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Original Article

Quantification of dental plaque in oral cavity was enabled by a novel algorithm of image processing

Naho Sueishi [a], Tomoko Ohshima [b,*], Takashi Oikawa [a], Hiroshi Takemura [c], Mai Kasai [c], Nobuko Maeda [b], Yoshiki Nakamura [a]

[a] Department of Orthodontics, Tsurumi University School of Dental Medicine, 2-1-3 Tsurumi, Tsurumi-ku, Yokohama, Kanagawa 230-8501, Japan
[b] Department of Oral Microbiology, Tsurumi University School of Dental Medicine, 2-1-3 Tsurumi, Tsurumi-ku, Yokohama, Kanagawa 230-8501, Japan
[c] Department of Mechanical Engineering, Faculty of Science and Technology, Tokyo University of Science, 2641 Yamazaki, Noda City, Chiba 278-8510, Japan

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ABSTRACT

Objectives: Quantitative analysis of the biofilm has been critical for pathogenicity of oral diseases such as caries and periodontal disease. Currently, the plaque control record (PCR) is used most frequently to evaluate the presence of plaque on teeth, but no quantification methods exist so far. The aim of this study was to establish a method to quantify plaque adhesion on teeth.
Methods: A novel algorithm and executing program was developed to calculate the staining volume of biofilm on plastic disks or extracted teeth inoculated by *Streptococcus mutans* from photographic image data. The biofilm's volume on plastic disks was determined using a confocal microscope, and correlations between image analysis data were analyzed. Subsequently, the amount of plaque in the oral cavity was clinically evaluated using this algorithm.
Results: A strong correlation was observed between the biofilm's volume and the image analysis data. Similar results were also obtained with the biofilm model using extracted teeth. Clinically, it was revealed that an assessment of the amount of plaque adhesion for every tooth was possible, which could not be determined by PCR in the oral cavity.
Conclusions: We successfully developed a novel method, designated “plaque volume ratio,” to quantify the plaque accurately from photographic image data. Our findings indicated that this method may be useful for the evaluation of the amount of plaque on the surfaces of not only teeth but also dental materials in the oral cavity.

1. Introduction

Indigenous oral bacteria coexist with humans and are vital for preventing colonization by foreign pathogenic microorganisms. They proliferate with time, and together with the extracellular polymeric substance that they produce, form a biofilm known as dental plaque [1–3]. Quorum sensing (QS) signals, a communication system that allows microorganisms to sense each other's presence, were recently found to control the formation of the biofilm [4–6]. The microorganisms constituting the biofilm have also been shown to activate the expression of pathogenic factors when QS signaling molecules, also called “autoinducers,” act as transcription factors [6].

The oral plaque contains microorganisms responsible for caries and periodontal disease, which also have a potential for pathogenicity even when they are a part of the biofilm [7–10]. Plaque control record (PCR) is commonly used to evaluate the plaque adhesion level to human teeth. The PCR method was proposed by O'Leary et al. [11] for the purpose of showing patients the effects of plaque control. Briefly, every tooth is divided into four surfaces: mesial, distal, labial (buccal), and palatal (lingual). The presence of plaque adhering to the gingival margin on each tooth surface is calculated as the number of surfaces with plaque/number of test tooth surfaces × 100 (%). The ideal goal set for patients is reported to be less than 10%, above which periodontal surgical procedures are not indicated [11]. However, a PCR below 20% is considered to be fairly good.

Currently, PCR is the most popular evaluation method for plaque and is very useful for providing oral hygiene instructions. However, this method only determines the existence of plaque and cannot evaluate its quantity. In addition, as only cervical plaque is evaluated, PCR is generally suitable for risk assessment of periodontal disease, but not for caries, because plaque that does not

* Corresponding author.
E-mail address: ohshima-t@fs.tsurumi-u.ac.jp (T. Ohshima).

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come into contact with the tooth cervix is considered negative (zero). In patients undergoing orthodontic treatment, tooth cleaning is often difficult and results in an increased risk of caries. However, there are no methods to evaluate the plaque around a multi bracket appliance. Therefore, a method is required for quantitative evaluation of plaque, which can be used in various settings including oral hygiene instructions, guidance for patients with aspiration pneumonia, and application of dental materials to the quantification of plaque deposition. Thus, the aim of this study was to establish a quantification method of the plaque adhering to the teeth in the oral cavity.

2. Materials and methods

2.1. Bacterial strain and culture conditions

Frozen stock of *Streptococcus mutans* (ATCC 25175) was thawed and cultured in brain-heart infusion (BHI) broth (Becton, Dickinson and Company) overnight under anaerobic conditions at 37 °C. After inoculation of 100 μL to 10 mL of fresh BHI, bacterial suspension was subjected to shaking culture under aerobic conditions overnight at 37 °C. Subsequently, a portion of the bacterial suspension was further inoculated into a 1000-fold volume of BHI and subjected to 6 hof shaking culture under aerobic conditions to obtain $10^{8–9}$ CFU/mL as a pre-culture.

2.2. Determination of the most sensitive color channel in the biofilm images

We used plastic disks (Cell Disk LF1, Sumitomo Bakelite Co., Ltd., Tokyo, Japan) with a diameter of 13.5 mm and a thickness of 0.1 mm. In order to create a biofilm on a plastic disk, the latter was immersed in human saliva (filtered with Milex 0.22 μm; Merck Millipore, Darmstadt, Germany) overnight at 4 °C, inoculated with 1×10^8 CFU/mL of pre-cultured *Streptococcus mutans* in BHI broth supplemented with 5% sucrose, and cultured with shaking for two days at 37 °C. The biofilm on the plastic disk was stained with a staining solution (PROSPEC; GC Co., Ltd., Tokyo, Japan), washed with water, and fixed in 99.9% ethanol. A photograph of the stained disk was acquired and the image was converted to RGB (for Red, Green, and Blue) color space [12,13].

2.3. Evaluation of the biofilm of Streptococcus mutans on plastic disks

The thickness of the formed and stained biofilm in 2.2 was measured at 10 randomly selected spots on each disk with a confocal laser scanning microscope (OLS3000; OLYMPUS, Tokyo, Japan). A photograph of the plastic disk was acquired with a color code (color chart; Pantone LLC, NJ, USA) to calculate the staining level of the biofilm.

The relationship between the biofilm thickness and the color intensity of the stained area was investigated. A quantitative staining value of the biofilm was calculated based on the color intensity of an inscribed rectangle on the plastic disk. Briefly, the acquired photograph was converted to RGB and the color intensity was evaluated using the following Stain Value equation:

$$sv = \sum \sum (255 - G_{i,j})/N$$

$$SV = \sum^{\text{top } 10} sv/10$$

Where *sv* is the Stain Value of a small square area, *SV* is the Stain Value of the plastic disk, $G_{i,j}$ is the Green channel value of pixel (i, j), and N is the number of pixels $(i \times j)$ in the square area. Due to the variable biofilm thickness, the inscribed rectangle of the plastic disk was divided into 25 small square areas. SV of the plastic disk was an average of the top 10 sv of the small square area. To compensate for the lighting conditions, the values of the Green channel of the color code were used for intensity calibration of the different photography conditions. The following equation was used for calibration.

$$G'_{i,j} = (G_{i,j} + G_c)/(G_r - G_c) * (GR - GC) - GR,$$

where $G'_{i,j}$ was the calibrated value, G_r was the average value of the right side color code of the image, G_c was the average value of center color code of the image, *GC* and *GR* were constant values depending on the center color code and the right side color code, respectively.

2.4. Evaluation of the biofilm of Streptococcus mutans on extracted teeth

After informed consent, 20 maxillary first premolars of patients were used. The study was approved by the Ethics Committee at Tsurumi University (Approval no. 802).

The extracted teeth were polishing and autoclaved. Subsequently, the teeth were fixed with paraffin wax (Paraffin wax; FEED Co., Ltd., Kanagawa, Japan) on a plastic cap, and dipped in 2.5 mL of filtrated human saliva in a 24-well plate overnight at 4 °C to acquire a pellicle on the surface.

The dental crown was immersed in a pre-cultured *Streptococcus mutans* suspension in BHI containing 5% sucrose of each concentration of the 10-fold serial dilution. A biofilm was formed by agitating the culture. The tooth on which the biofilm had formed was subsequently dyed with a plaque staining solution (PROSPEC), fixed with methanol, washed with water, assigned a color code, and photographed.

Maxillary first premolar images were cropped from the photographs and subsequently converted to RGB color space. The tooth area images were distinguished using Otsu's binary method [14] on the Red channel in the cropped image. The stained area was extracted using the k-means clustering method [15,16] on the Green channel values from the tooth area image. The cluster number of the k-means method was determined manually (k = 2–5). The quantitative value of the plaque amount in the tooth area was defined as plaque volume ratio (PVR), which is the area ratio of the plaque volume, and was calculated using the following equation:

$$\text{plaque volume ratio (PVR)} = \sum (255 - G_{i,j})/A_t$$

where $G_{i,j}$ is the Green channel value of pixel (i, j) and A_t is the area of the tooth area. To compensate for the lighting conditions, the values of the Green channel and the biofilm plastic disk were used for calibration. Differences in the biofilm thickness were indicated by pseudo-color images of the tooth stained area.

After acquiring a photograph, the stain incorporated in the biofilm on the tooth surface was extracted with 99.9% ethanol overnight, and the optical density of the extracts was measured using an absorbance meter (Varioskan; Thermo Fisher Scientific K. K, Yokohama, Japan) at OD540 nm to quantify the biofilm [17,18].

2.4.1. Clinical study

This study was carried out under the approval of Tsurumi University Ethics Committee (Approval no. 1145) and with informed consent from the participants. Ten participants (5 male) aged 25–37 years (mean, 28.3 years) volunteered in this study. Our

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