Red fluorescent dental plaque: An indicator of oral disease?
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Citation for published version (APA):
Volgenant, C. M. C. (2016). Red fluorescent dental plaque: An indicator of oral disease?
General introduction
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Oral diseases are the most common diseases in the world with a prevalence of caries of nearly 100% among adults, depending on age and country (Petersen et al., 2005; WHO, 2012). These diseases have large effects on the quality of life of individuals and cost a substantial amount of money (Listl et al., 2015). Dental caries and periodontal inflammatory diseases are the main diseases caused by microorganisms in the oral cavity and are strongly associated with dental plaque (Löe et al., 1965; Axelsson and Lindhe, 1978; Marsh, 1994).

Dental plaque is a biofilm, which is a diverse microbial community on the tooth surface embedded in a matrix of polymers of bacterial, salivary and dietary origin (Marsh and Bradshaw, 1995; Hall-Stoodley et al., 2004). When plaque is not timely removed, the biofilm produces substances like acids or proteinases that can cause disease (Löe et al., 1965; Axelsson and Lindhe, 1978). This production also depends on environmental factors such as the frequent intake of carbohydrates, the frequency of fluoride use, the host’s inflammatory response. When a biofilm becomes older, the microbial composition shifts from aerobic to facultative anaerobic bacteria and, eventually, to strict anaerobic bacteria (Ritz, 1967; Syed and Loesche, 1978; Marsh and Bradshaw, 1995). Dependent on the oral conditions, proteolytic ‘amino acid-degrading’ bacteria or saccharolytic ‘carbohydrate-degrading’ bacteria take over (Takahashi, 2015). Prolonged exposure to old plaque leads to a clinically visible inflammation of the gingiva within days to weeks (Löe et al., 1965). Without removal of the plaque and within a susceptible host, the inflammation of the gingiva may progress to inflammation of the underlying supporting tissues. Acid produced by the bacteria in old plaque can, after a period of time, result in demineralization of the hard oral tissue (Featherstone, 2000). This first sign of caries becomes clinically visible as white areas on the enamel of a tooth, which are therefore called white spot lesions. When the oral conditions do not change, these white spots will develop into caries with cavitation.

For years, specific bacteria have been related to dental diseases, like Porphyromonas gingivalis to gingival inflammatory disease (Haffajee et al., 2008) and Streptococcus mutans (Loesche, 1986), lactobacilli (Badet and Thebaud, 2008) and (more recently) bifidobacteria (Mantzourani et al., 2009b) to caries. Currently, the paradigms for oral diseases shifted from the specific plaque hypothesis to the ecological plaque hypothesis (Marsh, 1994). This hypothesis states that synergy is present in the dental plaque with (relative) homeostasis that can turn into (polymicrobial) dysbiosis, influenced by environmental changes. In dysbiosis, an imbalance occurs in the relative abundance of microbial species within the ecosystem. This imbalance leads to disease or can be the consequence of disease (Hajishengallis and Lamont, 2012;
Hajishengallis, 2014; Simón-Soro and Mira, 2015). The microbial species of which the relative abundance increases can be either a ‘classical’ pathogen or a pathobiont; a normally harmless symbiont, which can become pathogenic under specific conditions (Hajishengallis, 2014).

Therefore, the prevention of both caries and gingivitis focuses on plaque management (Kidd, 2011; Chapple et al., 2015). This plaque management consists of the removal and disturbance of plaque (in combination with the use of fluoride toothpaste) to maintain microbial homeostasis and prevent disease. However, it is difficult to determine who is at risk for the development of disease before clinical signs are visible. Especially for caries, the recognition of these first manifestations are important, since the first clinical signs are not completely reversible, which is in contrast to gingivitis. Therefore, reliable prognostic models or tools are needed to identify who harbours characteristic(s) leading to disease, allowing intervention before disease manifests (Zero et al., 2009; Tellez et al., 2013).

At present, dentists inspect patients for clinical manifestations of disease during periodic dental exams. For gingivitis detection, they use a probe to assess bleeding on probing and pocket depth and also register the presence or absence of calculus (Van der Velden, 2009). For caries detection, dentists visually assess the dental hard tissues by looking at the colour, lesion reflectance and surface texture (visual-tactile assessment, preferably with a ball-ended probe). This examination is complemented with dental radiographs if required. Unfortunately, these methods are using clinical characteristics of disease presence to detect patients at risk of disease, while prevention of even these early stages of disease is preferred.

Several diagnostic tools have been developed to detect caries already in a very early phase of the process, while for gingivitis this was less a priority due to its reversible nature and easy detection. In the 1980s, the principle of fluorescence was explored for its potential use for (early) diagnostics of caries lesions in the oral cavity using autofluorescence (Bjelkhagen and Sundstrom, 1981; Bjelkhagen et al., 1981). More recently, this technique was suggested to be valuable for the assessment of dental plaque. Dental hard tissues fluoresce green, while dental plaque has been reported to fluoresce red (Heinrich-Weltzien et al., 2003). Fluorescence is photoluminescence: the excitation with light (photons) of a specific wavelength brings electrons of molecules from a ground state to an excited state. These electrons release this acquired energy rapidly by emitting light. Since a small amount of energy is lost during this process, the emitted light has lower energy and therefore a longer wavelength, than the wavelength of excitation. Specific molecules emit fluorescence without intervention other than light excitation. The green fluorescence from teeth and the red fluorescence from plaque are an intrinsic (endogenous) property and
hence called autofluorescence, unlike extrinsic (exogenous) fluorescence that is induced by using e.g., a fluorescent dye. Without further notice, the fluorescence in this thesis is autofluorescence.

When the oral cavity is illuminated with blue-violet light (370 - 500 nm), teeth fluoresce green (510 - 535 nm) (Stübel, 1911; Benedict, 1928). White spots emit less fluorescence, which enhances their visibility; they become visible as a dark area compared to the surrounding healthy tissue, which is fluorescing brightly (Figure 1.1) (Angmar-Mansson and Bosch, 2001). Further development of these systems in the 90s led to the use of a lamp with bandpass filter ($\lambda_{ex} = 405$ nm) in the first Quantitative Light-induced Fluorescence (QLF) systems (the QLF/Clin and Inspektor Pro, Inspektor Research Systems B.V., Amsterdam, the Netherlands). Subsequently, other fluorescent diagnostic systems were introduced onto the market using different excitation wavelengths (Gimenez et al., 2013), such as the DIAGNOdent ($\lambda_{ex} = 655$ nm; KAVO, Biberach, Germany, Lussi et al., 2004), the VistaProof ($\lambda_{ex} = 405$ nm; DÜRR DENTAL, Bietigheim-Bissingen, Germany, Jablonski-Momeni et al., 2014) and the Sopro-Life ($\lambda_{ex} = 450$ nm; Sopro-Aceton, La Ciotat, France, Tassery et al., 2013).

When using the QLF-system ($\lambda_{ex} = 405$ nm) in clinical studies, red fluorescence has been observed from (older) plaque (Heinrich-Weltzien et al., 2003; Coulthwaite et al., 2006; Van der Veen et al., 2006), calculus (Buchalla et al., 2004a; Qin et al., 2007), non-cavitated (Buchalla et al., 2004b; Buchalla, 2005; Felix Gomez et al., 2016) and cavitated caries lesions (König et al., 1993; König et al., 1999; Lennon et al., 2002) as well as from cracks in the enamel (Jun et al., 2016). Furthermore, a patent application was filed, suggesting the use of fluorescence for monitoring gingival inflammation (Kanbara et al., 2006).

The previous QLF-devices were optimized towards a single-lens reflex (SLR) camera with LED-lighting to look at a larger field of the oral cavity per image compared to the earlier intra-oral camera systems. With the use of dedicated filters, the contrast between the red plaque and green enamel fluorescence signals was enhanced. The green fluorescing teeth were given a more natural appearance on the photographs as well (Figures 1.2 and 1.3). This new QLF-D camera was equipped with filters that transmit nearly 100% of the red part of the spectrum, 15% of the green part of the spectrum and 15% of the blue part of the spectrum. The contrast between sound and carious dental tissues was enhanced by this differential optical filtering.

The red fluorescence from plaque is considered to originate from porphyrin-like molecules, which are co-enzymes or factors that play a role in electron transport for different enzymatic reactions. Porphyrins are associated with many metabolic and
Chapter 1

Figure 1.1 A white light image (a) and fluorescence image (b) of a tooth. The white spot lesion is clearly visible on the right image due to fluorescence loss of the enamel of the starting lesion. Images courtesy of M.H. van der Veen.

Figure 1.2 A white light photograph of the anterior teeth (a) and its accompanying fluorescence photographs (b). No red fluorescent plaque is visible on the teeth.

Figure 1.3 A white light photograph (a) and fluorescence photographs (b) of teeth with a substantial amount of red fluorescent plaque and calculus.

catabolic pathways of bacteria and they are known to fluoresce red when illuminated with near-UV light (König, 1994b; König et al., 1998; Buchalla, 2005; Buchalla et al., 2008). It has been suggested that red fluorescence is related to mature dental plaque, but up to now this is based only on circumstantial evidence.

Outline of this thesis
The aim of this PhD thesis was to understand why some dental plaque fluoresces red and to determine the applicability of red fluorescent plaque as a prognostic factor for
oral microbial diseases. Part one of this thesis describes *in vitro* studies concerning red fluorescence and Part two of this thesis describes the clinical (*in vivo*) studies.

**Part one** - Oral microbial diseases are traditionally attributed to specific bacteria present in plaque. In *chapter 2*, the red fluorescence properties of some of these bacteria that are related to gingivitis or caries are described. Since bacteria in a biofilm are exposed to environmental influences, different nutrients were studied to explore their influence on the ability of single bacteria to fluoresce.

More recent studies about caries and periodontal diseases concern biofilm studies, which is a situation that is more comparable with the situation in the oral cavity. Dental plaque is present at different locations of a tooth: at smooth surfaces, occlusal grooves and approximal spaces. The thickness of dental plaque varies due to sheer forces of the tongue, the depth of inapproachable fissures and other local differences, while the accessibility also differs per location, potentially resulting in differences in the age of the plaque. *Chapter 3* discusses the effect of some of these parameters on biofilm fluorescence, while in *chapter 4* the green and red fluorescence from early biofilm development is described in a biofilm under continuous flow.

**Part two** - In a daily dental practice where the focus lies on prevention of oral diseases, the presence of dental plaque is made visible to the patient using a dedicated disclosing solution (such as erythrosine or a two-tone plaque disclosing solution). Since this is a well-known method to assess the oral hygiene levels, *chapter 5* discusses the correlation between the total amount of disclosed dental plaque and red fluorescent dental plaque in a study with 48 participants. The relationship between red fluorescence and bleeding on marginal probing (BOMP) is discussed as well. This BOMP measurement quantifies gingival health and is often used as an indication of the long-term oral hygiene. Red fluorescent plaque in relation to BOMP is assessed in *chapter 6*, which describes an experimental study where the participants are asked not to perform any oral hygiene for the duration of 14 days. *Chapter 7* investigates the bacterial composition of saliva in parent-child pairs with and without red fluorescent dental plaque. This study also gives new insights in the association between the oral flora of a parent and his/her child. Considering that red fluorescent plaque is suggested to be related either with specific bacteria or with the maturation of dental plaque, a comparison of the composition of red fluorescent dental plaque and not-red fluorescent dental plaque was performed in *chapter 8*. Plaque from areas with and without red fluorescence was collected from 50 children. Their caries status was assessed using the International Caries Detection and Assessment System (ICDAS) and related to the red fluorescent plaque score at site level as well as at subject level.

In *chapter 9*, a general discussion and directions for future research are provided, after which a summary of the results of this thesis is presented.