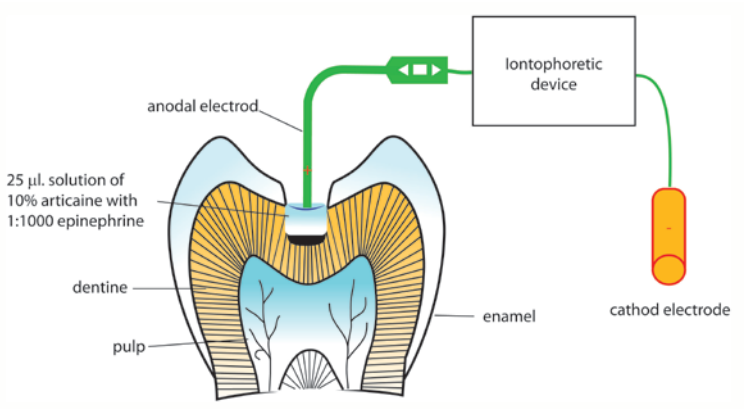


Fig. 1. Diagram of iontophoresis technique

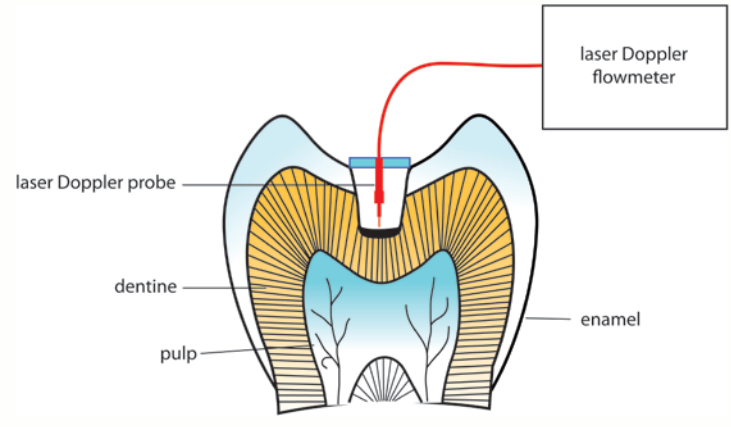


Fig. 2. Diagram of the method for recording the pulpal blood flow from the exposed carious dentine surface.

Experimental design and procedure

The 18 carious molar and premolar teeth were iontophoresis 25 µl 10% articaine with epinephrine (1:1,000). The teeth were selected randomly which isolated with an opaque black rubber dam sheet and soft caries were removed with a spoon. Then, the cavity was prepared with an airotor until the subject complained of pain. Then it was etched with 35% phosphoric acid for 15 seconds, rinsed with water and 0.12% chohexidine for 1 minute. Baseline pain sensation caused by drilling, air blast and probing stimuli was assessed on 100 mm. Visual Analogue Scale (VAS) was recorded. After that, the cavity was filled with 25 µl of 10% articaine with 1:1,000 epinephrine. Then, the drug was delivered with the iontophoretic device (Dentaphore-II model 611 D, Life-tech, Inc. USA) with current of 0.12 mA for 2 minutes. Pain assessment to all stimulation was done again. Then, cavity preparation and restoration were completed with Filtek ™ Z350 (3M Dental product, USA). If the pulpal anesthesia was not obtained (VAS score ≠ 0) after iontophoresis. Supplement dose was perform for another 1 minute. Then pain assessment was performed again. If this technique was used more than 5 minutes the standard injection anesthesia was performed. Pulpal blood flow was monitored before and after iontophoresis with a laser Doppler flowmeter on 15 teeth. Subjects were assessed satisfaction and recall 3, 6 months

Statistical analysis

The differences of flux and VAS pain scores to all forms of stimulus under different conditions were tested using Paired t-test in parametric data and Wilcoxon Signed Rank Test in non-parametric data at $P < 0.05$.

Evaluation of effectiveness

The effect of iontophoresis using articaine and epinephrine solutions on pulpal anesthesia was determined by pain stimulation and pain assessment before and after iontophoresis. The effectiveness of iontophoretic anesthesia was decided from VAS score = 0 or subjects have no pain between caries removal, airblow and probing. Assumption of success or unsuccess for inontophoresis of drugs was

- Success = obtained pulpal anesthesia after iontophoresis </= 5 min.
- Unsuccess = no pulpal anesthesia or after iontophoresis > 5 min.

Recall period

Patient were ask to come back at 3 months, 6 months, 12 months and 18 months recall for evaluate the clinical sign and symptoms, the vitality of the pulp via EPT and radiograph.

Results

A total of 18 teeth were treated in 9 subjects aged between 15 to 30 years old (mean age 20.8 ± 5.3 years old). The baseline mean VAS scores obtained from all subjects (N= 18). The examples of the records radiographs, pulpal blood flow and bar charts of VAS scores obtained from the subjects before and after iontophoresis with 10% articaine with 1:1,000 epinephrine were shown in Figs 3 and 4. The mean VAS scores from airblast were significantly decreased from 4.833 ± 1.581 to 0±0 cm. ($p<0.001$, Paired t-test) corresponding to drilling which decrease from 4.056 ± 2.127 to 0±0 ($p<0.001$, Paired t-test) after iontophoresis of drug for 2 or 3 minutes. The data were summarized in Table 1 and Fig. 5. In 9 teeth, the pulpal blood flow were monitored, the means pulpal blood flow at baseline were 2.3467 ± 1.6478 P.U. After iontophoresis of 10% articaine with 1:1,000 epinephrine, they were significantly decrease to 1.0722 ± 0.9105 P.U. ($P<0.005$, N=9, *Paired t-test*). The data were summarized in Fig. 6. The pulpal anesthesia was obtained in all tested teeth. There were 72.22 % of them pulpal anesthesia were obtained with iontophoresis time of 2 minutes while the Other 27.78% needed another 1 minute suplement. The results were shown in Fig. 7.

During follow up period of 3 months, 6 months, 1 year and 18 months in all 18 teeth had no clinical sign or symptom. All subject had no signs and symptoms on the teeth. The positive response to EPT and no sign of radiographic failure. There were 5 teeth from 4 subjects that loss follow up at 1 year but subjects came back for 18 months recall. The example VAS and graphs of pulpal blood flow obtained from subjects were shown in Fig. 8 to Fig. 13.

Table 1 The mean VAS scores (cm) from all subjects (N = 18). The records were obtained under the following conditions: baseline, after drug application at 2 and 3 minutes (T2 and T3)

	Baseline (mean±SD)	**T2 (mean±SD)**	**T3 (mean±SD)**	**P-value**
VAS scores from drilling	4.833 ± 1.581	0.6667 ± 1.1376	0±0*	P < 0.001
VAS scores from air blast	4.056 ± 2.127	0.0556 ± 0.2357	0±0*	P < 0.001

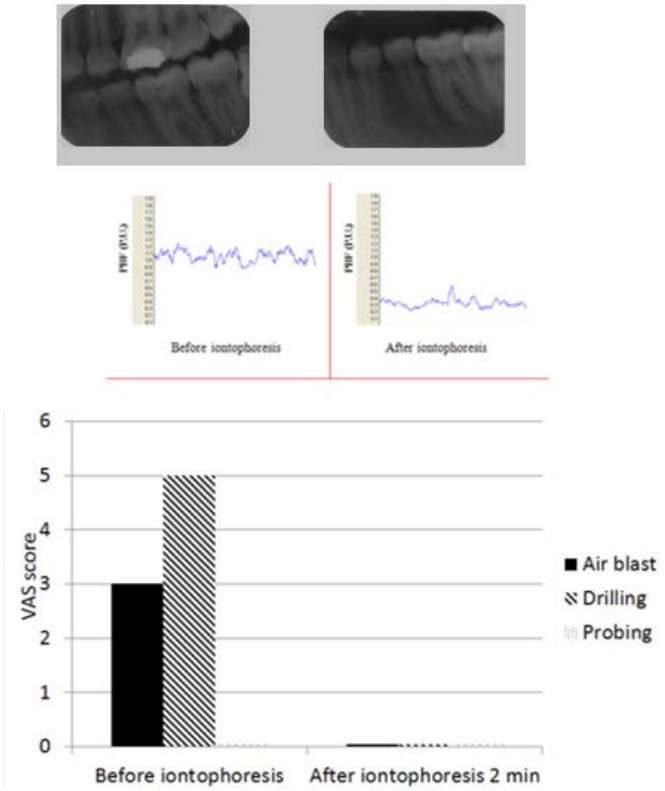


Fig. 3. The records of radiographs, pulpal blood flow and bar charts of VAS scores obtained from from tooth 36 in one subject. The records were obtained under following conditions: baseline and after iontophoresis with 10% articaine with 1:1,000 epinephrine

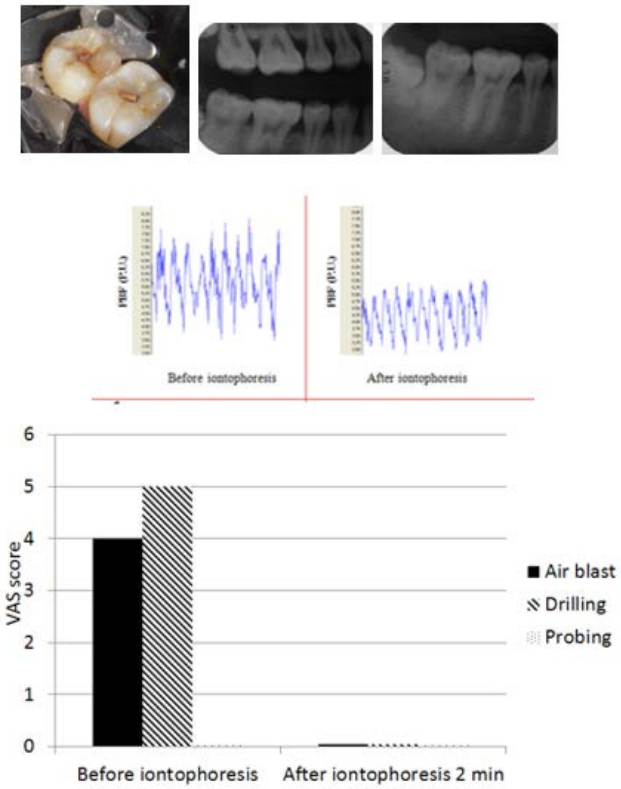


Fig. 4. The records of radiographs, pulpal blood flow and bar charts of VAS scores obtained from from tooth 46 in another subject. The records were obtained under following conditions: baseline and after iontophoresis with 10% articaine with 1:1,000 epinephrine

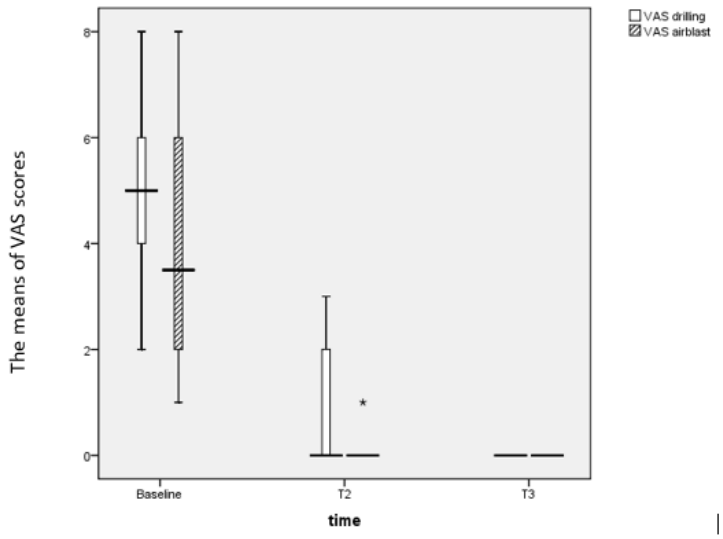


Fig. 5. The mean VAS scores recorded with drilling and air blast stimulation of carious dentine obtained from all subjects (N=18) under the following conditions: baseline, after iontophoresis of 10% articaine with 1:1,000 epinephrine for 2 and 3 minutes

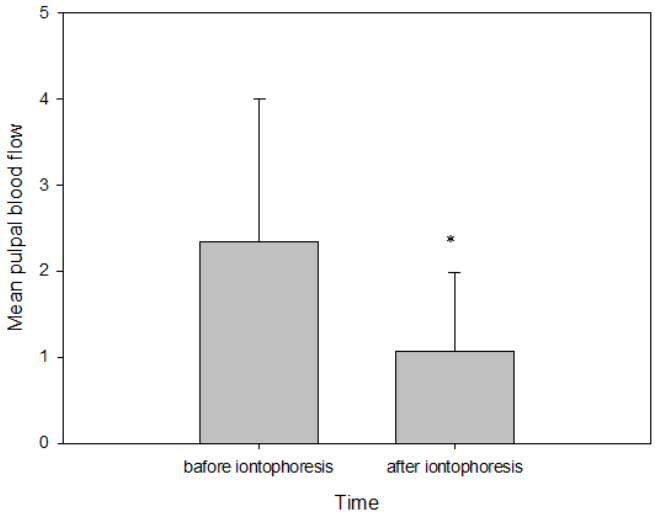


Fig. 6. The means pulpal blood flow in 9 teeth were monitored under the following conditions: before iontophoresis and after iontophoresis.

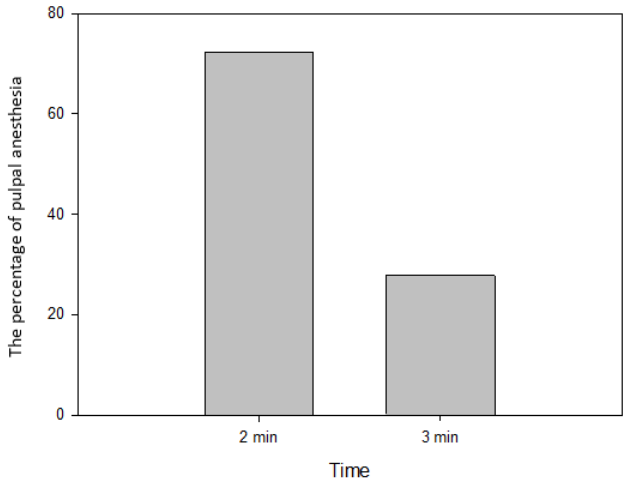
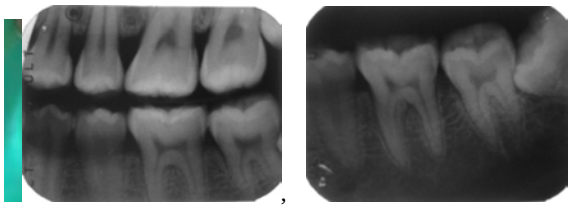
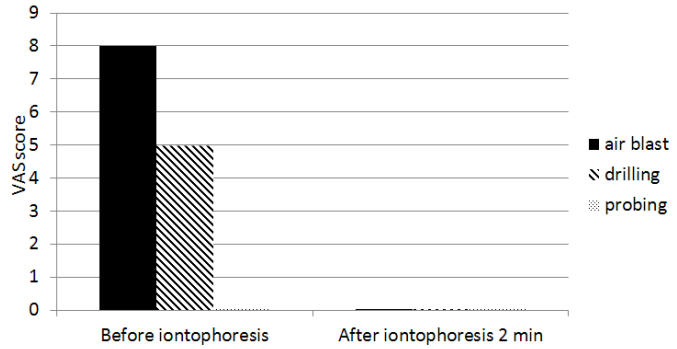


Fig. 7. The percentage of teeth that pulpal anesthesia was obtained after iontophoresis of 10% articaine with epinephrine 1:1,000. Pain was caused by drilling. The iontophoresis time used to obtain pulpal anesthesia were 2 minutes and 3 minutes.

Baseline





3 month recall

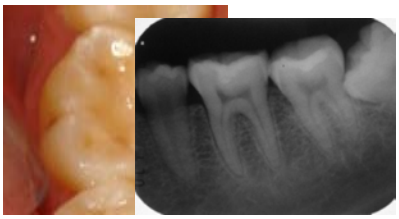
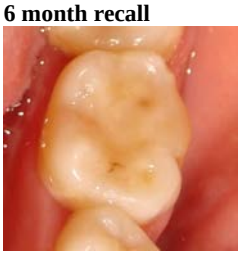
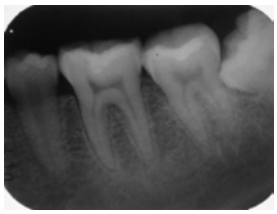


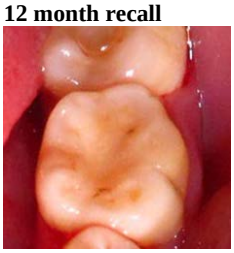
Fig. 8 The records VAS scores obtained from subject in cavity 36(O). The records were obtained under following conditions: baseline and after iontophoresis with 10% articaine with 1:1,000 epinephrine and The picture, radiographic finding at 3 months recall**.**

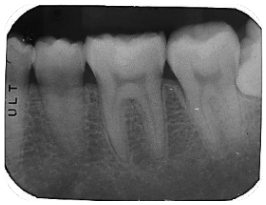
6 month recall



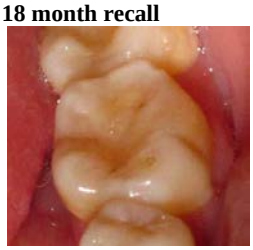


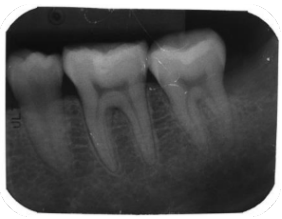
12 month recall





18 month recall

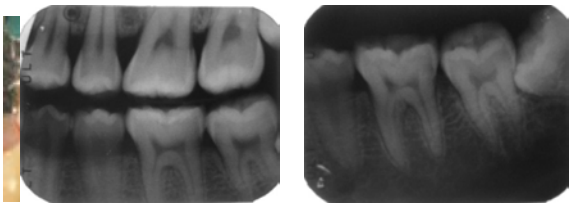


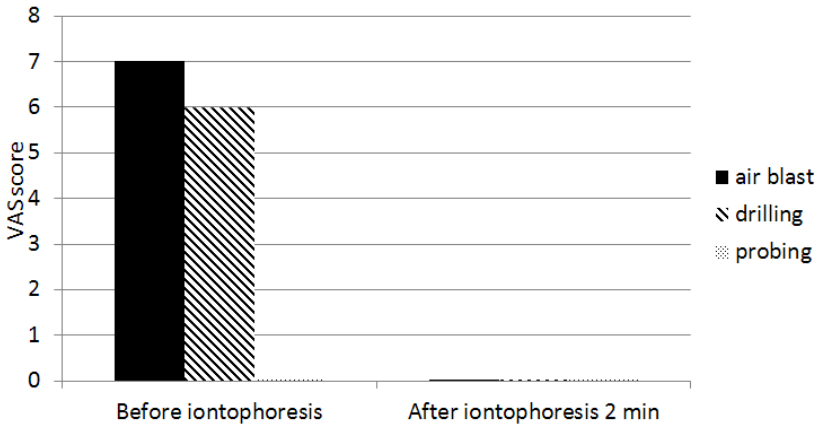


	Baseline	**3 month**	**6 months**	**12 months**	**18 months**
Clinical	No	No	No	No	No
Gingiva	Normal	Normal	Normal	Normal	Normal
Percussion	Negative	Negative	Negative	Negative	Negative
Mobility	No	No	No	No	No
Vitalometer	Positive	Positive	Positive	Positive	Positive

Fig. 9. The picture, radiographic findings of tooth 36 (same tooth as Fig. 8) and clinical sign & symptom during 18 months recall visits.

Baseline





3 month recall

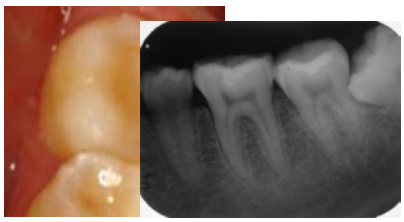
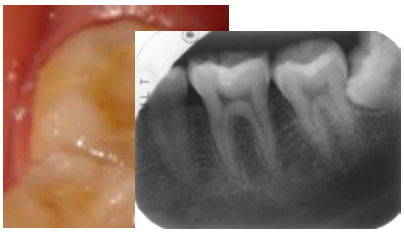
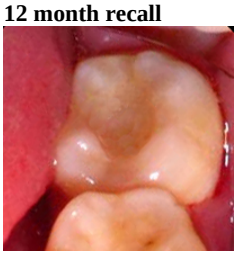


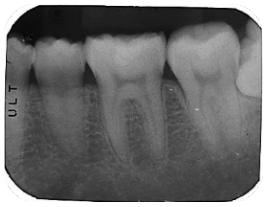
Fig. 10. The records VAS scores obtained from subject in cavity 37(O). The records were obtained under following conditions: baseline and after iontophoresis with 10% articaine with 1:1,000 epinephrine and The picture and film of tooth 37 after 3 months recall visits.

6 month recall

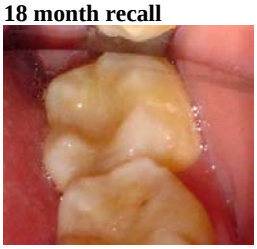


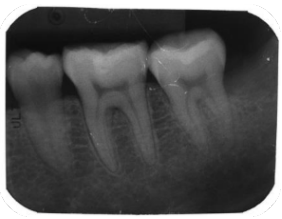
12 month recall





18 month recall

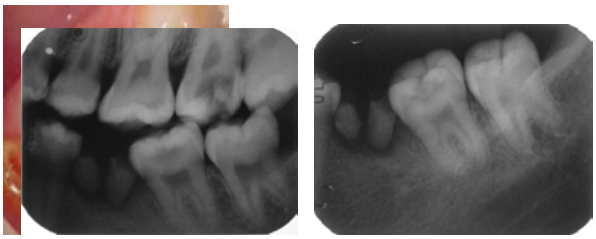


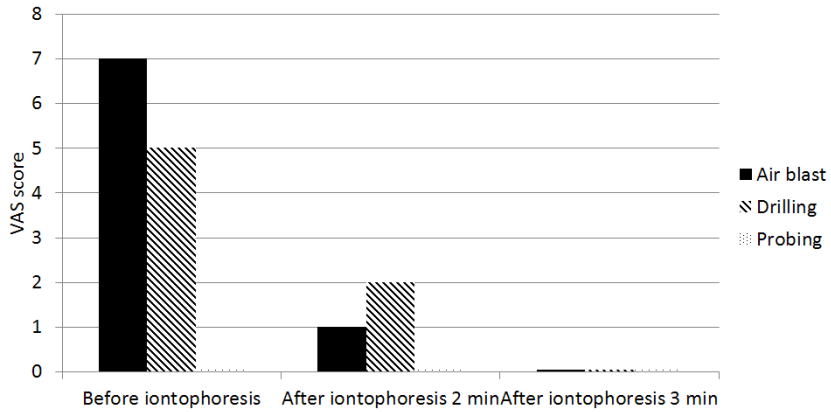


	Baseline	**3 month**	**6 months**	**12 months**	**18 months**
Clinical	No	No	No	No	No
Gingiva	Normal	Normal	Normal	Normal	Normal
Percussion	Negative	Negative	Negative	Negative	Negative
Mobility	No	No	No	No	No
Vitalometer	Positive	Positive	Positive	Positive	Positive

Fig. 11. The picture, radiographic findings of tooth 37 (same tooth as Fig. 10) and clinical sign & symptom 18 months recall visits.

Baseline





3 month recall

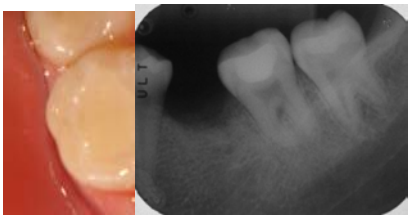
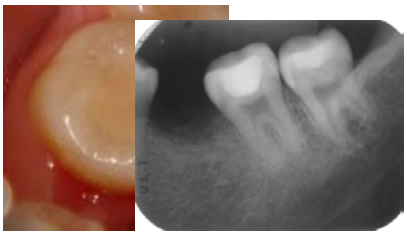
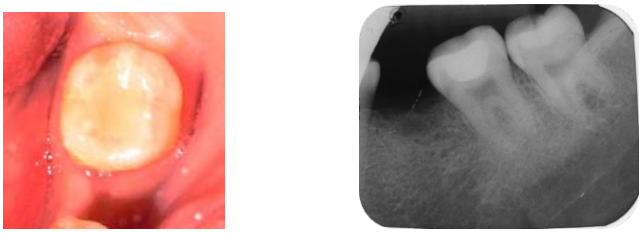


Fig. 12. The records VAS scores obtained from subject in cavity 36(O). The records were obtained under following conditions: baseline and after iontophoresis with 10% articaine with 1:1,000 epinephrine and The picture and film of tooth 37 after 3 months recall visits.

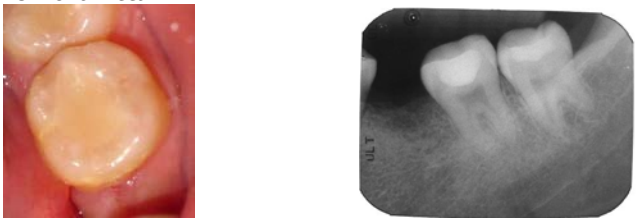
6 month recall



12 month recall



18 month recall



	Baseline	**3 month**	**6 months**	**12 months**	**18 months**
Clinical	No	No	No	No	No
Gingiva	Normal	Normal	Normal	Normal	Normal
Percussion	Negative	Negative	Negative	Negative	Negative
Mobility	No	No	No	No	No
Vitalometer	Positive	Positive	Positive	Positive	Positive

Fig. 13. The picture, radiographic findings of tooth 36 (same tooth as Fig. 12) and clinical sign & symptom during 18 months recall visits.

Discussion

The result from this study demonstrated that pulpal anesthesia could be obtained after iontophoresis of 10% articaine with epinephrine (1: 1,000; 1mg/ml) through etched carious dentine in human subjects. The technique was proof that this method could be used for pain control for cutting the dentine without injection of local anesthetic drug or needle anesthesia. The success rate of pulpal anesthesia was 100 % that all of the teeth tested the carious dentine could be completely removed and sealed with composite restoration.

Thongkukiatkun *et al*. (2015) was the first to use of the technique of iontophoretic delivery of 20% lidocaine with epinephrine through exposed dentine for only 90 seconds could produce pulpal anesthesia in premolar teeth. However, when similar method was used in carious teeth and the time used were up to 10 minutes, the pulpal anesthesia were obtained only 87% of cases (Smitayothin, et al., 2015). The unsuccessful group was thought that the intradental nerves in the pulp beneath carious dentine were sensitized and developed the so called tetrodotoxin resistant Na^+ channels (TTX). Recently, Tetrodotoxin resistant voltage gate sodium channel was reported in irreversible pulpitis in permanent teeth which were NaV 1.8 and NaV 1.9 (Suwanchai et al., 2012; Renton et al., 2005). Lidocaine was demonstrated that inferior to block tetrodotoxin resistant voltage gate sodium channels (Scholz & Vogel, 2000). Attempt have been made to better local anesthetic drug that could block tetrodotoxin resistant Na^+ channel that was bupivacaine (Scholzet al. 1998; Gioia Luigiet al., 1999). However, when bupivacaine was used in the Akkarachaneeyakorn *et al*. (2009) model, it was found that bupivacaine had longer onset which might not appropriate to use clinically. Articaine was used in this study because it was reported that it was superior than lidocaine to block tetrodotoxin resistant voltage gate sodium channel. Articaine could block NaV 1.8 and also had rapid onset (Wang et al., 2009).

Articaine was one of the safe local anesthetics used in dentistry due to its rapid metabolism into inactive metabolite and decreasing the risk of systemic toxicity and also overdose (Malamed, 2004). Articaine is inactivated in the liver as well as by hydrolyzation in the tissue and the blood. Articaine is also the only local anesthetic agent which is inactivated in both mechanisms. The hydrolization is very fast process and start immediately after injection, about 85 to 90% of administerd articaine is inactivated in this way. Main metabolic product is arti-cainic acid (or more accurately: articainic carboxylic acid), which is untoxic and inactive as local anesthetic. Less than 10% of articaine are metabolized in the liver, about 5% are excreted unchanged. The elimination half-time of

articaine is about 15 to 20 min. it is also safe because it is a high clearance drug with clearance of 6,000 ml/min (Malamed, 2004; Oertel et al., 1997). At present few of adverse reaction to articaine has been report, including hypersensitivity, ophthalmologic complications, ischemic skin necrosis and fever, chills and arthralgia (Malanin & Kalimo, 1995; Penarrocha-Diago & Sanchis-Biela, 2000). The most controversy issue about articaine was the safety following non-surgical dental procedures with inferior alveolar nerve block. Garisto *et al*, (2010) reported that articaine having higher incidence of paresthesia (persistant anaesthesia or an abnormal or unprovoked sensation) than lidocaine. However, some of evidences found that no significant difference in post-injection paresthesia incidences between both local anesthetic drugs. Recently, Katyal *et al*., (2010) used a meta-analysis to compare efficacy and safety of articaine and lidocaine also shown that no difference in post-injection adverse events between them.

In this study no incidence of paresthesia was found in all volunteers. The amount of articaine used in this study was only 2.5 mg that was much less than 4% articaine in 1 cartridge (68 mg). The articaine solution in this study has pH 3.4 that had aciduric properties but in normal pulp had buffer capacity so the small amount of articaine wouldn't damage pulpal tissue. After 18 months follow up, there was no signs and symptoms in all teeth tested. However, further clinical studies was need to evaluate in longer period of time. Iontophoresis was an alternative noninvasive technique to deliver ionized drugs into pulp. The drug diffusion was more increased by the effect of electric current on repelling ionized drugs and electrochemical potential gradient across the dentine (Gangarosa, 1983). Paupichardumrong *et al*., (2003) demonstrated that iontophoresis could enhance the delivery of ionized drug for example metronidazole, sodium salicylate, naproxen sodium through both intact and carries-affected dentine in vitro. However, when the positive current was applied to the dentine, it also caused inward flow of fluid which called "electro-osmosis" (Vongsavan &Matthews, 1995). The inward flow of fluid through dentine has the advantage of more drugs would be delivered to the dental pulp but it had also the disadvantage that it might push toxin of bacteria in the carious dentine into the dental pulp. In this study, soft carious was remove by spoon and then the cavity was rinsed with water and filled 0.12% chlohexidine for 1 minute before iontophoresis procedure. Chlohexidine was significant reduction in the number of bacteria. The cariogenic bacteria were also reduced after incompleted caries removal (Nakratok et al., 2020). Moreover, Phonghanyudh *et al*., (2012) reported that the teeth that had partial soft caries removal and

seal with GIC, the survival rate of pulp after 12 months follow up did not significant difference from the standard procedure.

Constant current (0.12 for 120 second) was chosen in the present study because it was used in earlier study and also did not cause any pain in the subjects. It seemed that the amount of this current did no harm to the dental pulp. Previous reported found that current up to 2 mA for 2 minutes caused neither histological nor ultrastructure alterations in the dog tooth pulp (Walton et al, 1979). In vitro studies have used current of 0.1 – 2 mA to demonstrate current increased permeation of ions through dentinal tubules. In human, 1 mA has been used in trials to increase diffusion of fluoride ion into dentine. Such current intensities are likely to excite pulpal nerves caused pain when it was applied to exposed dentine in humans. In our studies, we have used lower current intensities (0.12 mA) which made the patients felt comfortable.

The result from Kittiyanpanya *et al*., (2012) shown that iontophoresis of distilled water did not alter the pain sensation to air blast and probing stimuli. This indicate that electrical current could not make pulpal anesthesia. Thongkukiatkun *et al*. (2015) also reported that iontophoresis of epinephrine 1: 1,000 did not produce any anesthesia indicated that in the condition the pulpal vasoconstriction was not in the stage of ischemia enough to cause pulpal anesthesia. In this experiments, a laser Doppler meter had used to record blood flow in the dental pulp tissue. The result was shown that the pulpal blood flow was significantly reduced after iontophoresis of 10% articaine with epinephrine. This result confirmed that 10% articaine with epinephrine was reached into the pulp. The benefit of adding epinephrine into the articaine solution was that it produced pulpal vasoconstriction and lead to increase the duration of pulpal anesthesia.

In our study we used iontophoresis of 10% articaine with epinephrine through the exposed carious dentine for 3 minutes and the success rate is 100%. This procedure could be benefit tool in clinical use especially in children because its non-invasiness and shortening the time for drug application. It might also be alternatively use to make pulpal anesthesia, such as in the hot tooth, drilling the cavity in hypersensitive teeth and pulp treatment in children. That may useful in needle phobia patient and reduce complications after needle injection such as lip biting or hematoma. All of the patients in these experiments have class I dental caries. That mean we still don’t have enough information in the other class of dental caries. Further clinical study in class II dental caries and the others are needed.

In our experiments, all the subjects were followed up for 18 months to evaluate that there was any adverse effect in this procedure. All the teeth had no clinical sign or symptom, positive to electric pulp test and no sign of pulp necrosis from radiograph. Even none of resent tooth vitality test represents the histologic health of the pulp. In this study combination of clinical sign and symptoms, electric pulp test and radiograph which were commonly use in dental practice to confirm the tooth vitality.

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ตอบคำถามผู้ทรงคุณวุฒิ

คำถาม: การใช้วิธี iontophoresis จะทำให้ผลักเชื้อ bacteria ที่อยู่ใน carious dentinal tubules เข้าไปสู่ dental pulp หรือไม่?

ตอบ: การใช้วิธี iontophoresis จะไม่ผลักเชื้อ bacteria ที่อยู่ใน carious dentinal tubules เข้าไปสู่ dental pulp ด้วยเหตุผลและหลักฐาน 2 ข้อคือ

1. การใช้วิธี iontophoresis จะไม่ผลักเชื้อ bacteria ที่อยู่ใน carious dentinal tubules เข้าไปสู่ dental pulp เนื่องจาก bacteria จะมี net charge เป็นประจุลบ และ electrode ส่วนที่ carious cavity เป็นบวก (Anode)(+) ดังนั้นจะไม่ผลักเชื้อ bacteria ไปสู่ส่วน deeper dentinal tubules นอกจากจะไม่ผลักแล้วน่าจะมีผลดึงเชื้อมาสู่ carious dentin surface

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2. จากการใช้วิธีศึกษาของ Nakratok et al., (2020) ศึกษาในฟันผุที่ถอนแล้ว พบว่าการใช้วิธี iontophoresis ดังกล่าวไม่ทำให้ the distance from exposed dentine surface ที่พบ bacteria ที่อยู่ใน dentinal tubules beneath caries แตกต่างอย่างมีนัยสำคัญกับ กลุ่มที่ไม่ได้ใช้วิธี iontophoresis

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Nakrathok P, Kijsamanmith K, Vongsavan K, Rirattanapong P, Vongsavan N. The effect of selective carious tissue removal and cavity treatments on the residual intratubular bacteria in coronal dentine. J Dent Sci. 2020 (inpress).

Outputs จากโครงการวิจัยที่ได้รับทุนจาก สกว.

1. ผลงานที่ตีพิมพ์ในวารสารระดับนานาชาติ

1.1 ผลงานที่ได้รับการตีพิมพ์ในวารสารระดับนานาชาติแล้ว

Kijsamanmith K, Vongsavan N, Matthews B. Pulpal blood flow recorded from exposed dentine with a laser Doppler flow meter using red or infrared light. *Archives of Oral Biology* 2018;87: 163-167.
(impact factor = 1.663, From Journal Citation Reports®, 2018)

2. ผลงานที่ส่งเพื่อตีพิมพ์ในวารสารระดับนานาชาติ (Manuscript)

Kijsamanmith K, Vongsavan N, Matthews B. Electroosmosis in human dentine in vitro. Submitted to *Archives of Oral Biology*. ได้รับการตอบรับในเบื้องแรก ต้องปรับปรุงแก้ไข ขณะนี้อยู่ระหว่างการแก้ไขบทความเพื่อให้ได้รับการยอมรับลงตีพิมพ์

ภาคผนวก

1. Reprints ของ

Kijsamanmith K, Vongsavan N, Matthews B. Pulpal blood flow recorded from exposed dentine with a laser Doppler flow meter using red or infrared light. *Archives of Oral Biology* 2018;87: 163-167.
(impact factor = 1.663, From Journal Citation Reports®, 2018)

2. Manuscript ของ

Kijsamanmith K, Vongsavan N, Matthews B. Electroosmosis in human dentine in vitro. Submitted to *Archives of Oral Biology.*