

Confocal Laser Microscopic Images of *In Vitro* Cariogenic Biofilm and Resulted Demineralized Dentin

Khairul Matin^{1-3*}, Thwe Zin Ei¹, Ryoko Otsuka⁴, Ayako Okada⁵, Kenichi Nebuka^{2,3}, Nobuhiro Hanada⁴ and Junji Tagami¹

^{*1}Department of Cariology and Operative Dentistry, Department of Oral Health Sciences, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo, Japan

²Department of International Oral Health Sciences (Affiliated with the Department of Translational Research), School of Dental Medicine, Tsurumi University, Japan

³Medoc International Co. Ltd., Tokyo, Japan

⁴Department of Translational Research, School of Dental Medicine, Tsurumi University, Japan

⁵Department of Operative Dentistry, School of Dental Medicine, Tsurumi University, Japan.

Received July 25, 2018; Accepted August 04, 2018; Published October 27, 2018

Keywords: Cariogenic biofilm, Confocal laser scanning microscope (CLSM), Demineralized dentin, Dental tubules

INTRODUCTION

Dental caries or tooth decay is a bacterium induced disease process that initiates with the adhesion, proliferation of oral bacteria, eventual biofilm formation and acid secretion causing dental hard tissue demineralization [1-3]. *Streptococcus mutans* (*S. mutans*) is known as the leading bacteria involved in developing dental caries and even secondary caries as per more than five thousand clinical study and in vitro research reports [4-6]. A good number of researchers all around the world use laboratory strains of *S. mutans* to form biofilms in vitro to find answers to the global issue of tooth decay [6-8]. An oral biofilm reactor (OBR) in our facilities is being used for in vitro formation of cariogenic biofilms aiming to contribute in preventing caries or secondary carie [9,10]. Several methods of analysis have been employed to investigate the *S. mutans* biofilms after formation on enamel or dentin surfaces in those studies - including imaging with light microscope, fluorescence microscope and scanning electron microscope [8-10]. However, images from a confocal laser scanning microscope (CLSM) capable of reproducing surface topography of unstained biofilms with color differentiated depth profile has not been reported.

MATERIALS AND METHODS

Rectangular shaped dentin specimens were prepared from the bovine incisor roots. The specimens' surfaces were ground flat and then polished by diamond slurries with particle sizes up to 0.25 μm . Specimens were washed in an

ultrasonic bath with deionized water, ethanol and again with deionized water each for 15 min to remove smear plugs and micro-biomes. Scanning was carried out using a non-contact profilometer (NCP) with a red laser light source (VK-X150/X160, Keyence Co., Ltd., Osaka, Japan) and images were saved.

S. mutans biofilms were formed on the dentin specimens using *S. mutans* MT8148; a laboratory strain, as described elsewhere [8,9]. In short, sucrose supplemented (1% sucrose in Heart Infusion medium) biofilms were grown on the dentin specimen surfaces inside two identical water jacket-encircled chambers of an oral biofilm reactor (OBR) for 24 h. All through the biofilm formation period the pH was continuously recorded. After biofilm formation inside the OBR chambers, each specimen containing the biofilms was gently removed from the Teflon holder.

Corresponding author: Khairul Matin, Department of Cardiology and Operative Dentistry, Tokyo Medical and Dental University, 1-5-45, Yushima, Bunkyo-ku, Tokyo 113-8549, Japan, Tel: 81-(0)3-5803-5483; Fax: 81-(0)3-5803-0195; E-mail: matope@tmd.ac.jp; matintmd@gmail.com

Citation: Matin K, Ei TZ, Otsuka R, Okada A, Nebuka K, et al. (2018) An Introduction to Surgical Correction of Gingival Recession. J Oral Health Dent, 1(2): 39-41.

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One set of specimens with the undisturbed biofilms were immediately scanned by the CLSM and 3D images were saved (1D biofilm). Then the biofilms were cleaned using 0.25 M NaOH and the specimens were washed with distilled water to remove the biofilms. CLSM images were taken again to record the demineralized dentin surfaces (1D biofilm attacked specimen). Another set of specimens were transferred to a 24-well culture plate and were incubated at 37°C in sucrose-HI medium (changed in every alternate day) to demineralize further for 7 days. Thereafter, scanned by CLSM with the undisturbed biofilms on their surface and 3D images were saved same as mentioned above (7D biofilm). Biofilms were then removed using the same method and CLSM images of the 7D demineralized dentin were taken (7D biofilm attacked specimen). All CLSM scanning were carried out using a non-contact profilometer (NCP) with a red laser light source (VK-X150/X160, Keyence Co., Ltd., Osaka, Japan) and Multifile analyser (VK-150-H1XM) software was used for 3-D image construction.

RESULTS AND DISCUSSION

The biofilms that were formed on the dentin specimens in 24 h appeared creamy-white slimy substances on necked eyes and top surface was not smooth characteristically. CLSM 3D images confirmed that showing mountains, valleys and river-like channels running in between (**Figure 1**, left). In this particular image, the height of the biofilm (or vertical thickness) appeared to be more than 60 µm as color gradation displayed unevenness between the biofilm clusters. Most interestingly, the top-most surface of individual clusters was not essentially smooth; porous appearances were remarkable in between already smoothed parts. CLSM 3D images of 7D biofilm have shown further maturation; in vertical direction in exceeded 240 µm (**Figure 1**, right). Color gradation clearly indicated that mountains, valleys continued growing - though river-like channels were disappearing and most of the top surface became significantly smooth. Also, porosity could not be detected - instead they appeared as low-pitched rough surfaces at this stage. Deep ditch-like whole could be detected.

Root dentin surface images are shown in **Figure 2**. The surface was remarkably smooth with open dentinal tubules - open tubule size was very small as obvious in the dentin before biofilm attack (left). After 1D biofilm attack the surface became rougher and tubular orifice became wider which are easily detectable on the image (middle). As biofilm attack continued for 7 days the surface roughened further and tubular orifice widened remarkably as demineralization continued (right). Some notable information with 3D color gradation of the biofilm and dentin demineralization was acquired with this VK-X series of CLSM. The VK-X achieves high-resolution sensing by using a 16-bit photomultiplier as its laser receiving element. Also, it is capable of nanoscale measurements, even from a-

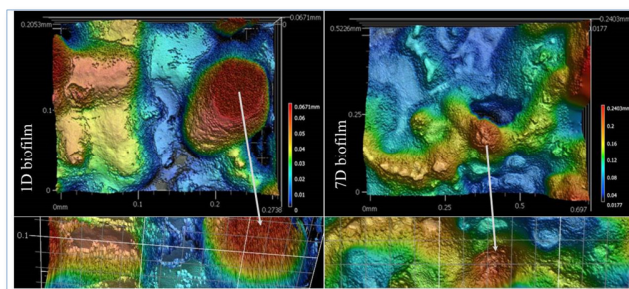


Figure 1. Confocal laser scanning microscopic (CLSM) images of *S. mutans* biofilms formed on dentin surfaces. Left-image is '1 day biofilm' and right-image is '7 day biofilm'. The reverse-sides of respective images are shown below (partially); arrows indicate porous part on 1D biofilms, are not seen on 7D biofilm.

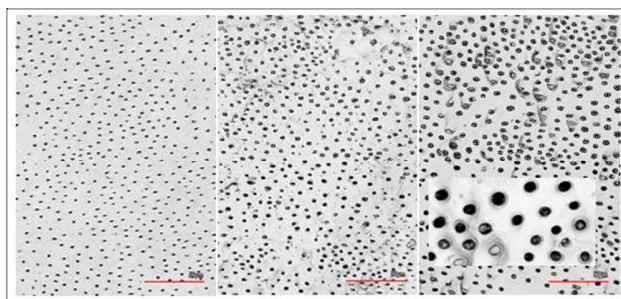


Figure 2. Confocal laser scanning microscopic (CLSM) images of demineralized dentin surface (bars are 50 µm). From left to right images are 'Before biofilm attack', 'After 1 day biofilm attack' and 'After 7 day biofilm attack' respectively. Inset is a high magnification image. In all images many clear open dentinal tubules are seen, some partially or completely blocked dentinal tubules are also seen.

-distance. The non-contact profilometric image of the unstained biofilm could be taken in less than a minute unlike other biofilm imaging reported so far [11]. Therefore, biofilm maturation can be monitored at any stage without disturbing their growth. Also, the same biofilm specimen can be analyzed using any other method as the partially air dried biofilm remains unharmed. Identification of porous areas was possible due to the penetration and return of CLSM laser through premature or loosely condensed matrix of the biofilms and that would help in determining the time of application of antibacterial agents during in vitro studies. Clear images of normal dentin and demineralized dentin are also helpful for many in vitro experiments; e.g. testing degree of demineralization and remineralization of dental hard tissues [12].

REFERENCES

1. Featherstone JDB (2008) Dental caries: A dynamic disease process. Aust Dent J 53: 286-291.

2. Scheie AA, Petersen FC (2004) The biofilm concept: Consequences for future prophylaxis of oral diseases? Crit Rev Oral Biol Med 15: 4-12.
3. Black GV (1886) Gelatine forming micro-organisms. Independent Practitioner 7: 546-548.
4. Loesche WJ (1986) Role of *Streptococcus mutans* in human dental decay. Microbiol Rev 50: 353-380.
5. Bourbia M, Ma D, Cvitkovitch DG, Santerre JP, Finer Y (2013) Cariogenic bacteria degrade dental resin composites and adhesives. J Dent Res 92: 989-994.
6. Hayati F, Okada A, Kitasako Y, Tagami J, Matin K (2011) An artificial biofilm-induced secondary caries model for in vitro studies. Aust Dent J 56: 40-47.
7. Ajdic D, McShan WM, McLaughlin RE, Savic G, Chang J, et al. (2002) Genome sequence of *Streptococcus mutans* UA159, a cariogenic dental pathogen. Proc Natl Acad Sci 99: 14434-14439.
8. Li J, Wang W, Wang Y, Zeng AP (2013) Two-dimensional gel-based proteomic of the caries causative bacterium *Streptococcus mutans* UA159 and insight into the inhibitory effect of carolacton. Proteomics 23-24: 3470-3477.
9. Gyo M, Nikaido T, Okada K, Yamauchi J, Tagami J, et al. (2008) Surface response of fluorine-polymer incorporated resin composites to cariogenic biofilm adherence. Appl Environ Microbiol 74: 1428-1435.
10. Oyanagi T, Tagami J, Matin K (2012) Potentials of mouthwashes in disinfecting cariogenic bacteria and biofilms leading to inhibition of caries. Open Dent J 6: 23-30.
11. Bowen WH, Koo H (2011) Biology of *Streptococcus mutans*-derived glucosyl transferases: Role in extracellular matrix formation of cariogenic biofilms. Caries Res 45: 69-86.
12. Fernández CE, Tenuta LMA, Del Bel Cury AA, Nóbrega DF, Cury JA (2017) Effect of 5,000 ppm fluoride dentifrice or 1,100 ppm fluoride dentifrice combined with acidulated phosphate fluoride on caries lesion inhibition and repair. Caries Res 51: 179-187.