



RUJUKAN ✓

Bahagian Penyelidikan dan Pembangunan
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11800 Minden. PULAU PINANG

Laporan akhir projek penyelidikan jangka pendek.

- 1). Ketua Penyelidik: Dr Farid Bin Che Ghazali
- 2). Penyelidik Bersama: P.M Dr Syed Hatim Noor @ Nyi Nyi Naing
- 3). **Tajuk projek penyelidikan:**

Human molar dentinal tubules and its odontoblast process. A combined investigation using variable pressure scanning electron microscope and image analysis.

- 4). **Pusat pengajian:** Sains Kesihatan (PPSK).
- 5). **Penemuan projek / abstrak:**

Introduction: Dentine is a biological mineralized tooth structure that is densely perforated with dentinal tubules that extend from the pulp chamber to the enamel-dentine junction. The presence of dentinal tubules is an attributing factor to dentine permeability (Thomas, 1985). VPSEM was used based on the inquisitive recognition of the tissue morphological characteristics in their natural hydrated state. No prior critical-drying or coating is needed. In conventional high vacuum scanning electron microscopy, specimens need to be coated with conductive material like gold to dissipate charge build up.

Objective: It is hope that insights gained from this study will further update knowledge of use to oral biology especially to the understanding of dentine sensitivity.

Methodology: The study gives an anatomical overview of the dentine morphology of partially demineralised molar human tooth preparations with special emphasis to its dentinal tubules diameter and numbers located at the middle part of the coronal end of the pulp chamber. The spatial arrangement and diameter was examined using a LEO 1455VP VPSEM without the tedious conventional scanning electron microscope tissue preparation protocols. Five caries free adult human molar tooth were used. The teeth were chemically fixed in 10% formalin immediately after extraction for 24 hours, than demineralised in 10% EDTA at pH 7.3 for 4 weeks, with regular changes of the demineralization solution. On the 4th week enamel were macroscopically absent leaving occlusal dentine exposed. The partially demineralised dentine was divided into two halves using a surgical scalpel; then rinsed with repeated changes of Ringer's solution. Each halves was examined under the VPSEM, using its peltier

cooling sub-stage at 35 Pascal pressure at an accelerating voltage of 15 kV. They were examined from occlusal to the pulpal border. Photomicrographs images of the dentinal tubules were transferred to Leica imaging workstation to enable accurate location of the study areas. Grey image processing was used to extract dentinal tubules. Grey level threshold was set to detect only the tubules. Binary image processing was used to remove little artifacts. Resultant image was measured and colour-coded in accordance to histogram classes. Statistical results for area, length, breadth, perimeter and equivalent diameter were tabulated

Results and conclusions: Demineralised dentine undergoes morphological shrinkage during normal dehydration post-demineralization and thus provides a false value of its tubule density and diameter (Garberoglio and Brannstrom 1976). In our partially demineralised dentine preparations the tubules appeared intact and were mostly empty.

References:

Thomas, H.F. (1985). The dentine-predentine complex and its permeability; Anatomical overview. *J. Dent Res.* 64: 607-612.

Garberoglio, R., Brannstrom, M. 1976. Scanning electron microscopic investigation of human dentinal tubules. *Archs oral Biol.* 21. 355-362.

6). **Senarai kata kunci yang digunakan di dalam abstrak:**

- Dentine
- Dentinal tubules,
- Odontoblast processes
- Variable pressure scanning electron microscopy (VPSEM).

7). **Output dan feadah projek**

Penerbitan

International Micrographs competitions

Micrograph competition '**Science and engineering visualization challenge**', National Science Foundation, Virginia USA April 2003. Micrograph title: Odontoblast process in undemineralised human dentine-VPSEM Photomicrograph image. Multimedia category. Co researcher: En Jamaruddin Mat Asan.

Journal/Proceeding/Abstract

Farid Che Ghazali, Ismail Abdul Manan. (2002). The coronal pulp chamber dentinal tubules; A combined investigation using variable pressure scanning electron microscopy and image analysis. First Asean Conference on Medical Sciences. 18 – 21 May, Abstract proceeding no: p-57, p83.

Farid Bin Che Ghazali, (2003). Dentine Permeability. *Malaysian Journal of Medical Sciences*. January, vol 10, no 1. 27-36. (Manuscript no: 07/2003/09).

Farid Bin Che Ghazali, (2003). High-resolution microscopy and microanalysis in oral biology and dentistry. Abstract proceeding from seminar on microscopy and microanalysis 2003. Universiti Putra Malaysia. P1-4.

8). **Faedah lain seperti perkembangan projek, prospek komersialisasi dan pendaftaran paten.**

The VPSEM morphological characterization observed and reported from this study should be an excellent database and of beneficial to the oral care worker especially research workers in dentine hypersensitivity and permeability. The information's gathered can be used to increase awareness and scientific-evidence data's thus reducing controversies in craniofacial sciences.

9). **Latihan gunatenaga manusia:**

The project has initiated training in high-resolution microscopical techniques and evidence-based microscopy to technologists based at the school of Health Sciences, USM Kubang Kerian.

The school graphic and computer officers were also exposed to high-resolution imaging especially to usage of high-end microscopical software and dye sublimation printing. These officers were exposed to micrographs preparations and competitions at the highest level in the national and international academic and research circuit.

10). **Peralatan yang telah di beli**

Peralatan (alat radas/plastik)

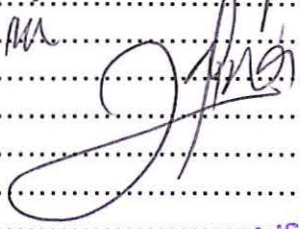
1. Stainless steel tweezers halus dan tajam	RM 400.00
2. Specimen bottles (vials) 3.8 ml	RM 200.00
3. Stationeries (A4)	RM 350.00
4. Imation super disk	RM 100.00
5. Imation super disk drive	RM 500.00
6. Ink for laser and colour printer	RM 400.00

Bahan kimia

RM 6000.00

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Timbalan Dekan
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Pusat Pengajian Sains Kesihatan
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Kampus Kesihatan



USM Short term grant final / completion report
304/PPSK/6131185

Human molar dentinal tubules and its odontoblast process. A combined investigation using variable pressure scanning electron microscope and image analysis.

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December 2004

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Author Keywords and Abbreviations: Dentine, Dentinal tubules, Odontoblast processes and Variable pressure scanning electron microscopy (VPSEM).

Abstract

Electron microscopy studies of the dentinal tubules are mainly investigated using the high vacuum conventional scanning electron microscope (SEM). We aimed to investigate its morphology using the variable pressure SEM. Three almost caries free intact adult human molar tooth were prepared as unembedded demineralised whole dentine preparations that were initially chemically fixed with 10% formalin for 24 hours. They were demineralised in 10% EDTA solution for 4 weeks following, which they were then sectioned into two equal halves. The uncoated partially demineralised dentine half tissue was inspected systematically under a LEO VPSEM using the peltier cooling sub stage at 15 Pascal pressure with an accelerating voltage of 15 kV. An occlusal and longitudinal observation was made from its occlusal surface to the pulpal border. Both low and high magnification images were prepared from each specimen observed.

Introduction

Dentine is a biological mineralised tooth structure that is densely perforated with dentinal tubules that extend from the pulp chamber to the enamel-dentine junction. The presence of dentinal tubules is an attributing factor to dentine permeability (Thomas, 1985). There are numerous literatures descriptions of odontoblast processes in dentine tubules (Ten Cate, 1967; Brannstrom and Garberoglio, 1972; Garberoglio and Brannstrom, 1976). These earlier studies measured the diameter and number of the dentinal tubules in animal and human dentition using both the light and the conventional scanning electron microscope but still the extent of the process and the volume of the dentinal tubule it occupies especially in human tooth model are some features that still remain until now controversial. At present the odontoblast layer is being suggested as a barrier. Little knowledge is known now to suggest the mechanism orchestrated by this layer. To understand the barrier there is a need to acknowledge the functional odontoblast cellular kinetics that maintains its intactness as a layer and its ultrastructural significant. This will further understanding of the role of the intercellular junctional complexes of the odontoblast layer towards exogenous permeation of fluid and substances.

In mineralised dentine there are thousands of microscopic tubules that extend from the pulp-dentine border to the enamel-dentine junction (EDJ) or cement-dentine junction (CDJ). A morphometric assessment of the cross sectional area of coronal dentine occupied by dentinal tubules in human third molars gave an incidence of 22,000 dentinal tubules per square millimetre near the enamel-dentine junction while midway between the pulp chamber and the enamel-dentine junction the number of tubules was 37,000 mm⁻². Closer to the pulp the number of dentinal tubules was 48,000 mm⁻² (Dourda, Moule and Young, 1994). Hence the numbers of tubules present per square millimetre increased from the

EDJ to the pulp (Mjor & Nordahl, 1996; Dourda, Moule & Young, 1994; Berkovitz, Holland & Moxham, 1992;).

Variable pressure scanning electron microscope is new introduction to high-resolution microscopy. The microscopical investigation using the variable pressure scanning electron microscope (VPSEM) is based on the inquisitive recognition of the tissue morphological characteristics in their natural hydrated state. No prior knowledge in staining reagents or coating is needed. In conventional high vacuum scanning electron microscopy, specimens need to be dehydrated in critical drying and then coated with a layer with conductive material like gold to dissipate charge build up. Thus VPSEM allows image to be studied uncoated and fully hydrated and reduced sample preparation requirements, in such a situation its application in particular to forensic science applications have been significantly noted.

Hals1983, and Dyngeland *et al.*, 1983, described the presence of wide diameter 'giant tubules' that was observed in human coronal dentine. The presence of these giant tubules was supported by a series of paper that was presence in animal and human tooth dentin model but a review of the literatures showed that these studies were largely investigated in unerupted anterior tooth. In a pilot study Farid *et al.*, (2001) studied on five caries free adult human molar with special emphasis to its dentinal tubules diameter and numbers located at the middle part of the coronal end of the pulp chamber. The statistical summary showed that the mean and standard deviation were: area 4.88 ± 2.43 , length 3.22 ± 0.94 , breadth 2.08 ± 0.58 , perimeter 9.93 ± 3.22 , and equivalent diameter 2.41 ± 0.62 which indicates normally distributed data.

Objective

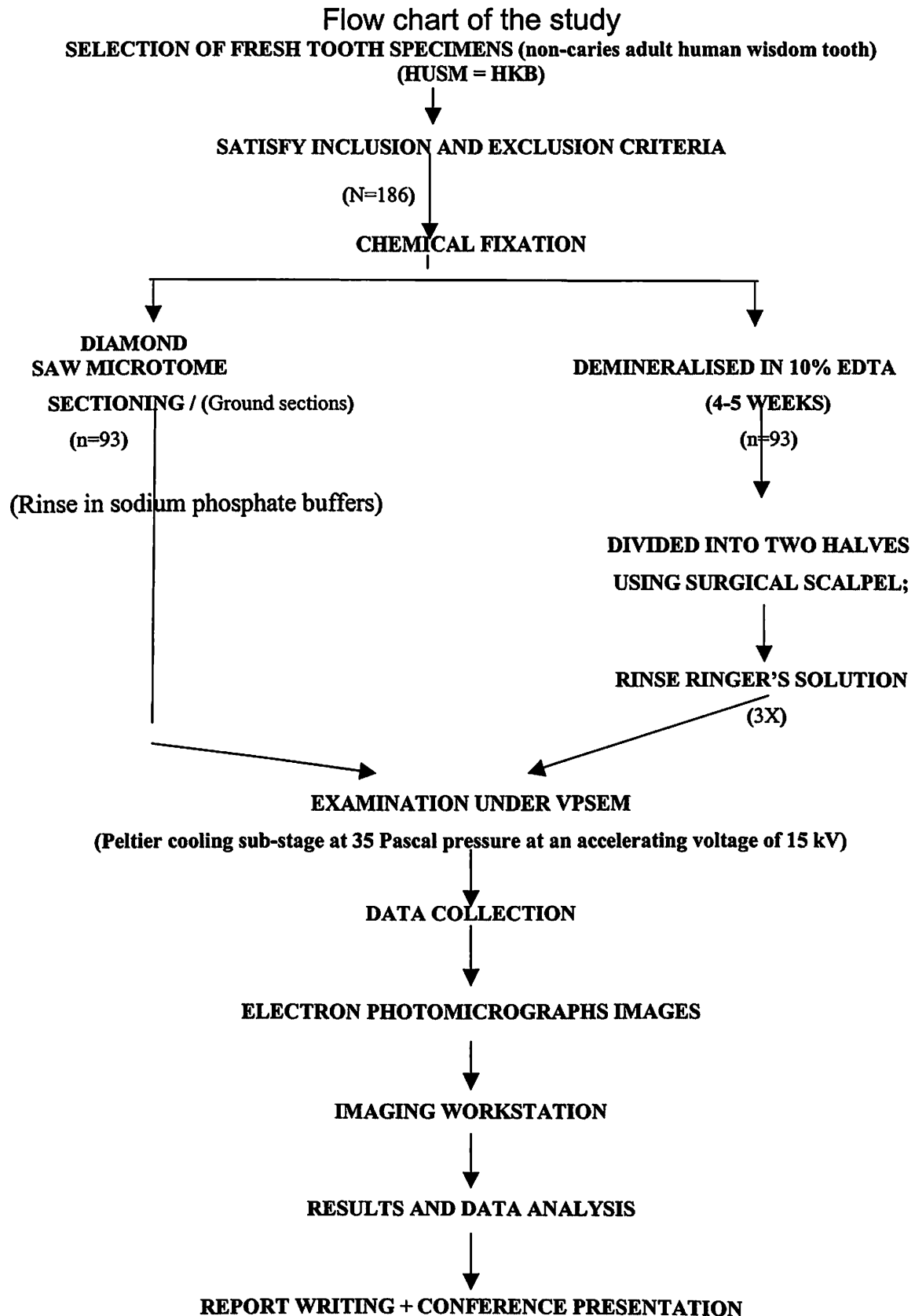
1. To gain insights of use to oral biology especially to the understanding of dentine sensitivity.
2. To observe human tooth dentinal tubule density and diameter as measured at various distances from the enamel dentine junction to the predentine-pulp interface.
3. To observe the dentinal tubule density and numbers observed under the VPSEM in almost its hydrated natural condition.
4. To observe extension of the odontoblast process in its almost hydrated natural condition in unerupted mandibular tooth /wisdom tooth'.
5. To observe the different levels of mineralisation of dentine using backscattered electron-imaging (BSE) technique of the VPSEM.
6. To compare ground and demineralised dentinal tubule's preparations measurements.

Methodology

This study was focused to recognize the natural hydrated anatomical overview of the dentine morphology in non-carious molar human tooth preparations with special emphasis to its dentinal tubules diameter, incidence and numbers located over the whole length of the dentine proper. The dentinal tubules spatial arrangement and diameter will be examined using a variable pressure scanning electron microscope LEO 1455VP and the dentine tooth preparation will be prepared without the tedious conventional scanning electron microscope tissue preparation protocols. The criteria for sample inclusion are normal healthy non-painful fresh tooth whereas exclusion of sample are caries, painful, old extracted tooth. A minimal number of 30 non-carious adult human mandibular third molar teeth will be selected. The tooth will be collected fresh from HUSM operating theatre or HKB dental surgeries. The teeth will be selected from those indicated for wisdom tooth removals especially those that is indicated for orthodontics requisite. The investigator will not make the decision for extraction. The teeth will be chemically fixed in full strength Karnovsky's immediately after surgical removal for 24 hours, then either sectioned with a diamond rotary saw microtome as ground sections or demineralised in 10% EDTA at pH 7.3 for 4 weeks, with regular changes of the demineralisation solution. Based from previous works by the 4th week enamel will be macroscopically absent leaving occlusal dentine exposed. The demineralised dentine will be divided into two equal halves using a surgical scalpel; each halves will then be rinsed with repeated changes of Ringer's solution. Each demineralised halves and ground sections will be examined under the VPSEM, using its peltier cooling sub-stage at 35 Pascal pressure at an accelerating voltage of 15 kV. They will be examined from their occlusal surface to the pulpal border. Photomicrographs images of the dentinal tubules will be transferred to Leica imaging workstation to enable accurate location of the study areas. Grey image processing was used to extract dentinal tubules. Grey level threshold will be set to detect only the tubules. Binary image processing will be used to remove little artefacts. Resultant image will be measured and colour-coded in accordance to histogram classes. Statistical results for area, length, breadth and diameter will be expected to be derived and tabulated.

Imaging Methodology:

Electron microscopy studies of the dentinal tubules are mainly investigated using the high vacuum conventional scanning electron microscope (SEM). We aimed to investigate its morphology using the variable pressure SEM. The specimens will be examined using a LEO 1455VP Variable Pressure Scanning Electron Microscope (VP SEM). In variable pressure mode, neutralization of charge build up in insulating samples was achieved by adjusting chamber pressure (range from 1Pa to 400Pa). In general, greater pressure will be used for more insulating specimens. Without variable pressure mode, the specimens will be difficult to be examined using conventional high vacuum SEM. In conventional high vacuum, specimens need to be coated with a layer with conductive material like gold to dissipate charge build up.



Sample size calculation

$$N = \frac{2\sigma^2 (Z\alpha + Z\beta)^2}{\Delta^2} \cong$$
$$N = \frac{2 \times (2.43)^2 (1.96 + 0.84)^2}{(1.0)^2} = 92.58$$

=93 per group

($Z\alpha = 1.96$, $Z\beta = 0.84$, $\sigma = 2.43$, $\Delta = 1.0$)

Reason for selecting just mandibular third molar tooth.

1. It's the last tooth to erupt into the oral cavity hence it is expected that the selected tooth specimen will be of minimal or caries free.
2. The tooth will be least exposed to mastication activities hence attrition and erosion that leads to crown fracture and present of micro leakage should be minimal or absent.
3. Medical and dental history of consenting patients will be bettered assessed on the day of surgery.
4. Finally the tooth specimen can be obtained fresh.

Outcome:

1. By measuring the diameter of the tubules better understanding of dentine permeability in human model preparation (ground and demineralised) will be derived. Thus providing further background data for further scientific researches in dentine permeability and endodontics.
2. It will enhanced better overview of the morphological changes as observed in dentine tissue especially in their hydrated state.
3. Promote mineralised tissue research especially in tooth ultrastructural hence thus to encourage collaborative research with overseas centres.
4. Transfer expertise in mineralised tissue and high-end resolution microscopy to University Sciences of Malaysia.

Results and Discussions

Demineralised dentine undergoes morphological shrinkage during normal dehydration post-demineralisation and thus provides a false value of its tubule density and diameter (Garberoglio and Brannstrom 1976). In our partially demineralised dentine preparations the tubules appeared intact and were mostly empty. Occasionally a cylindrical whitish tread like substance was observed transversing along the major length of the dentinal tubules they were suggestive of putative odontoblast processes. The dentinal tubule diameter measured at random, was approximately within 4 μm to 5 μm in diameter. Summary statistics showed that mean and standard deviation were: area 4.88 ± 2.43 , length 3.22 ± 0.94 , breadth 2.08 ± 0.58 , perimeter 9.93 ± 3.22 , and equivalent diameter 2.41 ± 0.62 which indicates normally distributed data. No intratubular dentine was

observed, both in the coronal and pulpal ends. Arends *et. al.*, (1995) ⁽¹⁾; measured the diameter of the dentinal tubules at a distance of about 1.5 mm from the pulp and found that the diameter was affected by the critical point drying techniques regularly used in conventional scanning electron microscope. In this study, the change in diameter observed in the preparations was quite minimal. In conclusion VPSEM offers a promising horizon and new challenges for further understanding especially in the extent of odontoblast process and lamina limitans morphology within dentine structure.

Conclusion

Variable pressure scanning microscopy when used to study mineralised tissue such as dentine or bone, in their hydrated natural state and with the present of other soft tissues offers a very promising potential and new challenges for further probing and understanding of various controversies still lingering especially in the extent of odontoblast process and lamina limitans morphology within dentine structure.

References

- Arends, J., Stokross, I., Jongebloed, W. G., Ruben, J. (1995). The diameter of the dentinal tubules in human coronal dentine after demineralisation and air-drying. A combined light and SEM study. *Caries Res.* 29. 118-121.
- Berkovitz, B. K. B., Holland, G. R., Moxham, B. J. (1992). Secondary dentine and post eruptive features. In: A colour atlas and textbook of oral anatomy, histology and embryology. 2nd Eds, Wolfe Publishing Ltd. 142-143.
- Brannstroms, M., Garberoglio, R. (1972). The dentinal tubules and the odontoblast process; A scanning electron microscopic study, *Acta Odontol Scand* 30: 291.
- Dourda, A. O., Moule, A. J. and Young, W. G. (1994). A morphometric analysis of the cross sectional area of dentine occupied by dentinal tubules in human third molar teeth. *Int. Endod.J.* Jul. 27 (24): 184-189.
- Dyngeland T, G. Fosse, N.P, B. Justesen (1983), Histochemical study of giant tubule content in dentine of unerupted cow incisors. *Scand J Dent Res* 92, 177-182.
- Farid Bin Che Ghazali, Nyi Nyi Naing, Ismail Abdul Manan (2001). The coronal pulp chamber dentinal tubules. A combined investigation-using variable pressure scanning electron microscopy and imaging analysis. Accepted abstract proceeding Asean Medical Conference.
- Garberoglio, R., Brannstrom, M. (1976). Scanning electron microscopic investigation of human dentinal tubules. *Archs oral Biol.* 21. 355-362.
- Hals. (1983), Observations on giant tubules in human coronal dentin by light and micro-radiography. *Scand J Dent Res* 91, 1-7.

- Mjor, I.A. Nordahl, I. (1996). The density and branching of dentinal tubules in human teeth. *Archs oral Biol.* 41, (5), 410-412.
- Ten Cate, A. R. (1967). A histochemical study of the human odontoblast. *Archs oral Biol.* 12. 963-969.
- Thomas, H.F. (1985). The dentine-predentine complex and its permeability; Anatomical overview. *J. Dent Res.* 64 (spec no): 607-612.

Acknowledgement.

The authors would like to acknowledge that the funding of this research project was supported by a USM short-term grant: 3045/PPSK/6131217. And thank Dr Endang Purwani for providing the fresh surgically removed tooth that was used in this experiment.

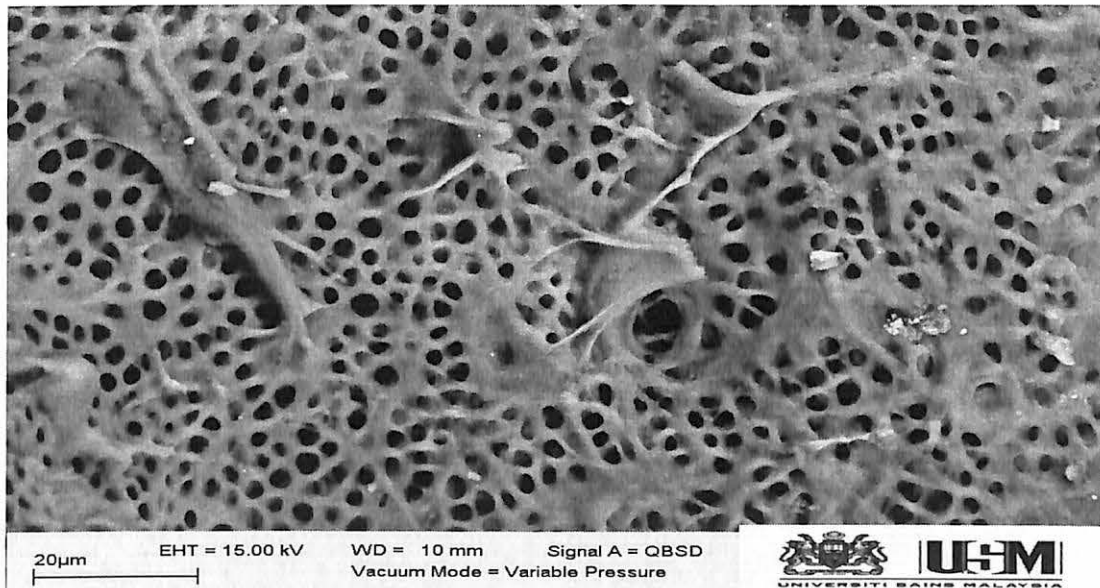


Figure 1: Dentinal tubules orifices observed at the coronal pulpal end of the dentine preparation. No intratubular dentine was observed.

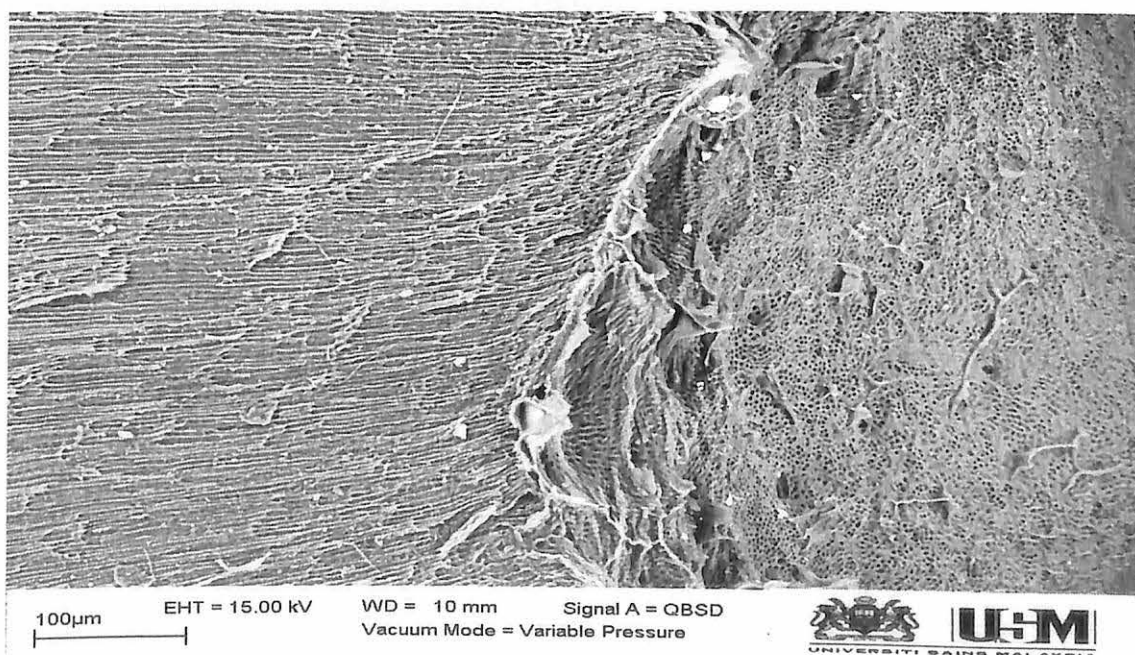


Figure II: Pulpal-predentine border showing the longitudinally running dentinal tubules and its orifice at the pulp end.

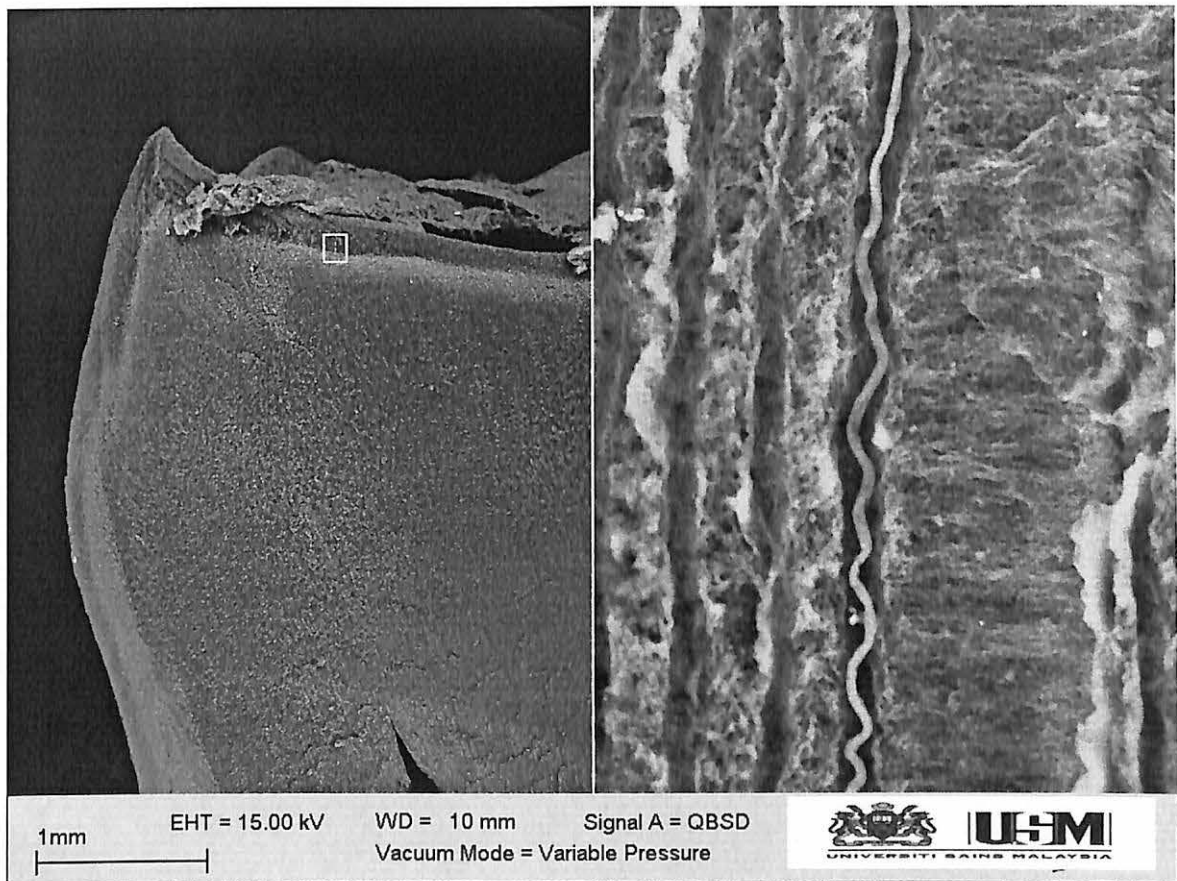
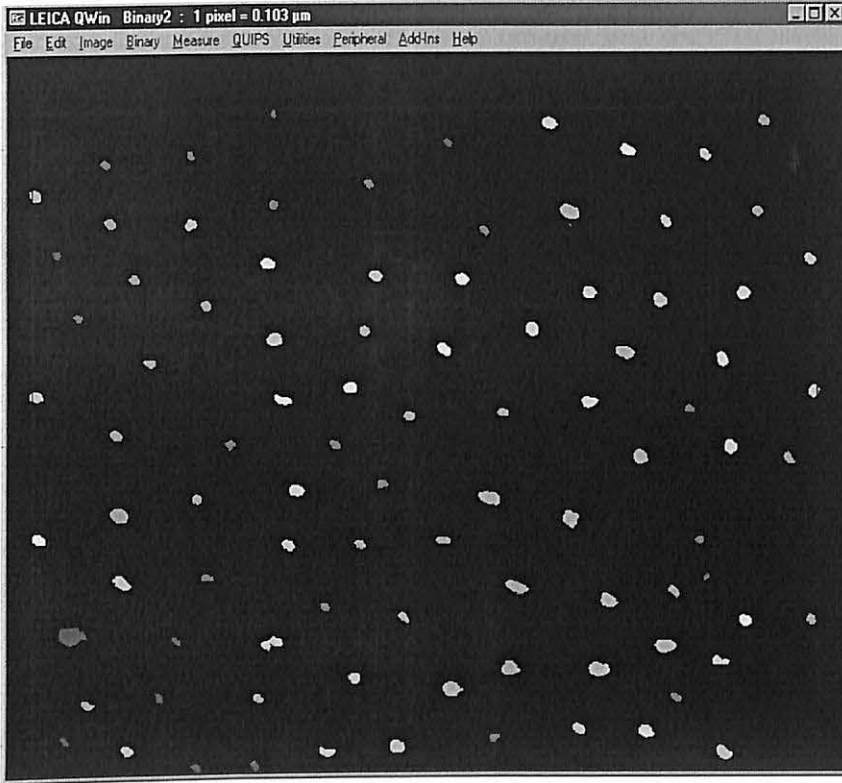
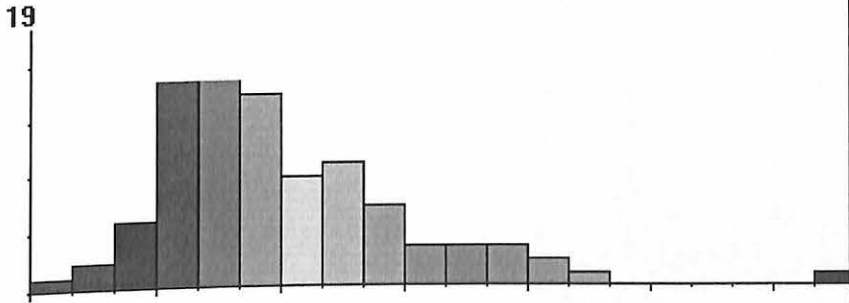


Figure III: Whitish tread like material suggestive of a putative odontoblast process observed in the coronal end of the dentine.

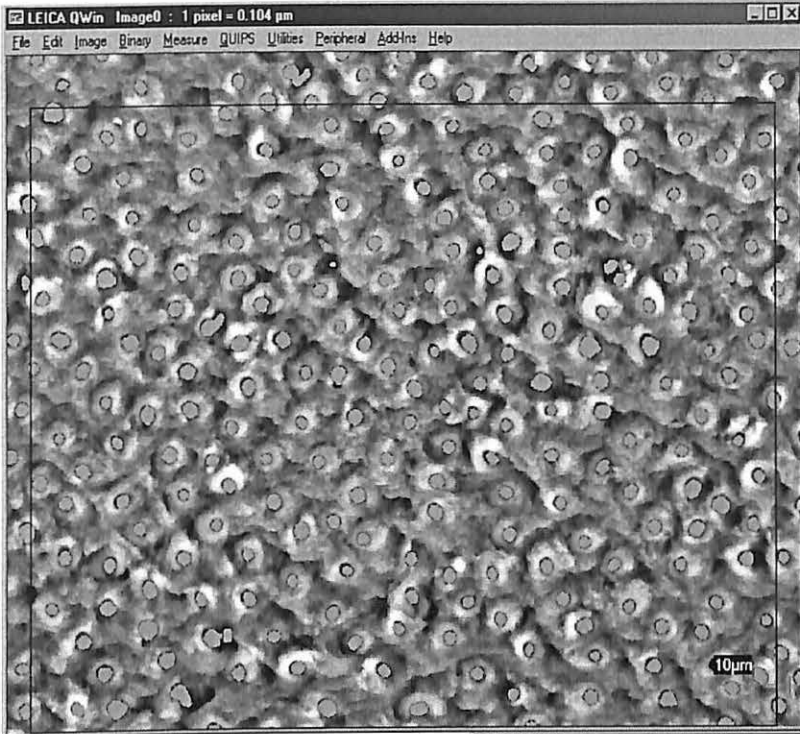
Feature Measurement



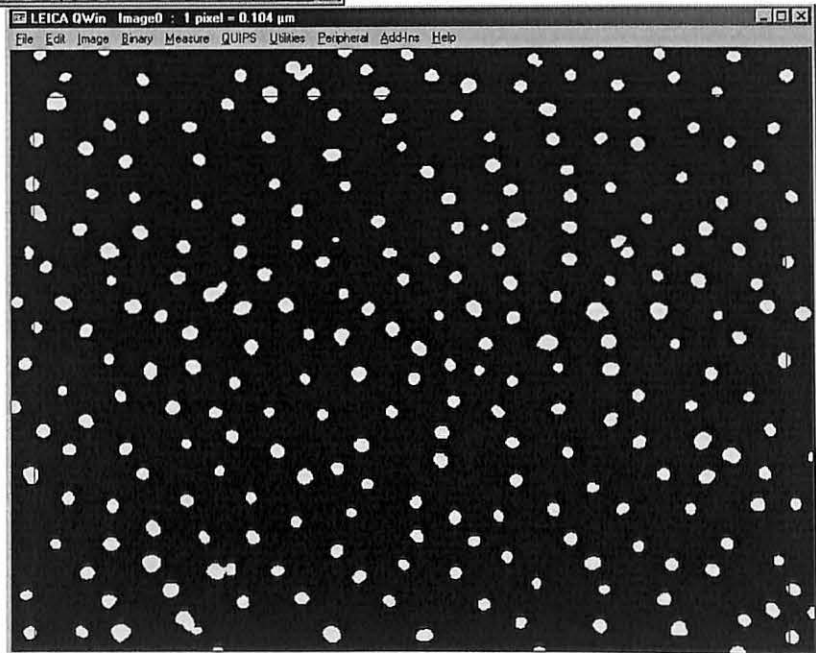
Bin	Area (μm ²)	Count	Count (%)	
1	0	0.25	1	1.06
2	0.25	0.5	2	2.13
3	0.5	0.75	5	5.32
4	0.75	1	17	18.09
5	1	1.25	19	20.21
6	1.25	1.5	14	14.89
7	1.5	1.75	8	8.51
8	1.75	2	9	9.57
9	2	2.25	6	6.38
10	2.25	2.5	3	3.19
11	2.5	2.75	3	3.19
12	2.75	3	3	3.19
13	3	3.25	2	2.13
14	3.25	3.5	1	1.06
15	3.5	3.75	0	0
16	3.75	4	0	0
17	4	4.25	0	0
18	4.25	4.5	0	0
19	4.5	4.75	0	0
20	4.75	5	1	1.06
Undersize	0		Total Count	94
Oversize C	0			
Total	140.54			
Mean	1.5			
Std Dev	0.75			
Std Error	0.08			
Max	4.93			
Min	0.12			
2-s Range	3.01			
Median	1.38			
Mode	1.13			
Skewness	1.45			
Kurtosis	3.44			
Features	94			
Spec. Area	7188.96			
Norm. Cou	13075.6			



Dentine-mid-lower-third



Area	377.722
Count	220
Area Fraction	0.053
Area Fill	0.055
Area Percent	5.254
Measure Frame	7188.965



Number	Area (µm ²)	Length (µm)	Breadth (µm)	Perimeter (µm)	Roundness
Total	381.554	358.856	295.686	1105.991	248.25
Mean	1.734	1.631	1.344	5.027	1.128
Std Dev	0.674	0.366	0.251	1.022	0.091
Std Err	0.045	0.025	0.017	0.069	0.006
Max	4.446	3.807	2.263	11.523	2.234
Min	0.296	0.72	0.617	2.263	1.045
2-s Ran	2.697	1.465	1.004	4.088	0.365
Features	220				

PERMEABILITY OF DENTINE

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This is an update on the present integrated knowledge regarding dentine permeability that assumed a role in dentine sensitivity and contribute clinically to the effective bonding properties of restorative dental materials. This paper will attempt to refer to *in vivo* and *in vitro* studies of dentine permeability and the various interrelated factors governing it.

Key words : dentinal tubules, permeability, odontoblast and fluid movement.

Introduction

Dentine forms the bulk of the tooth and is perforated by tubules that extend from the pulp to the enamel-dentine or cement-dentine junction. Dentine is a permeable mineralised tissue, which often becomes exposed by disease or restorative procedures. Once exposed the dentine can be very sensitive and any fluid within its tubules can affect the bonding properties of restorative materials. Interest in the permeability of dentine arises partly from the hydrodynamic hypothesis of dentine sensitivity. This hypothesis states that fluid movement occurs within the tubules when dentine is stimulated. The movement is thought to be rapid enough to stimulate nerve endings in the inner parts of the tubules. There is evidence for fluid movement through dentine both *in vivo* (1, 2) and *in vitro* (3).

The permeability of a material may be defined as its capacity to allow the passage of a solvent or solution through it. The permeability of a membrane to water may be total i.e. all water may pass through. On the other hand solutes in water may or may not be able to pass through depending upon the size of the solute particles with respect to the pore size in the membrane. The term reflection coefficient is used to denote the extent to which solute particles may pass across a membrane and it is assigned a numerical value of 1.0 when no particle may pass across and 0.0 when all such particles may pass through.

'The first description of permeation of substances across the dentine was given by Fritsch in 1914' (4). The relationship between differences

in the permeability of dentine and its topography has been investigated. The permeability coefficient, which is the opposite of reflection coefficient, measures the ease with which solutes can diffuse across membranes down a chemical gradient in the absence of bulk fluid movement. These terms have been taken from physiology (where the permeability of e.g. capillary membranes is of great interest) and have been extrapolated to research in dentine permeability mostly by Pashley and his colleagues (5, 6, 7). In dentine the permeability coefficient of glucose, sucrose and dextran are all different, because their molecular size and intrinsic diffusibilities are different (6). The permeability coefficient and flow rates vary widely with the highest permeability observed over the regions of the pulp horns and the lowest in the center of the occlusal dentine (7). The density and diameter of the dentinal tubules increases with dentine depth from the EDJ (Enamel dentine junction) to the pulp region. The permeability of dentine is lowest at the EDJ and highest at the pulpal ends. The permeability of dentine has been said to be related to the functional diameter of the dentinal tubules; the greater the functional diameter, the higher the flow rate and thus the rate of permeation. The permeability increases (hydraulic conductivity increases) rapidly as the pulp chamber is approached as permeability of dentine is proportional to the product of tubule numbers and diameter both of which increase as the tubules converge on the pulp (8). Thus the lower permeability of central dentine compared with that over the pulp horn might be due to fewer tubules per unit of the cross sectional area and that central

tubules may have smaller diameters because they are further away from the pulp. Each tooth appears to have its own permeability characteristics that differ from other teeth and there are also permeability differences between different age groups (6, 9).

Factors that may reduce the permeability of dentine include a) normal appositional growth of intratubular dentine, b) deposition of calcium phosphate crystals e.g. whitlockite in the tubules in the translucent zone ahead of the demineralised areas of caries, sometimes referred to as caries crystals, c) the coagulation of plasma proteins (fibrin) from pulpal blood vessels in the tubules beneath a recently cut cavity (10), d) pathological precipitation of intratubular materials i.e. mineral deposits, collagen fibrils, proteoglycan linings and bacteria and e) the formation of a smear layer of dentine debris on its cut surface during cavity preparation. All these factors can contribute to intratubular occlusion that reduces permeability.

Flow in dentine shows similarities to systems of porous media in which flow occurs through an assembly of channels that vary in diameter and shape, and such systems include membranes, plant cell walls, wood and soils. As flow in such systems is complicated by interruptions between the solvents and solutes, so flow in dentine is influenced by the presence of organic material, cellular elements and the residual dentinal fluid. Pashley, (11), has shown that evaporative, thermal and osmotic stimuli applied to the dentine surfaces can induce fluid movement. He postulates that fluid lost is replaced by transudates that arise from pulpal vessels. However, the influence of the odontoblast layer on dentine permeability has effectively been ignored in studies of fluid movement. Information on permeability and its influence on the ultrastructure of the odontoblast layer in humans is lacking.

The basic concept of fluid movement in the tubules was proposed long ago (12, 13). A summary of the forces that are thought to affect fluid flow through dentine today include the following: (1) diffusion involving concentration and electrical gradients. (2) filtration flow down a hydrostatic pressure gradient. (3) capillarity forces. (4) evaporation. (5) osmotic pressure.

An understanding of dentine permeability depends upon knowledge of fluid flow characteristics under controlled conditions. Among the factors that influence the conditions are the tissue morphology. Although there is considerable information on fluid flow, however only little information has been focused on the morphology

related to fluid flow experiments. This pre review attempts to address this issue.

Dentine permeability studies *in vitro*.

In recent decades efforts have been made study fluid flow phenomena in detail. *In vitro* studies on fluid movement provide means of studying diffusion of chemicals through dentine and thus assist the safe undertaking of clinical trials through dentine (14, 15, 16, 17). Brannstrom's significant contributions include studies on whole extracted teeth in which the pulp has been removed and the chamber filled with physiological saline. The crown was either heated or cooled with water and the solution in the chamber moved out of the apex with heat and into the tooth on cooling. It was concluded that heating the tooth induced centripetal fluid movement in the tubules i.e. towards the pulp and centrifugal fluid movement on cooling (18).

Anderson, *et al.*, (19) examined the possibility that osmotic stimuli can induce fluid movement through dentine. They placed saturated calcium chloride solution in cavities prepared in extracted teeth and observed that saline placed in the pulp chamber moved towards the cavity preparation. The fluid was observed in a capillary tube attached to the pulp chamber and movement was said to have occurred because the meniscus moved towards the pulp. This was among the early publication attempts to examine the influence of osmotic gradients on fluid movement in dentine.

The hydraulic conductance of a tissue expresses the ease in which fluid can move across unit surface area under a unit pressure per unit time (5). The gradient can be driven by hydrostatic or osmotic forces. The measurement of hydraulic conductance *in vitro* has provided information about the physical factors governing tubular fluid movements and dentine permeability. The hydraulic conductance of dentine is determined by a number of variables that include the pressure moving fluid across the dentine, the length of the dentinal tubules, the viscosity of the fluid and the radius of the tubules. In 1974 Outhwaite, *et al.*, (20), established their first *in vitro* method to measure the hydraulic conductance of dentine. The measurements were derived from experiments with a split chamber device that uses slices of coronal dentine of 1 mm thickness from extracted human third molars. These slices were placed on a piece of plexiglass with one end connected to a source of hydrostatic pressure. The other end was used to collect the fluid in order to measure the flow rate. This set-up was the

modified. (one end of the specimen was acid etched) to study convection and diffusion across dentine with active pressure with the help of a pressure transducer that created a hydrostatic pressure to activate fluid flow.

The calculation of the hydraulic conductance (L_p) of dentine was based on a mathematical equation as follows,

$$L_p = J_v / At (P)$$

where

$$L_p = \text{Hydraulic conductance in } \mu\text{L.cm}^{-2} \cdot \text{min.}^{-1} \cdot \text{cm.H}_2\text{O}^{-1}$$

$$J_v = \text{Fluid flow in } \mu\text{L.min.}$$

$$A = \text{Surface area of the dentine in cm}^2$$

$$t = \text{time}$$

$$P = \text{Hydrostatic pressure applied in cm. H}_2\text{O.}$$

The values for hydraulic conductance (L_p) in various different specimen preparations e.g. cryo preserved, unpreserved and freshly extracted teeth ranged from $1.5 \times 10^{-3} \mu\text{L.cm}^{-2} \cdot \text{min.}^{-1} \cdot \text{cm.H}_2\text{O}^{-1}$ to $8.97 \times 10^{-3} \mu\text{L.cm}^{-2} \cdot \text{min.}^{-1} \cdot \text{cm.H}_2\text{O}^{-1}$. These results have been obtained using a range of hydrostatic pressure gradients (21, 22, 23, 24). Between different research groups there has been little standardisation of hydrostatic pressure and time of application.

Pashley and his colleagues (23) have investigated numerous dentine permeability phenomena in extracted teeth and dentine disc preparations. For example they have shown that resistance to fluid movement across crown segments under pressure is composed of three parts, the pulpal resistance, the intratubular resistance and the surface (smear layer) resistance (6). According to this early study the smear layer accounted for 86% of the total resistance (25). The pressure applied across the crown segment in these experiments was high (non-physiological) and a lot of faith was placed in the integrity of the odontoblasts and pulp despite lack of chemical fixation or control of their vitality. In other experiments using dentine discs Pashley, *et al.*, (23), have demonstrated the effects of temperature and acid etching on fluid flow. An increase in temperature of 400 gave a 1.8 fold increase in fluid flow in 'unetched' (one side etched) dentine and an increase of 4 fold in dentine that had been etched on both sides. In more recent work Pashley and Matthews, (29) have shown that diffusion of molecules can occur across dentine in the opposite direction to forced convective flow.

Physiological studies by Pashley, *et al.*, (23)

and other workers (26, 27) did suggest that the hydraulic conductance of the dentine is governed by its biological variability but surprisingly there is yet to be published a proper morphological description of the dentine preparations used. In addition, there are important questions that need to be answered; these include the level of preservation of the tissue and whether it represents the condition *in vivo* or not. It would also be interesting to study the ultrastructure of the tissue following dentine permeability studies and compare them with non-experimental tissues. Another issue that is also of concern is the permeability of acellular dentine in which all the organic elements have been removed during tissue preparation. Among the methods used to remove the organic components is the maceration technique using 2.5N NaOH aqueous solution (28). Comparison of fluid flow either spontaneous or pressure induced supported by morphological investigation could offer valuable insight into the understanding of dentine permeability.

Diffusion or passive transport is the net movement of particles from an area of high concentration within a medium such as water to an area of low concentration due to the random molecular movement of particles. According to Pashley & Matthews (29) diffusion is a relatively slow process across dentine requiring 30-120 minutes depending on the size of the molecules across a thickness of 1-2 mm of dentine. Very little detailed information is available about the diffusion of drugs across dentine. Knowledge of diffusion properties in dentine may be useful for studying the diffusion of drugs across the dentine. For example is it possible for local anesthetics or medicaments to move into the pulp by intratubular diffusion? Diffusion of radionuclides such as ^{14}C -urea, ^3H -cortison, ^{65}Co -hydroxycobalamin and ^3H -tetracycline in dentine has been studied using autoradiography (30) and the diffusion of epinephrine and methylene blue through dentine by Bitter (31) and Ciarlone *et al.*, (32). Epinephrine permeability was observed to increase with decreasing thickness of the dentine disc used and increased significantly with the removal of the smear layer.

As the investigation of dentine permeability reaches new frontiers, the measurement of dentine permeability becomes more specialised. Hughes, *et al.*, (33) used scanning electro-chemical microscopy (SECM), a relatively new chemical imaging technique that can be used to measure fluid flow across individual dentine tubules. They scanned

a microscopic electrode near the surface of a dentine disc in a raster pattern. Potassium ferrocyanide was placed in a chamber below the dentine slab and allowed to diffuse across open dentinal tubules to reach the scanning electrode tip, where it was electrochemically oxidized. After obtaining the baseline diffusional transport rates across dentine they then increased the pressure of the pulpal side of the chamber to 20 cm H₂O to induce convective transport of potassium ferrocyanide along with the diffusive transport. They were then able to detect and analyse from images created to localise the fluid flow of individual dentinal tubules. The conclusion is that the fluid flow across that dentine was far from uniform and those dentinal tubules that permitted fluid flow did so at a very high velocity.

Other methods of studying dentine using complex mathematics have been introduced. Examples are an impedance technique (34), an electrochemical technique (35), and an alternating current impedance technique (36, 37, 38) but a proper description of the histology of the specimens used is still lacking.

Another possible approach to the study of dentine permeability *in vitro* is to remove the pulp from the odontoblast layer leaving the latter in situ. Evidence that such an approach might be possible is provided in studies by Kramer, (39) and Rockert (40). Chadha and Bishop (41), Ghazali and Bishop (42) showed excellent retention of postmitotic human odontoblasts after pulp removal from third molars. Recently Ghazali and Bishop (43) have improved the technique of pulp removal, as compared to the earlier study with light microscope

by Chadha and Bishop (41), odontoblasts with well defined morphology (cell bodies and cytoplasmic processes) was well retained in the dentinal tubules of third molar crowns following pulp removal. Transmission electron microscope. JEOL JEM 12 EXII The results of this study creates possibility maintaining the viability of odontoblasts retain in situ without the pulp and of investigating the permeability of that layer.

Dentine permeability studies *in vivo*.

In, Pashley *et al.* (44), published study on the hydraulic conductance of dentine in dog teeth *in vivo*; they applied positive or negative hydrostatic pressures to the exposed dentine of canines and molars. The pressure was applied by means of a conical chamber cemented to the dentine. The base of the chamber was attached to a reservoir of Dulbecco's phosphate buffered saline via a micropipette. Positive pressure was applied when the reservoir was raised above the tooth (up to 24 cm) and negative pressure was applied when the reservoir was lowered beneath the level of the teeth (up to 85 cm below). Fluid movement was observed by the movement of an air bubble within the micropipette. The bubble moved towards the tooth when the pressure was positive and away from the tooth when the pressure was negative. This implies that fluid can move into or out from the dentine and it was the first time that dentinal fluid movement was demonstrated in a live animal. At the same time these workers calculated the hydraulic conductance of the dentine and found that it was significantly

Figure 1: Transmission electron photomicrograph of chemically fixed odontoblast processes in dentinal tubules in human third molar. (JEOL JEM 1200 EXII)

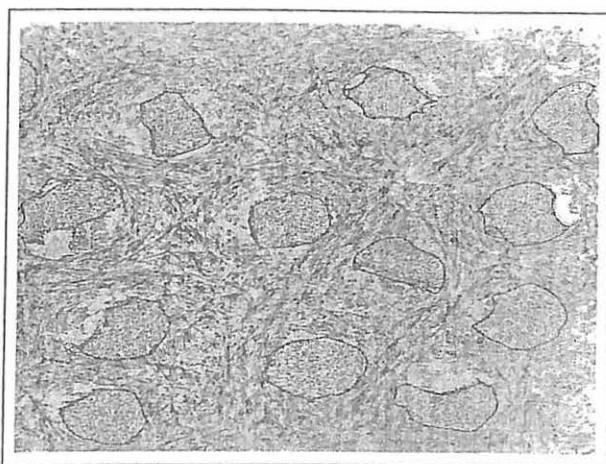
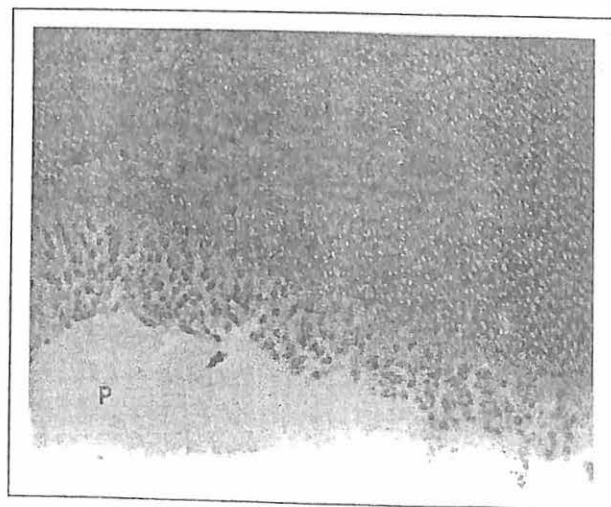


Figure 2: Light photomicrograph of odontoblast cell bodies lining the coronal surface of pulp chamber. The pulp tissues have been removed. (P=Pulp Chamber)



higher in the molars than in the canines even though the dentine thickness was comparable.

In later experiments it was shown that the smear layer influences permeability of dentine (45). Pashley, *et al.*, (46) then showed that the permeability of dentine in live dogs decreased with time. This was found to be due to the accumulation of inflammatory products including plasma protein and possibly fibrin in the tubules. Next the permeability of dentine in fibrinogen-depleted dogs was shown to be greater than that in which no fibrinogen depletion had been induced (10). Although this work is very significant it also shows that fluid movement has been demonstrated under conditions that seem to be pathological. No attempt seems to have been made by Pashley and his colleagues (44, 45, 46) to examine the morphology of their preparations. Byers, *et al.*, (47), and others (48, 49) have shown that the odontoblasts can easily be disrupted if the dentine is drilled and if deeper preparations into dentine is made the damage could be severe. In the light of the evidence for a permeability barrier consisting of tight junctions between the odontoblasts (50, 51) it is difficult to see how such rapid fluid movement can occur across dentine without its disruption.

Another significant contribution to knowledge of dentinal fluid movement has been made by Vongsavan and Matthews (1, 2). Among the most significant findings is the demonstration that fluid pours out of the cut ends of dentine tubules in cat canines (1, 2). They observed under a light microscope the procession of fat droplets in a micropipette that was connected to an exposed dentine surface of an anaesthetized cat. Thus a novel technique for measuring fluid flow across cat dentine *in vivo* has been devised. This fluid flow has been carefully monitored and its rate has been shown to increase following electrical stimulation of the sensory supply to that tooth and to decrease following sympathetic stimulation. The latter stimulus can also stop the flow or even reverse its direction. In the case of sensory stimulation it seems possible that the axon reflex may be initiated. As such stimulation of the sensory supply electrically in addition to stimulating the nerve endings may stimulate nearby vessels supplied by a branch of the same axon. This use of the axon reflex pathway results in vasodilatation of the vessels involving presumably the production of more vascular transudate all assisting to produce more fluid movement out through the tubules. By observing the movement of lipid droplets in dilute milk solution

they established that the resting outward fluid flow is around 13pl/s. The mean flow per tubule is 0.6fl/s, which corresponds to a mean tubular fluid velocity of 1.4 $\mu\text{m/s}$ at the outer dentine surface (1, 2, 52).

Vongsavan and Matthews (11) showed that the rate of outward fluid movement in cat dentine *in vivo* was sufficient to prevent the inward diffusion of Evans blue dye. This study gave evidence that the rate of inward diffusion of chemicals through exposed dentine is affected by the rate of outward flow of fluid through the dentinal tubules. In attempting to test the hydrodynamic theory of dentine sensitivity directly in cats by inducing known fluid flow rates across dentine while simultaneously recording intradental nerve activity, they reported that outward fluid movement activates intradental nerves at lower velocities than inward fluid flow (52).

Evaporation of water from dentine.

Dentine exposed to air is likely to lose water by evaporation. Air currents or air blasts such as those used clinically are probably effective in evaporating water from dentinal fluid if the humidity of the air is below 100%. The lower the humidity, the higher is the rate of water evaporation. Among the variables involved in evaporative water loss from dentinal fluid are the humidity of air, velocity of air, clinically the distance of an air syringe from a tooth and the temperature of the air (52). In 1993, Matthews and colleagues (53) reported that spontaneous evaporative water loss from dentine *in vitro* is about 1 $\mu\text{l. cm}^{-2} \cdot \text{min}^{-1}$, both in the presence or in the absence of a smear layer. It was observed that smear plugs and layers permit water vapour to permeate almost as easily as if they were absent.

The smear layer.

The smear layer is an aggregation of inorganic and organic substances or debris produced by cutting or burnishing the dentine (53, 54, 55, 56, 57). It is found on the surface of the cut dentine forming a carpet lining which covers the exposed tubules and intratubular zones. The smear layer has been found to be 1-5 μm thick and can be identified as a thin white coat in the SEM (58). It adheres tenaciously to the underlying dentine.

The presence of the smear layer has been said to have a profound influence on the permeability of dentine (11, 59). Its removal can lead to increases in the rate of fluid permeation across dentine (60, 61). In some cases the presence of the smear layer has been reported to reduce the flow rate to zero

(56). It had been shown previously by Stevansson, (62), that grinding the dentine *in vitro* markedly reduced its permeability. This reduction may have been due to the formation of a smear layer. The permeability of dentine was reported to decrease up to 35% in the presence of the smear layer (25). Other studies by Pashley and colleagues (6, 63), on the permeability of slices of human dentine with and without the smear layer showed that the presence of smear layer on both sides of the discs reduced the rate of transport of $3\text{H}_2\text{O}$ by 72% and 86% respectively. Hence it was calculated that removal of the smear layer from one side of the disc would produce a decrease in the permeability of dentine by about 40%.

Some workers have observed that following cavity preparation a number of the orifices of the cut dentinal tubules contain plugs of dentinal debris, which they called, smear plugs (64). The smear plugs extend varying distances into the dentinal tubules (65, 66) and are usually much longer than the thickness of the smear layer. The use of dilute concentrations of EDTA has been shown to remove the smear layer without removing the smear plug. The smear plug alone can prevent adhesive resin penetration into the dentinal tubules (67). On the other hand if sufficient etching time is permitted, the acid will dissolve both the smear layer and the entire length of the smear plug. While this would permit the penetration of restorative resins into the tubules, it would also permit the outward flow of dentinal fluid that may interfere with the bonding of dental restorative resin to the walls of the tubules.

The permeability of normal dentine increases following acid etching (24, 68, 53, 69). However, Kurosaki, *et al.*, (70) demonstrated that the tubules in acid etched excavated carious dentine was occluded by plugs that would make dentine impermeable. Recently Pashley, *et al.*, (71), confirmed that extremely low values for permeability in studies of excavated carious dentine.

In normal dentine which has been cut the combination of smear layer and smear plug forms a continuum that is responsible for reducing the permeability of dentine.

Bacteria, caries and dentine permeability.

Dental caries is a multifactorial tooth disease that results in various mineralisation changes in the tooth structure. There has been intense interest in the permeability of sclerotic and tertiary dentine because it can form beneath carious dentine in a symptom free tooth. Studies with the use of dyes,

solvent and radioactive ions have shown that sclerotic dentine production can cause a decrease in the permeability of dentine (72). The presences of caries crystals are also believed to be effective in blocking fluid movement within the tubules (71, 73, 74). Some workers (75, 76) believed that these two types of dentine were permeable but Pashley in 1989 (73), suggested that the presence of sclerotic dentine would enhance the role of dentine as a barrier. The irregular reparative dentine was said to reduce permeability and fluid flow in dentine and thus decrease dentine sensitivity by blocking the pulpal ends of the tubules. Regular secondary dentine on the other hand only affected the permeability by increasing diffusion distances (77).

Over the years bacteriological studies have also demonstrated that dentine can act as an effective barrier to bacterial infiltration (64, 78). On an exposed cut surface of dentine, generally it was thought that the presence of a smear layer would prevent microorganisms from invading into the dentinal tubules but these studies have shown that even in its absence, if the microorganisms are able to penetrate into the tubules, their advancement will be halted. The microorganisms have great difficulty penetrating the whole thickness of the dentine due to the varying shape and diversity of the tubules. The physical presence of the microorganisms entrapped within the dentinal tubules is thought to reduce dentine permeability. Trowbridge, (79) suggested that the any entrapped bacteria can interact with the local intratubular dentine. This, Trowbridge believed would result in the formation of caries crystals and sclerotic dentine that would reduce the permeability of that dentine. The permeability properties of the dentinal tubules indicate that functionally they have much smaller dimensions than their actual microscope dimension (5, 74). Although the microscopic diameter of the dentinal tubules at the EDJ has been reported to be $0.5\ \mu\text{m}$ to $0.9\ \mu\text{m}$; it was suggested that they have a diameter of $0.1\ \mu\text{m}$. To support this, Pashley in 1985 (55) reported that dentine was able to remove 99.8% of a bacterial suspension that had diameters of approximately $0.5\ \mu\text{m}$.

Effect of storage medium on permeability.

Investigators have used many different types of storage solutions and storage time periods for teeth prior to their permeability studies. Dentine discs or crown preparations have also been stored before permeability experiments were performed. The type of storage medium affects the permeability

of dentine *in vitro*. However there is no standard storage protocol for either whole teeth or prepared specimens. The solutions used for storage have included distilled water (80, 81), formalin (82, 83), ethanol (84, 85) saline solutions (86, 65) and phosphate buffer's solutions (8). Most of the data have not compared the effects of one storage solution to another and therefore it is possible that the results of any one study cannot be compared directly to any other study (87). Salt precipitation has been observed to occur in dentine stored in sodium phosphate buffered saline, which could explain its decrease in permeability over time (46). Elsewhere the decrease in permeability due to type of storage medium used was due to dissolution and redeposition of either mineral or organic components of the dentine into the lumen of the tubules (88, 89).

The use of fixatives was reported to give low but consistent permeability values (87). The fact that fixed dentine showed little variation in permeability suggests that the residual material in the tubules such as the odontoblast process and collagen were controlled (preserved). Outhwaite, *et al.*, (8) reported that there was a modest increase in dentine permeability between hour one two days after extraction. No further changes were observed over the following 3-4 weeks. Reeder, *et al.*, (24) used various storage times between 2 days and 1 month in their studies to determine the hydraulic conductance across dentine but did not mention whether the different times of storage had any significant influence on conductance. Goodis, *et al.*, (90) stated that individual specimens have variable permeability in different solutions and thus different solutions affect permeability differently.

Pashley and Matthews (29) reported that steam sterilisation did not alter dentine permeability. Apparently neither high nor low temperatures can alter the functional properties of dentine as long as the teeth are intact and dentine is mineralised. (91). On the other hand refrigerated storage with antiseptics modifies dentine permeability by intratubular precipitation of mineral components (92) or organic components such as odontoblast processes, or intratubular collagen fibres (87, 90).

In any dentine permeability experiment performed *in vitro* the variables should be controlled as well as possible. It is quite clear that much work in this field has been done in specimens that have been stored.

Conclusion

The understanding of the importance morphological role of the odontoblasts and its processes within the dentinal tubules and the tubules physiological characteristics is a structural linked asset to dentine fluid flow and its permeability. Controlled clinical trials should not underestimate this role as a gold standard in clinical trial to effective prompt treatment of dentine hypersensitivity especially with the usage of dentine desensitizing agents.

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References

1. Vongsavan, N., Matthews, B. The permeability of the cat dentine *in vivo* and *in vitro*. *Archs oral Biol.*, 1991; 36, 9: 641-646.
2. Vongsavan, N., Matthews, B. Interaction between neural and hydrodynamic mechanisms in dentine and pulp. *Archs oral Biol.*, 1994; 39 Suppl., 87s-95s.
3. Maita, E., Simpson, M.D, Tao, L., Pashley, D. H. Fluid and protein flux across the pulpo dentine complex of the dog *in vivo*. *Archs oral Biol.*, 1991; 36: 103-110.
4. Fritsch, C. Untersuchungen uber den Bau und die innervierung des Dentins. *Arch. Mikrosk. Anat.*, 1914; 84: p307.
5. Pashley, D. H. Dentine permeability: Theory and practice. In: Experimental endodontics. (Ed. Spangberg L) CRC press inc., Boca Raton, Fl. 1990: p19-49.
6. Pashley, D. H., Livingston, M. J, Greenhill, J. D. Regional resistance to fluid flow in human dentine *in vitro*. *Archs oral Biol.*, 1978; 23: 807-810.
7. Pashley, D. H., Andringa, H. J., Derkson, G. D., Derkson, M. E. Kalathoor, S. R. Regional variability in the permeability of human dentine. *Archs oral Biol.* 1987; 32(7): 519-523.
8. Outhwaite, W. C., Livingston, M. J., and Pashley, D. H. Effect of changes in surface area, thickness, temperature and post extraction time on dentine permeability. *Archs oral Biol.*, 1976; 21: 599-603.
9. Mjor, I. A, Heyeraas, K. J., Pulp-dentin biology in restorative dentistry. Part I: Normal structure and physiology. *Quintessence International* 2001; vol., 32, 6: 439-400.

10. Pashley, D. H., Galloway, S. E., Stewart, F. Effects of fibrinogen *in vivo* on dentine permeability in the dog. *Archs oral Biol.*, 1984; 29: 725.
11. Pashley, D. H., Smear layer: overview of structure and function. *Proc Finn Dent Soc.*, 1992; 88 Suppl 1: 215-24.
12. Blandy, A. A. On the sensibility of teeth. *Am. J. Dent. Sci.*, 1850; 1: 22-28.
13. Gysi, A. An attempt to explain the sensitiveness of dentine. *Br J. Dent. Sci.*, 1900; 43: 865-868.
14. Hume, W. R. A new technique for screening chemical toxicity to the pulp from dental restorative materials and procedures. *J. Dent. Res.*, 1985; 64(11): 1322-1325.
15. Gerzina, H.T., Hume, W.R., Effects of hydrostatic pressure on the diffusion of monomers through dentine *in vitro*. *J. Dent Res.*, 1994; 74(1): 369-373. January.
16. Ciarlone, A. E., Pashley, D. H. Permeability of root dentine to epinephrine released from gingival retraction cord. *Oper Dent.*, 1992; 17: 106-111.
17. Hanks, C.T, Wataha, J.C, Parsell, R.R, Strawn, S. E, Fat, J. C. Permeability of biological and synthetic molecules through dentine. *J. Oral Rehabilitation*. 1994; 21: 475-487.
18. Brannstroms, M., Johnson, G. Movements of the dentine and pulp liquid on application of thermal stimuli. An *in vitro* study. *Acta Odontol. Scand.*, 1970; 28: 59-70.
19. Anderson D.J., Matthews B., Gorretta C. Fluid flow through human dentine. *Archs oral Biol.*, 1967; 12: 209-216.
20. Outhwaite, W. C., Mc Kennzie, D. M. and Pashley, D. H. A versatile split chamber device for studying dentine permeability. *J. Dent. Res.* 1974; 57: 1503.
21. Camps, J., Martin, P., Ladeque, P., Rieu R., Fuser, J., Influence of tooth cryopreservation on human dentin permeability. *Dent Mater*, May 1994; 10: 210-214.
22. Ozok, A. R., Wu, M. K., Wesselink, P. R., The effects of post-extraction time on the hydraulic conductance of human dentine *in vitro*. *Arch Oral Biol*. Jan., 2002; 47(1): 41-6.
23. Pashley, D. H., Thompson, S. M., Stewart, F. P. Dentine permeability: Effects of the temperature on hydraulic conductance. *J. Dent. Res.*, 1983; 62 (9) Sept. 956-959,
24. Reeder, O. W. Jr., Walton, R. E., Livingston, M. J., Pashley, D. H. Dentine permeability: Determination of hydraulic conductance. *J. Dent Res.*, 1978, Feb; 57(2): 187-193.
25. Dippel, H. W., Borggreven, J. M., Hoppenbrouwers, P. M. M. Morphology and permeability of the dentinal smear layer. *J. Prost. Dent.*, 1984; 52, 5: 657-662,
26. Brown, L. R., Wachtel, L. W., Wheatcroft, M. G. Diffusion of niacin through extracted human teeth and its effects on bacterial penetration into dentine. *J. Dent. Res.*, 1969; 41: 684.
27. Fogel, H M., Pashley, D H., Dentine permeability effects of endodontics procedures on root slabs. *Endodon.*, 1990; 16: 442-425.
28. Ohtani, O. Three dimensional organisation of the connective tissue fibers of the human pancreas: scanning electron microscopic study of NaOH treated tissue. *Arch. Histol. Jap.* 1987; 50(5): 557-566.
29. Pashley, D. H., Matthews, W. G. The effects of outward forced convective flow on the inward diffusion in human dentine *in vitro*. *Archs oral Biol.*, 1993; 38 (7) 577-582.
30. Haustein, K. O., Thiele, G., Stangel, U. Transport of various substances through human enamel and dentine. *Int. J. Clinical Pharm. & Therapeutics*. 1994; 32 (9) 483-487.
31. Bitter, N. C. The effect of 25 % tannic acid on prepared dentine. A scanning electron microscope-methylene blue dye study. *J. Prosthet. Dent.*, 1990; 64: 12-16.
32. Ciarlone, A. E., Tao, L., Ziemer D. M., Pashley, D. H. Epinephrine permeation across dentine *in vitro*. *Endo. Dent. Traumatology*, 1991; 7: 5-9.
33. Hughes, N. P, Littlewood, D, Macpherson, I. V., Beeston, M. A., Unwin, P. R., Direct measurements of fluid flow rate in dentinal tubules using scanning electro-chemical microscopy. In: Dentin/pulp complex. Shimono M, Takahashi K, Editors. Tokyo, Japan: Quintessence Publishing 1996.
34. Hoppenbrouwers, P. P. M., Scholberg, H. P. F., Borggreven, J. M. P. M. Measurement of the permeability of dental enamel and its variation with death using an electrochemical method. *J. Dent. Res.* 1986; 65: 1322-1325.
35. Von Fraunhofer, T. J., Hammer, D. W. Microleakage of composite resin restorations. *J. Prosthet. Dent.*, 1984; 51: 209-213.
36. Levinkind, M., Vandernoot, T.J., Elliott, J. C. Electrochemical impedance characterisation of human and bovine enamel. *J. Dent. Res.*, 1990; 69: 1806-1811.
37. Levinkind, M., Vandernoot, T. J., Elliott, J. C. Evaluation of smear layers on serial sections of human dentine by means of electro-chemical impedance measurement. *J. Dent. Res.*, 1992; 71: 426-433.
38. Vandernoot, T. J., Levinkind, M. Impedance characteristics of human dental enamel and dentine. *J. Electroanal Chem.*, 1991; 300: 191-198.
39. Kramer, I. R. H. The isolation and examination of odontoblasts in fresh unfixed state. *Proc. Roy. Soc. Med.*, 1956; 49: 545-546.
40. Rockert, H. Methods for the isolation of odontoblasts and determination of intracellular potassium. *Acta Odontol Scand.*, 1964; 22: 373-378.
41. Chadha, S., Bishop, M. A. Effect of mechanical removal of the pulp upon the retention of odontoblasts around the pulp chamber of human third molars. *Archs oral Biol.*, 1996; 41, 8/9: 905-909.

42. Ghazali, F., Bishop M.A. Permeability of human dentine to water in odontoblast preserved and odontoblast free preparations. *Australian and New Zealand Journal Of Medicine*, 1996; Vol. 26, 3, June.
43. Ghazali, F., Bishop, M. A. Contents of tubules in relation to spontaneous water flow through human dentine. *Journal of Dental Research*, 1998; 77 (special issue): 651, 158.
44. Pashley, D. H., Nelson, R., Pashley, E. L. *In vivo* fluid movement across dentine in the dog. *Archs oral Biol.*, 1981; 26: 707-710.
45. Pashley, D. H., Kepler, E. E., Williams, E. C., Okabe, A. The effects of acid etching on the *in vivo* permeability of dentine in the dog. *Archs oral Biol.* 1983; 28(7): 555-559.
46. Pashley, D. H., Kepler, E. E., Williams, E. C., O'Meara, J. A. The effects on dentine permeability of time following cavity preparation in dogs. *Archs oral Biol.*, 1984; 29: 65-68.
47. Byers, M. R., Narhi, M. V., Mecifi, K. B. Acute and chronic reactions of dental sensory nerves to hydrodynamic stimulation or injury. *Anat Rec.*, 1988; 221: 872-883.
48. Turner, D., Marfurt, C., Sattelburg, C. Demonstration of physiological barrier between pulpal odontoblast and its perturbation following routine restorative procedures. A horseradish peroxidase tracing study in rat. *J. Dent. Res.* 1989; 68(8): 1262-1268.
49. Ohshima, H. Ultrastructural changes in odontoblasts and pulp capillaries following cavity preparation in rat molars. *Arch. Histol. Cytol.*, 1990; 53: 423-438.
50. Bishop, M. A. Evidence for tight junctions between odontoblasts in the rat incisor. *Cell Tissue Res.*, 1985; 239: 137-140.
51. Bishop, M. A., Yoshida, S. A permeability barrier to lanthanum and the presence of collagen between odontoblast in pigs' molars. *J. Anat.*, 1992; 181: 29-38.
52. Vongsavan, N., Matthews, B. The relation between fluid flow through dentine and the discharge of the intradental nerves. *Archs oral Biol.*, 1994; 39 Suppl: 140s.
53. Pashley, D. H., Galloway, S. E. The effect of oxalate treatment on the smear layer of ground surfaces of human dentine. *Archs oral Biol.*, 1985; 30 10: 731-737.
54. Matthews, W. G., Showman, C. D, and Pashley, D. H. Air blast-induced evaporative water loss from human dentine, *in vitro*. *Archs oral Biol.*, 1993; 38: 517-523.
55. Ruse, N. D., Smith, D. C. Adhesion to bovine dentine-surface characterisation. *J. Dent. Res.*, 1991; 70: 1002-1008.
56. Pashley, D. H. The effects of acid etching on pulpo-dentine complex. *Operative dentistry*. 1992; 17: 229-242.
57. Prati, C. What is the clinical relevance of *in vitro* dentine permeability tests? *J. Dent. Apt.*; 1994; 22(2): 83-88.
58. O'Connell M. S., Morgan, L. A., Beeler, W. J., Baumgartner, J. C., A comparative study of smear layer removal using different salts of EDTA. *J Endod.* 2000, Dec; 26(12): 739-43.
59. Eick J. D., Wilko, R. A., Anderson C. H., Sorenson, S. E. Scanning electron microscopy of cut surfaces and identification of debris by the use of the electron microprobe. *J. Dent. Res.*, 1970; 53: 1255.
60. Prati C, Venturi L, Valdre G, Mongiorgi R. Dentin morphology and permeability after brushing with different toothpastes in the presence and absence of smear layer. *J Periodontol*, 2002, Feb.; 73(2): 183-90
61. Boyer, D. B., Svare, C. W. The effect of rotatory instrumentation on the permeability of dentine. *J. Dent. Res.*, 1981; 60: 966-971
62. Tagami, J., Tao, L., Pashley, D. H. Correlation among dentin depth permeability and bond strength of adhesive resins. *Dent. Materials*. 1991; 6: 45-50.
63. Stevansson, T. S., Fluid movement in human dentine. *Archs oral Biol.*, 1965; 10: 935-944.
64. Pashley, D. H., Livingston, M. J., Outhwaite, W. C. Rate of permeation of isotopes through human dentine *in vitro*. *J. Dent. Res.*, 1977; 56 (1): 83.
65. Olgart, L., Brannstrom, M., and Johnson, G. Invasion of bacteria into dentinal tubules. Experiments *in vivo* and *in vitro*. *Acta. Odontol. Scand* 1974; 32: 61.
66. Pashley, D. H., Tao l., Boyd, L., King, G. E., Horner, J. A. Scanning electron microscopy of the substructure of smear layer in human dentine. *Archs oral Biol.*, 1988; 33: 265-270.
67. Van Meerbeck, B., Inokoshi, S., Braem, M., Lambrechts, P., Vanherle, G. Morphological aspects of the resin dentine interdiffusion zone observed with different dentine adhesive resins. *J. Dent. Res.*, 1992; 71: 1530-1540.
68. Hamlin, P., Samarawickrama, D., and Lynch, E. Effect of the new conditioning agent on dentine. *American J. Dent.*, 1990; 3: 119-124.
69. Pashley, D. H., Michelich, V., and Kehl, T. Dentine Permeability: Effects of the smear layer removal. *J. Prosth Dent.*, 1981; 46: 531-537.
70. Tagami, J., Sugizaki, J., Hosoda, H. Effects of the various pretreatments for dentine bonding on the dentine permeability. *Japanese J. society of dental materials and devices*, 1990; 9: 240-246.
71. Kurosaki, N., Kubota M., Yamamoto, Y., Fusayama, T. The effects of etching on the dentine of the clinical cavity floor. *Quintessence International*, 1990; 21: 87-92.
72. Pashley, E. L., Talman, R., Horner, J. A., Pashley, D. H. Permeability of the normal versus carious dentine. *Endodontics and Dental Traumatology*. 1991; 7: 207-211.

73. Bergren, G. Brannstrom, M. The rate of flow in dentinal tubules due to capillary attraction. *J. Dent. Res.*, 1965; 44: 408.
74. Pashley, D. H. Dentin: A dynamic substrate: a review. *Scan microsc.* 1989; 3: 161-176.
75. Pashley, D. H. Dynamic of the pulpo-dentine complex, *Crit Rev. Oral. Biol. Med.* 1996; 7(2): 104-133.
76. Langeland, K., Tissue response to dental caries. *Endo Debt Traumatol.*, 1987; 3: 149-171.
77. Tronstadt, L., Langeland, K., Electron microscopy of human dentine exposed by attrition. *Scand J Dent Res.* 1971; 79: 160-171.
78. Holland, G. Morphological features of dentine and pulp related to dentine sensitivity. *Archs oral Biol.*, 1994; 39 (suppl): 3-11.
79. Michelich, V. J., Schuster, G. S., Pashley, D. H. Bacterial penetration of human dentine *in vitro*. *J. Dent. Res.* 1980; 59(8): 1398-1403.
80. Trowbridge, H. O. Pathogenesis of pulpitis resulting from dental caries. *J. Endodontol.*, 1981; 7 (92): 52.
81. Barakat, M. M., Powers, J. M., *In vitro* bond strengths of cements to treated teeth. *Aust. dent. J.*, 1986; 31: 415-419.
82. Addy, M., Mostafa, P., Dentin hypersensitivity – I. Effects produced by the uptake *in vitro* of metal ions, fluoride and formaldehyde onto dentine. *J. oral Rehab.*, 1988; 15: 575-585.
83. Wenner, K. K., Fairhurst, C. W., Morris, C. F., Hawkins, I. R., Ringle, R. D. Microleakage of root restorations. *J. Am. Dent. Ass.*, 1988; 17: 825-828.
84. Sandoval, V. A., Cooley, R. L., Barnwell, S. E. Evaluation of potassium oxalate as a cavity liner. *J. Prosthet. Dent.*, 1989; 62: 283-287.
85. Boyde, A. Air polishing effects on enamel, dentin, cement and bone. *Br. Dent. J.*, 1984; 156: 387-391
86. Taiz, L., Zieger, E. The structure and properties water. In: *Plant physiology. Benjamin & Cummings publishers, California.* 1991; Chapter 3: 61-67.
87. Tao, L., Reynolds, J. M., Pashley, D. H. Effects of dye on dentine permeability. *Endod. Dent. Traumatolog* 1988; 4: 16-18.
88. Goodis, H. E., Marshall, G. W. Jr., White, J. M, Ge L, Hornberger, B, Marshall, S. J. Storage effects on dentin permeability and shear bond strengths. *De Mater.*, 1993; Mar. 9(2): 79-84.
89. Sakae, T., Mishima, H., Kozawa, Y. Changes in bovine dentine mineral with sodium hypochlorite treatment. *J. Dent. Res.*, 1988; 67: 1229-1239.
90. Barbosa, S.V., Safavi, K.E., Spangberg, L. S. W. Influence of sodium hypochlorite on the permeability and structure of cervical human dentine. *Int. Endo. J.* 1994; 27: 309-312.
91. Goodis, H. E., Mashall, Jr., G. W., White, J. M. The effects of storage after extraction of the teeth on human dentine permeability *in vitro*. *Archs oral Biol.*, 1991, 36 (8): 561-566.
92. McGuckin, R. S., Pashley, D. H. The effect of disinfection/sterilization treatments on Gluma mediated dentine shear bond strengths. *Am J Dent.*, 1990; 3: 278-282.
93. Greenhill, J. D., Pashley, D. H., The effects of desensitising agents on the hydraulic conductance of dentine *in vitro*. *Journal of Dental Research* 1981; 60: 686-698.



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Science

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Title of Work: Odontoblasts processes in Undemineralised human dentine

- A Variable pressure SEM investigation.

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Principal investigator

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Contact Person: Dr. FARID BIN CHE GHAZALI

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Descriptive Summary of Submission (100 words): Describe the final product and the basic approach used to create the final product. Also include a succinct description of the resources utilized and a rationale of why the visualization will be an aid to science or engineering and how the concepts are better communicated to the end user.

Odontoblasts are terminally differentiated distinctive cellular bodies, which line the periphery of the pulp chamber in a tooth. Each cell has a cytoplasmic process that extends into dentine. In the literature there are numerous descriptions of odontoblast processes in dentine tubules (Ten Cate, 1967; Brannstrom and Garberoglio, 1972; Garberoglio and Brannstrom, 1976) but the extent of the process and the volume of the tubule it occupies are some of the features, which remain controversial. The photomicrographs were taken from our study (Farid, 1999, 2002) on odontoblasts using VPSEM (LEO 1450VP) technique to promote new avenues and prompt viable results mimicking viable, hydrated nature of the dentine tissues without the vigorous tissue preparation protocols. Hence encouraging a simpler innovative approach to visual human odontoblasts processes, its extension and role. in dentine proper of human third molars.

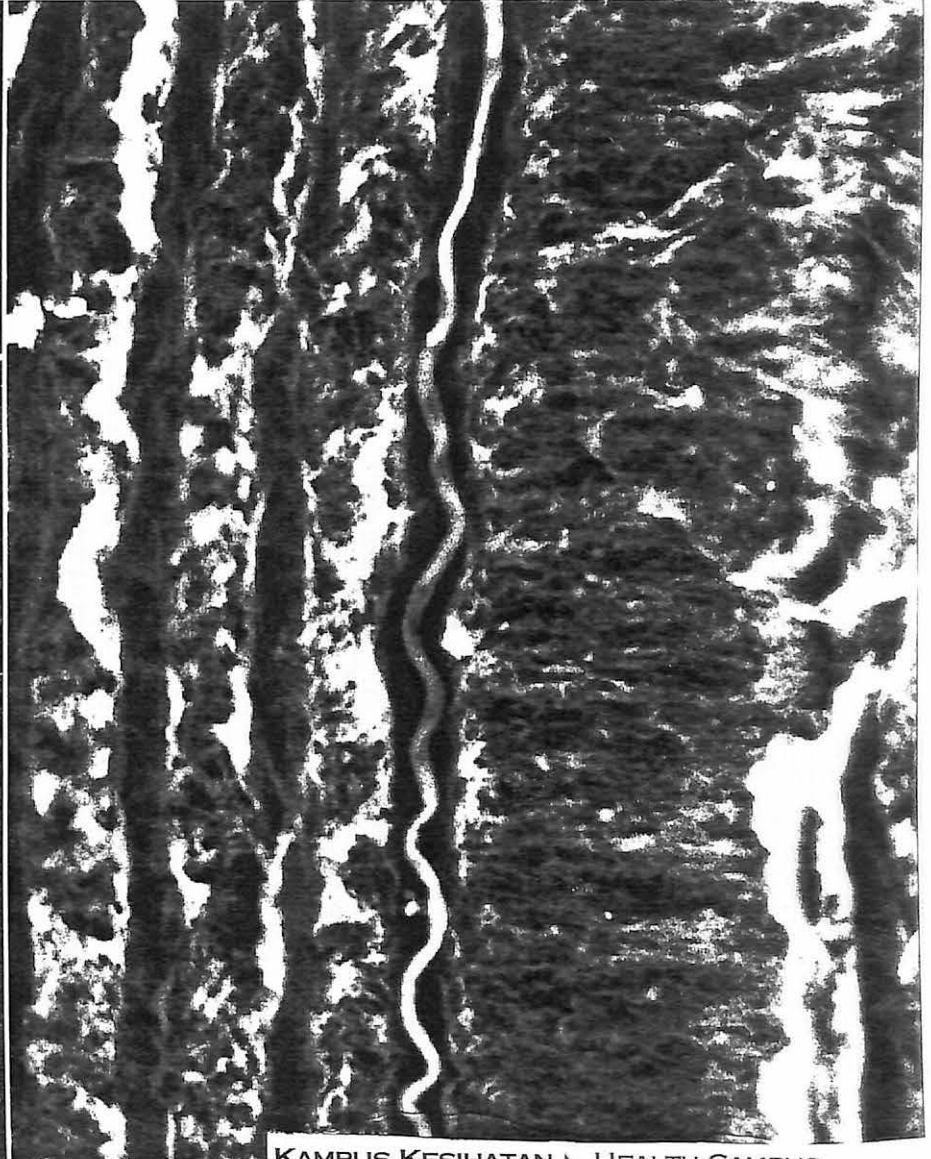
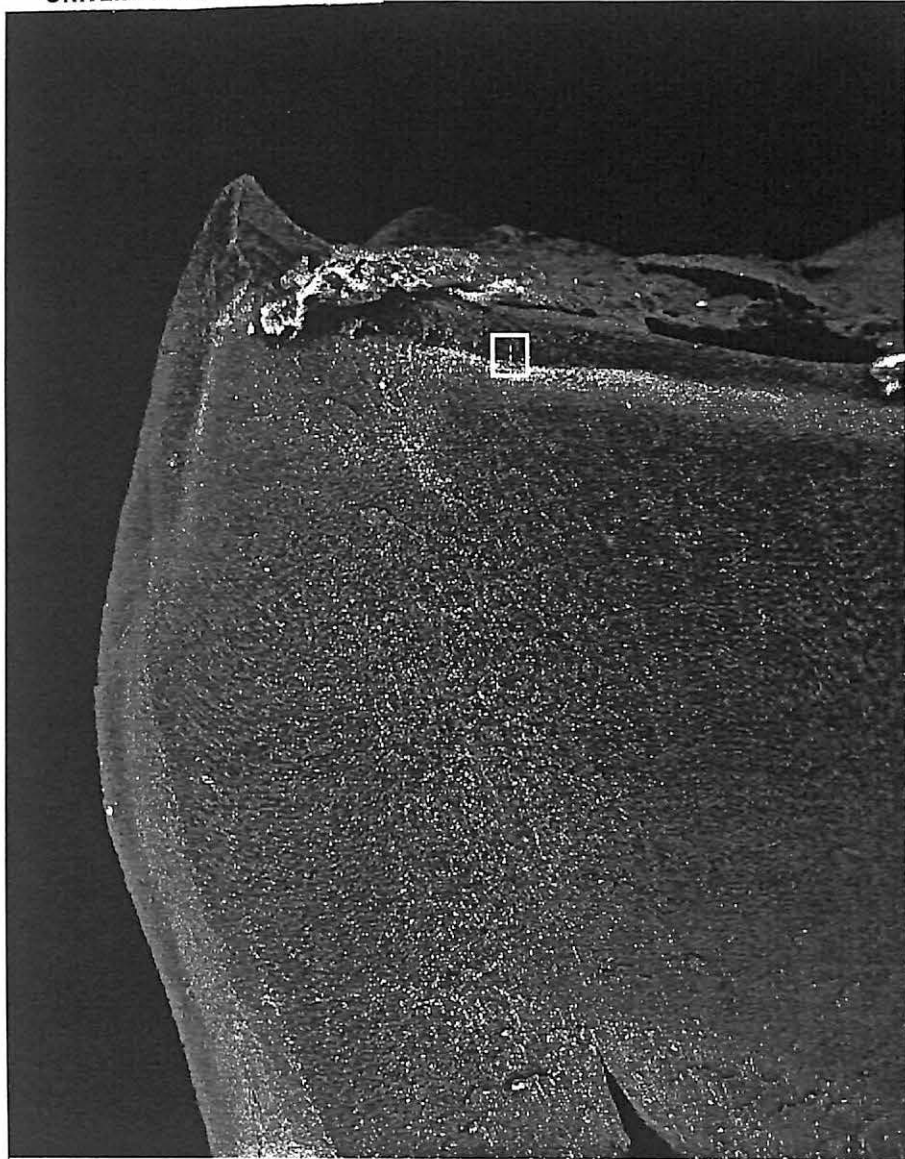
In this study, the combination of variable pressure microscopy (LEO 1450VP), image analysis system (Leica QWin) and dye sublimation printing have enhanced the visualization and probe the extent of the odontoblast processes into the dentinal tubules thus promoting further understanding of the odontoblasts role in dentine permeability and gives insights to controversies of its extent as observed in animals studies. In our study the visualization of odontoblast processes into mid dentine and enamel-dentine junction elucidated controversial belief that the odontoblasts is only found at the predentine and pulp-dentine interface. It is hope the study will revealing new insights and thinking of the role of odontoblasts to the dental clinician and basic oral biologist.

References

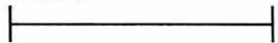
1. Brannstroms, M., Garberoglio, R. 1972. The dentinal tubules and the odontoblast process; A scanning electron microscopic study, *Acta Odontol Scand* 30: 291.
2. Farid Che Ghazali (1999). The Odontoblasts and its related structures: a review. *Malaysians Journal of Medical Sciences*. Vol 6 no: 1. P12-17, January.
3. Farid Che Ghazali, Ismail Abdul Manan. (2002). The coronal pulp chamber dentinal tubules; A combined investigation using variable pressure scanning electron microscopy and image analysis. *First Asean Conference on Medical Sciences*. 18 – 21 May, Abstract proceeding no: p-57, p83.
4. Garberoglio, R., Brannstrom, M. (1976). Scanning electron microscopic investigation of human dentinal tubules. *Archs oral Biol*. 21. 355-362.
5. Ten Cate, A. R. (1967). A histochemical study of the human odontoblast. *Archs oral Biol*. 12. 963-969.

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1mm



EHT = 15.00 kV

WD = 10 mm

Signal A = QBSD

Vacuum Mode = Variable Pressure

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Farid, C.G.

HIGH-RESOLUTION MICROSCOPY AND MICROANALYSIS IN ORAL BIOLOGY AND DENTISTRY

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The oral region contains all types of mineralised tissues found in the body. Its normal mucosa lining exhibits a greater variation in histo-structure than found in the entire skin surface. As such the oral cavity gives rise to diseases not found elsewhere in the body and additionally, a great variety of systemic diseases may express themselves in the oral cavity and paraoral region. High-resolution microscope applications in oral biology and dentistry, (1). brings structural biology understanding and maps the natural state of its biological cell e.g. odontoblasts, osteoclasts, cementoblasts etc under normal and pathological conditions-expanding research capabilities and ability to understand cellular structures and processes with direct impact to pain management (2). Its probing capabilities facilitate the characterization of the atomic structures of dental composites materials thus allowing observation of their dynamic behaviors under various different conditions. These observations are important especially to improve the quality, performance of the dental cosmetic composites and restorative materials and finally (3). the developments of microscopy and its techniques e.g. for isolation and growing cells under reproducible

conditions have contributed to the understanding of oral biology controversies thus providing better insight of actual descriptions. Dentine is a biological mineralised tooth structure that is densely perforated with dentinal tubules that extend from the pulp chamber to the enamel-dentine junction. The presence of dentinal tubules is an attributing factor to dentine permeability (Thomas, 1985). In the literature there are numerous descriptions of odontoblast processes in dentine tubules (Ten Cate, 1967; Brannstrom and Garberoglio, 1972; Garberoglio and Brannstrom, 1976 but the extent of the process and the volume of the dentinal tubule it occupies are some features, which remain controversial. Using fluorescent microscopy Byers and Sugaya, (1995), reported that the odontoblast process in rats does not extend into outer dentine except during the early stages of development. The study also reported that the processes present along the innervated regions of the crown were long and straight, whereas those in root dentine were extensively branched and shorter in length. In a scanning electron microscope study Szabo, *et al.*, (1984) showed the presence of the odontoblast process in inner, middle and peripheral dentine and reported that the odontoblast process observed in the inner third of the dentine was closely apposed to the tubule wall and was present in most tubules in that region. These earlier

studies measured the diameter and number of the dentinal tubules in animal and human dentition using both the light and the conventional scanning electron microscope but still the extent of the process and the volume of the dentinal tubule it occupies especially in human tooth model are some features that still remain until now controversial. At present the odontoblast layer is being suggested as a barrier. Little knowledge is known now to suggest the mechanism orchestrated by this layer. To understand the barrier there is a need to acknowledge the functional odontoblast cellular kinetics that maintains its intactness as a layer and its ultrastructural significant. This will further understanding of the role of the intercellular junctional complexes of the odontoblast layer towards exogenous permeation of fluid and substances.

In 1905, von Korff reported the presence of argyrophilic fibres between the odontoblasts in early stages of dentinogenesis in teeth of pigs and calves. Ever since then the existence of these corkscrew argyrophilic fibres has been very controversial. In 1994, Ohsaki and Nagata confirmed that the von Korff fibres seen in a developing mouse molar consisted mainly of type III collagen in which a non-collagenous protein fibronectin was found to be densely associated. The association of fibronectin with von Korff fibres may be related to the organisation and maintenance of their structural integrity. These von Korff fibres extend from the dentine between the apical ends of the odontoblast cell bodies into the interodontoblastic space and from the interodontoblastic space into the subodontoblast layer of the pulp (hence also known as interodontoblastic fibres).

There are various macroscopic changes observed on the tooth morphology, especially in its form and colour that occurs concurrently with age. These changes are usually associated related to wear and attrition. To date there is various high-grade research end light and electron microscope available that is of importance to dentistry, among them is the confocal true laser microscope, variable pressure scanning electron microscope and the fully automated analytical transmission electron microscope. The prevention and treatment of oral and dental diseases requires a thorough knowledge of the histological and biological variables influencing the aging and disease patterns.

References

- Brannstroms, M., Garberoglio, R. (1972). The dentinal tubules and the odontoblast process; A scanning electron microscopic study, *Acta Odontol Scand* 30: 291.
- Byers, M. R., Sugaya, A. (1995). Odontoblast process in dentine revealed by fluorescent Di-I. *J. Histochemistry and Cytochem.* 43(2). 159-168.
- Garberoglio, R., Brannstrom, M. (1976). Scanning electron microscopic investigation of human dentinal tubules. *Archs oral Biol.* 21. 355-362.
- Ohsaki, Y., Nagata, K. (1994). Type III is a major component of interodontoblastic fibers of the

- developing mouse molar root.
Anat. Rec. 240. 308-313.
- Szabo J., Trombitas K., Szabo I.
(1984). The odontoblast process
and its branches in human teeth
observed by scanning electron
microscopy. *Archs oral Biol.* vol.
29. 4. 331-333.
- Ten Cate, A. R. (1967). A
histochemical study of the human
odontoblast. *Archs oral Biol.* 12.
963-969
- Thomas, H. F., (1979). The extent
of odontoblast process in human
dentine. *Archs oral Biol.* 28. 465-
469.
- Von Korff, K. (1905). Die
Entwicklung der Zahnbein und
Knochengrundstanz der
Saugetiere. *Archiv fur
mikroskopische Anatomie und
Entwicklungsmechanik.* 67. 1-17.

PENYELIDIK : DR FARID CHE GHAZALI

NAMA PROJEK : "HUMAN MOLAR TOOTH DENTINAL TUBULES AND ITS ODONTOBLAST PROCESS.
 A COMBINED INVESTIGATION USING VARIABLE PRESSURE SCANNING ELECTRON MICROSCOPE & IMAGE ANALYSIS"

PENYATA PERBELANJAAN BAGI TEMPOR BERAKHIR PADA 31 DISEMBER 2003

PECAHAN KEPALA	PERUNTUKAN (RM)	PERBELANJAAN 2002	BAYARAN 2003	TANGGONGAN	PERBELANJAAN 2003	JUMLAH PERBELANJAAN	BAKI KESELURUHAN
11000 GAJI DAN UPAHAN	0 00	0.00	0.00	0.00	0.00	0.00	0.00
14000 ELAUN LEBIH MASA	0 00	0.00	0.00	0.00	0.00	0.00	0.00
15000 BONUS	0 00	0.00	0.00	0.00	0.00	0.00	0.00
21000 PERJALANAN & PENGANGKUTAN ORANG	5,000.00	1,894.10	0.00	0.00	0.00	1,894.10	3,105.90
22000 PENGANGKUTAN BARANG-BARANG	0 00	0.00	0.00	0.00	0.00	0.00	0.00
23000 PERHUBUNGAN DAN UTILITY	300 00	5.00	0.00	0.00	0.00	5.00	295.00
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25000 BAHAN-BAHAN MAKANAN & MINUMAN	0 00	8.50	0.00	0.00	0.00	8.50	(8.50)
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27000 BEKALAN BAHAN-BAHAN LAIN	7,000 00	11,682.40	3,813.50	0.00	3,813.50	15,495.90	(8,495.90)
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29000 PERKHIDMATAN IKTISAS & LAIN-LAIN PERKHIDMATAN & HOSPITALITI	600 00	195.00	301.00	0.00	301.00	496.00	104.00
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