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# Association between the cariogenicity of a dental microcosm biofilm and its red fluorescence detected by Quantitative Light-induced Fluorescence-Digital (QLF-D)

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ABSTRACT

*Objective:* This study evaluated whether Quantitative Light-induced Fluorescence-Digital (QLF-D) can detect the levels of cariogenicity of dental microcosm biofilms by assessing the red fluorescence intensity.

*Methods:* Dental microcosm biofilms were initiated from human saliva on bovine enamel discs. Biofilms with various levels of cariogenicity were then grown in artificial saliva supplemented with sucrose at different concentrations (0.05%, 0.1%, 0.2%, and 0.5%) in 24-well microplates. After 10 days, fluorescence images of the biofilms were captured by the QLF-D to analyse the red fluorescence intensity, which was quantified as the red/green ratio (*R/G* value). The supernatant pH was also measured, as well as the total and aciduric bacteria counts of the collected biofilms. Mineral loss in enamel was also evaluated by calculating the percentage of surface microhardness changes (%SHC).

*Results:* The *R/G* values of the biofilms differed significantly with the sucrose concentration ($p < 0.0001$), increasing consistently as the sucrose concentration increased from 0.05% (=0.91) to 0.5% (=2.56). Strong correlation was identified between the R/G value and the number of aciduric bacteria ($r = 0.83$, $p < 0.0001$), supernatant pH ($r = -0.95$, $p < 0.0001$), and %SHC ($r = 0.90$, $p < 0.0001$).

*Conclusions:* The red fluorescence as observed by the QLF-D was correlated with the cariogenic properties of dental microcosm biofilms *in vitro*, which indicates that this device can be used to detect the levels of cariogenicity of a dental biofilm.

*Clinical significance:* The QLF-D is able to assess the cariogenic levels of dental plaque based on the intensity of red fluorescence.



## 1. Introduction

Dental plaque represents a complex ecosystem composed of numerous species of bacteria inhabiting a tooth surface as a biofilm.[1] In response to changing environmental factors that induce the caries process, the microbial composition of dental biofilms changes from a balanced state to an imbalanced state, in which cariogenic bacteria, such as acidogenic and aciduric species, become dominant. The imbalances in the resident

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microflora result in the formation of cariogenic biofilms, which further induces demineralisation of dental hard tissue.[1–3] It would be helpful to be able to detect cariogenic dental biofilms and assess the metabolic activities of the resident microflora in these dental biofilms in order to successfully control and manage the development of dental caries.[4–6] Also, because the cariogenicity of the dental plaque is directly associated with the initiation and severity of dental caries,[5] knowledge of cariogenic biofilms based on scientific evidence and objective standards would facilitate the ability to predict the risk of dental caries.[4] In addition, the cariogenicity of dental plaque can vary between individuals and even at sites within the same oral cavity due to caries-related factors such as oral hygiene, dentition, diet, and saliva, which makes an understanding of the qualitative properties of the biofilms essential.[6]

Since plaque properties are determined by interactions between internal bacteria, it is vital to reproduce the natural microbial ecology in order to evaluate and research these properties. At the same time, the ethical issues and limited access of *in vivo* studies leads to a need for a laboratory model to allow investigations under controlled conditions.[7,8] However, dental biofilms composed of a single or only a few species do not represent the diversity, complexity, and heterogeneity of *in vivo* plaque. For these reasons, the dental microcosm biofilm formed from saliva inoculum, which is a resource of natural oral microflora has been used in various laboratory studies.[9–11]

A new type of Quantitative Light-induced Fluorescence (QLF) device called the QLF-D Biluminator™ (Inspektor Research Systems BV, Amsterdam, The Netherlands) has been newly developed to enable the detection and quantification of dental plaque by representing endogenous porphyrins produced by oral bacteria species as red fluorescence.[12–14] The auto fluorescence phenomenon of this equipment is known to be caused by certain oral bacteria that can synthesize high concentrations of endogenous metal-free fluorescent porphyrin.[15] The QLF-D Biluminator™ (henceforth referred to simply as the QLF-D) is an upgraded version of QLF devices that examines plaque more clearly as red fluorescence by strengthening these principles, making quantification of the plaque possible. This new device uses a narrow-band blue light source (centred at 405 nm) obtained by modifying the filter set (D007; Inspektor Research Systems BV, Amsterdam, The Netherlands) and consists of a Biluminator™ mounted on a high-specification digital single-lens reflex (SLR) camera fitted with a 60-mm macro lens, which is equipped with an illumination tube with white and blue light-emitting diodes positioned in a ring around the lens opening (Fig. 1). This device can produce high-quality photographs without any requirement for ambient light, visualize plaque more clearly, and detect subtle changes in plaque at a high resolution.[14] When viewed under its lighting conditions, regions where it is difficult to detect the presence of plaque with the naked eye are shown as red fluorescence, while the teeth themselves appear in their white natural state. These characteristics may make the new device useful as a diagnostic clinical tool for plaque control, as well as in the laboratory for the analysis of plaque.[14]

Previous studies found that the QLF could be used for the evaluation of dental plaque by presenting differences in fluorescence based on characteristic changes therein. When used under optimal conditions the QLF presented heavy dental plaque deposits on teeth as orange or deep red fluorescence, which indicated the presence of black-pigmented obligate anaerobes and secondary plaque colonizers.[16,17] Also, it was observed that the microbial composition of the plaque changed from Gram-positive to Gram-negative as well as from facultative to obligate anaerobes as it matured and also that the colour of the plaque fluorescence changed from being predominantly green to red or that the intensity of red fluorescence increased.[15,18] However, these findings were confirmed only in the previous QLF studies, and not in QLF-D studies; moreover, no previous study has investigated the ability of the new device to diagnose dental plaque. The intensity of red fluorescence seen during clinical investigations designed to observe dental plaque on tooth surfaces under the QLF-D has been found to vary between individuals and also between sites in the same oral cavity. Moreover, no study has identified any correlation of this red fluorescence intensity detected by the QLF-D with the cariogenicity of the dental biofilms. Thus, if the ability of the QLF-D to distinguish

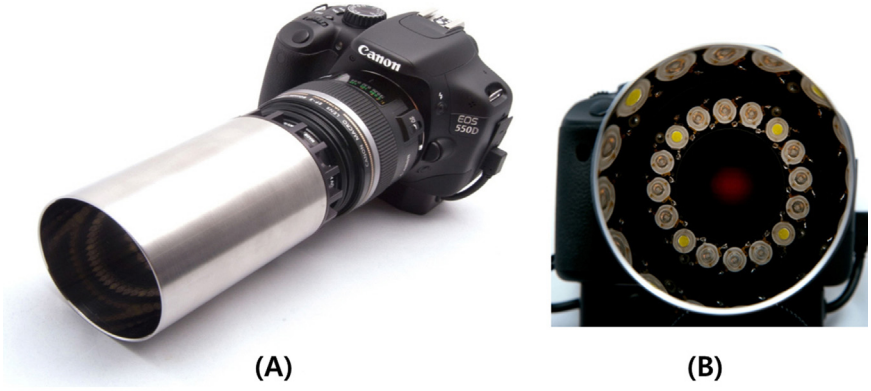


**Fig. 1 – The QLF-D Biluminator™ used in this study (image courtesy of Inspektor Research Systems). This device (A) is based on a full-sensor SLR camera (Canon 550D) equipped with an illumination tube with white and blue light-emitting diodes positioned in a ring around the lens opening (B).**