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A photochemical method for *in vitro* evaluation of fluid flow in human dentine

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ABSTRACT

Objective: To evaluate the flow dynamics of dentine fluid using a chemiluminescence method *in vitro*.

Materials and methods: Horizontally sliced coronal dentine specimens with thicknesses of 1.4, 1.6, 1.8, and 2.0 mm ($n = 10$ each) were prepared from extracted human third molars. After cleaning with EDTA, a mounted specimen was clamped between 2 acrylic chambers attached to both the occlusal and pulpal sides. The occlusal chamber, which was closed with a glass coverslip, was filled with a chemiluminescent solution (0.02% luminol and 1% sodium hydroxide in water). A trigger solution of 1% hydrogen peroxide and 1% potassium ferricyanide was injected into the pulpal chamber at a constant pressure of 2.5 kPa, and allowed to immediately flow into the patent dentinal tubules. Four consecutive measurements (T1–T4) were performed on each sample by recording the emission of chemiluminescence with a photodetector. The relationship between the crossing time of the liquid through the slice and dentine thickness was examined.

Results: An apparent time delay was detected between the starting points of the trigger solution run and photochemical emission at T1. Dt (Dt, s) values of each thickness group were 13.6 ± 4.25 for 1.4 mm, 18.1 ± 2.38 for 1.6 mm, 28.0 ± 2.46 for 1.8 mm, and 39.2 ± 8.61 for 2.0 mm, respectively. Dt significantly decreased as dentine became thinner towards the pulp chamber ($P < 0.001$).

Conclusions: The velocity of fluid flow increased both with increasing dentine depth or reduction of remaining dentine thickness.

1. Introduction

Slight thermal, tactile, and osmotic stimulations on the surface of exposed dentine evoke a sudden and stabbing pain in the teeth,[1] which is called dentine hypersensitivity. It only occurs when patent dentine tubules are exposed to external stimuli in the oral cavity. This phenomenon has been explained by the hydrodynamic theory,[2,3] which states that rapid shifts in dentinal fluid provoked by external stimuli stimulate odontoblast mechanoreceptors. Under normal conditions, intratubular fluid in patent dentine tubules moves

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towards the surface by pupal pressure.[4] However, an external stimulus applied to the dentine surface may initiate either inward or outward fluid flow in the dentine tubules depending on the type of stimulation.[5] A previous study reported that the flow rate of dentine fluid increased when the frequency of neural discharge in the pulp became higher.[6]

The structure of dentine, especially the number and diameter of tubules, affects the velocity of intratubular fluid. Other factors, such as pressure differences between the orifice and open end as well as the length and radius of tubules, also affect fluid velocity.[5,7] Fluid velocity was previously demonstrated to vary with the fourth power of the tubular radius, indicating that a small change in the tubule radius can have a profound affect on fluid flow.[8] Vongsavan and Matthews[9] reported that pulpal pressure affected the fluid volume transferred through the dentine of cat canines. However, the actual velocity of tubular fluid crossing through dentine has not yet been determined.

This aim of the present study was to investigate the characteristics of fluid flow in dentinal tubules. We developed a new measuring system to determine dentine permeability[10,11] and used it to investigate the time needed to transfer a liquid through the patent tubules of human dentine specimens.

2. Materials and methods

2.1. Preparation of dentine slices

The present investigation was conducted under the approval of the Research Ethics Committee of the Graduate School of Tohoku University, Japan. Forty extracted intact human third molars were obtained under written informed consent and used for specimen preparation. All teeth were mounted using adhesive material (Super Bond C and B: Sun-medical Co. Shiga, Japan) and self-curing resin on wooden blocks in order to prepare slices. Coronal dentine specimens were dissected to different thicknesses of 1.4, 1,6, 1.8, and 2.0 mm from the pulp horn perpendicular to the vertical axis (10 slices for each thickness) with a diamond wafer saw microtome (Model SP 1600; Leica Microsystems Nussloch GmbH, Nussloch, Germany) under copious water-cooling. All specimens were mounted in stainless steel rings by the adhesive material, and then cleaned with 0.5 M EDTA solution (pH 7.4) for 2 min to remove the smear layer and open the dentine tubules prior to thorough rinsing with deionized water.

2.2. Measuring device and process

To determine dentine permeability and detect liquid flow in dentine tubules, a modified split chamber column for photochemical measuring was fabricated as previously reported.[10,11] Two cylindrical acrylic chambers were sealed with O-rings on each sliced surface of the specimen and clamped in a metal frame connected with the ring. The chamber on the occlusal side of the sliced surface was sealed with a glass cover slip and filled with a chemical illuminant reagent (aqueous solution of 0.02% luminal [5-amino-2, 3-dihydro-1, 4-phtalazinedione] and 1% sodium hydroxide). The opposite chamber on the pulpal side was connected with a flask tube filled with an activator solution (1% potassium ferricyanide and 1% hydrogen peroxide) as a trigger for the chemiluminescence reaction. Following pressurizing at 2.5 kPa, the trigger solution was immediately injected into the pulpal chamber, from where it passed through the dentine tubules towards the occlusal chamber containing the illuminant reagent, resulting in the production of a luminescence reaction (Fig. 1). The photo signal was recorded with a photomultiplier tube detector (S10723, Hamamatsu Photonics, Hamamatsu, Japan) installed 5 mm above the cover slip on the occlusal chamber. All of the equipment was placed in a lightproof container to prevent any outer light signal that may interfere with the luminescence signal. The output voltage of the photodiode was recorded with an AD converter system at 1 kHz and stored in a CPU unit to control the system. Data at end of the experiment were transferred to a PC for further processing and analysis.

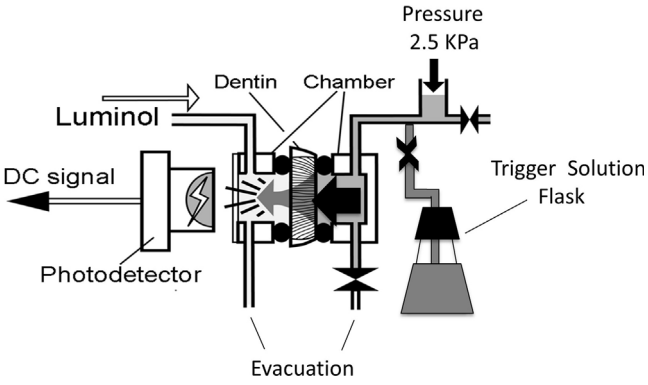

Fig. 1 – Schematic illustration of the device for measuring the velocity of fluid flow through dentinal tubules.

To identify the duration of transfer of the trigger solution through the patent tubules, we measured the delay time (Dt) to produce the chemiluminescence reaction after the trigger solution had been injected in four consecutive runs. Four consecutive run cycles were performed on each sample; each cycle consisted of 5 min of pressurization of the trigger solution at 2.5 kPa, followed by a 2-min pressure-free interval. Chemiluminescence was emitted when the trigger solution reached the opposite chamber containing the luminol solution, and Dt was defined as the time required for the trigger solution to pass through the dentine tubules of the slice. Dt values were used to calculate the velocity of flow passing through the thickness of each specimen.

2.3. Statistical analysis

All data were expressed as the mean ± standard deviation (SD) of each thickness group ($n = 10$). Graphs of box plots displayed the distribution of data based on a five-number summary: ten percent from the minimum, first quartile, median, third quartile, and 90 percent from the maximum. The central rectangle spanned the first quartile to the third quartile and was segmented inside at the median. Distribution of the data was analyzed using Kolgomorov-Smirnov test. When the data were not normally distributed non-parametric Kruskal-Wallis ANOVA was applied with Mann-Whitney *post hoc* testing to