Age-related changes of pulpal blood flow in primary teeth measured by laser Doppler blood flowmetry

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Abstract The purpose of the present study was to examine age-related change of the pulpal blood flow (PBF) in human primary teeth. PBF of 21 healthy upper primary central incisors in 12 children (age: 3 years 11 months–7 years 3 months) was measured by laser Doppler flowmetry (LDF). Recordings were made with and without opaque black rubber dam application. On 10 of the examined teeth, repeated measurements were made on different occasions: twice on 4 teeth (3 subjects) and 3 times on 6 teeth (3 subjects). The results obtained were as follows: 1) PBF with dam application was significantly decreased with increasing age of the subjects (\( P < 0.05 \), Spearman ranks order correlation, \( n = 21 \)). 2) In the repeated measurements, PBF showed a tendency of decrease with age. These results indicate that PBF in human primary incisors is reduced with age.

Introduction

The permanent tooth pulp in general shows age-related changes such as calcification, arteriosclerotic changes and dystrophic mineralization of nerve fibers\(^1\)\(^{-3}\). Regarding the primary tooth pulp, it is commonly accepted that the age-related changes are not similar to those in permanent tooth pulp. From the clinical standpoint, the pulpal tissue in a primary tooth is replaced by granular tissue together with root resorption with age. The colour of the residual pulp of the extracted primary tooth with root resorption generally shows normal appearance, which indicates the pulp is neither extremely vascularized nor non-vascularized.

Rapp\(^5\) examined the vascular pathways on 64 human primary tooth by a modified negative pressure suction technique. With advanced root resorption the number of fine arteriole-like structures arising within the coronal pulp was decreased, and capillaries and smaller venous structures were not detected. With further resorption of the root, smaller amounts of subodontoblastic capillary plexus and arteriole-like vessels were seen. Although very limited, the histologic study suggests that the pulp haemodynamics reduce with age.

Laser Doppler flowmetry (LDF) has been applied to pulpal blood flow (PBF) measurement in permanent\(^6\)\(^{-8}\) and primary\(^9\)\(^,\)\(^10\) teeth. The authors\(^10\) have examined the applicability of LDF to PBF measurement in human primary central incisors and the efficacy of an opaque black rubber dam on eliminating the signal contamination derived from periodontal blood flow. The authors also reported the age-related decrease of PBF in the human permanent upper central incisors\(^10\). To our knowledge, no evidence on the changes in the PBF in primary tooth pulp has been obtained in previous studies. Therefore, the purpose of the present study was to obtain information about age-related changes of PBF in primary teeth measured by LDF.
Materials and Methods

Subjects and examined teeth
This study was approved by the Tohoku University Graduate School of Dentistry Research Ethical Committee. The experimental purpose and methodology were explained to 15 children and their parents, and written informed consent was obtained from them. It was difficult to record blood flow signals in 3 children due to their uncooperative attitude. As a result, 21 healthy maxillary primary central incisors in 12 children (3 years 11 months–7 years 3 months, mean: 5 years 6 months) were examined. The examined teeth were diagnosed as healthy if they were free of caries, restoration, defects, attrition and discoloration. Teeth with a degree of mobility greater than 2 were excluded.

Prior to the recording, an individual resin cap was prepared for each tooth from a plaster model of the upper tooth arch of each subject so as to cover the labial and palatal surfaces of the tooth examined. A hole, 2.0 mm in diameter and with its centre approx. 1.5 mm from the gingival margin, was drilled in the labial side of the cap at right angles to the mesio-distal center of the tooth surface, and a stainless steel tube for the insertion of an LDF probe (o.d. 2.0 mm, i.d. 1.6 mm, length 3 mm) was fixed into the hole.

Laser Doppler flowmetry
A Moor blood flow monitor (type MBF3D, Moor Instruments, Axminster, UK; wavelength, 780–820 nm) was used with a dental probe (ext. diam. 1.1 mm, fibre diam. 0.2 mm, centres 0.5 mm apart). The probe tip was inserted into a polythene tube (o.d. 1.5 mm, i.d. 1.0 mm) to obtain a satisfactory fit into the stainless steel tube. The laser Doppler flowmetry was set up in the same way as described by Vongsavan and Matthews with a 0.1 second time constant and an upper band-pass limit of 14.9 kHz. The laser Doppler signal was also recorded from a stationary reflector with the same level of illumination as that present during recording from the tooth. This was used to determine the output equivalent to zero blood flow in the recording. The sensitivity of the blood flow monitor was adjusted using a standard suspension of polystyrene beads and measurements were made in arbitrary perfusion units (P.U.).

Recording procedure and rubber dam application (Fig. 1)
Each subject was placed in a supine position and the lips were retracted. An opaque black rubber dam (Four D Rubber Co., Ltd., Heanor, Derbyshire, UK; thickness 0.25 mm) was applied to the tooth being examined and to the adjacent teeth in order to examine its effects on eliminating signals derived from periodontal blood flow. The individual resin cap was fitted to the test tooth and was retained using a small amount of dental cement (Dycal, The L.D. Calk Division, DENTSPLY International Inc., Milford, USA) along the incisal edge, if necessary. The LDF probe tip was inserted into the labial hole of the cap.
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until the tips touched the tooth surface. LDF was recorded for about one minute with the dam in place. The dam was then removed using scissors without disturbing the tip of the LDF probetip in the cap, and recording was resumed. This procedure was then repeated for the second tooth examined in the same subject. The mean blood flow signals were obtained by personal computer (Power Macintosh G3, Apple Computer Inc., Cupertino, CA) and a laboratory interface (Power Lab, ADInstruments Pty Ltd., Australia).

Repeated measurement on the same tooth
In order to examine the blood flow changes in individual teeth with time, repeated measurements were made on 10 teeth in 6 subjects. Four examined teeth of 3 subjects were measured twice and 6 examined teeth of 3 subjects were measured 3 times on different days. The interval between the repeated measurements in each individual ranged between 7 months and 18 months (mean 13.3 months). In the course of the repeated measurement, satisfactory fitting of the resin cap was obtained, and no modifications of the cap were needed.

Data analyses
The relationship between the age of the subjects and the PBF signals with and without the dam were analyzed by Spearman ranks order correlation. For this analysis, regarding the subjects who participated in the repeated measurement of PBF, the mean value of the ages when the recordings were made was calculated as the age of the subjects. The effect of dam application was examined by paired t-test. Changes of the signals with time were analyzed using either Friedman analysis (3-times-examined teeth) or Wilcoxon signed ranks test (twice-measured teeth). P-values less than 0.05 were taken as indicating statistical significance.

Results
Figure 2 shows the relationship between the subjects’ ages and the blood flow signals with (filled circle) and without (open circle) the dam in place, respectively. There was a significant negative correlation between the subjects’ age and the blood flow signals with the dam (n = 21, P < 0.05, Spearman ranks order correlation). In contrast, no significant relationships were obtained between the age of the subjects and the signals without the dam (n = 21, P = 0.636, Spearman ranks order correlation). The dam reduced LDF signal amplitudes by between 38.1% and 96.7% (n = 21, mean ± SD, 75.3 ± 11.7%).
and this change was significant \((P<0.05, \text{Wilcoxon matched pairs test})\). In the repeated measurement (Fig. 3), there was a significant change of the signals with dam with time in the 3-times-examined teeth group \((n=6, \text{Friedman test, } P<0.01)\). In twice-examined teeth, the signals tended to decrease with time, but this result was not statistically significant \((n=4, \text{Wilcoxon signed ranks test, } P=0.067)\).

**Discussion**

The present results show age-related decrease of the PBF in human primary teeth. To the best of our knowledge this is the first such study. In the primary tooth pulp, a decrease of the volume of the coronal pulp due to the calcification with age, which is common in the permanent tooth pulp\(^{14}\), is not considered to occur. The decrease of PBF is considered to be closely related to the morphological changes of the blood vessels in the pulp; the number of the blood vessels decreases with age, and the decrease in number of fine arteriole-like structures and subodontoblastic capillary plexus proceeds to changes of larger vessels in the more central region of the pulp\(^{5}\). The variations of PBF among individuals in similar ages are considered to be in part due to the different degree of root absorption.

To date, when reviewing published reports on the use of LDF to record PBF in the human primary teeth, repeated measurement over a long period in the same subjects has not been reported. In the present study, the use of an individual resin cap allowed exact repositioning of the measurement probe and repeated measurement of PBF. This methodology will be beneficial in particular for the prognosis of traumatized teeth, because in such teeth the pulp does not respond to the vitality test and requires a relatively long period for confirmation of revascularization.

The PBF with opaque dam application significantly decreased with age, while that without dam application did not show any obvious changes. The authors have shown the efficacy of opaque black rubber dam application on the elimination of signal artifacts due to the periodontal blood flow\(^{15-18}\) in human primary upper central incisors. The present results again confirmed that the elimination of the signal outside the pulp is prerequisite.

The determination of the tooth pulp vitality in adult humans is made by clinical examinations such as visual examination, radiographs, electric pulp tests and thermal tests\(^{19,20}\). In these methods, electric and thermal tests rely on patients’ pain sensation, which is unreliable in primary teeth in children. Furthermore, the pain sensation produced by these tests may disturb further dental treatment. In the twice-examined teeth, there was a non-significant tendency of decrease in the signal amplitudes with age. This lack of statistical power may have been due to the small number of observations. If there were a significant relationship between the signal amplitudes and the status of root resorption, radiographic examination might be to some extent replaced by PBF measurement, which would reduce exposure to X-rays and benefit the patients. This possibly merits further study.

**Acknowledgments**

We are grateful to Prof. M.P. Hector, Queen Mary University of London for the valuable discussion. We are grateful to D. Mrozek for English proofing of this manuscript. This study was supported by a Scientific Research Grant from the Ministry of Education, Culture, Sports, Science and Technology in Japan (No.13771253) to Hideji Komatsu.

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