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RECENT ADVANCEMENTS IN PLAQUE AND CALCULUS DETECTION USING FLUORESCENCE TECHNIQUE

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BACKGROUND
The oral cavity is an open growth system with uninterrupted interactions of microbes and their nutrients. Deposition of dental plaque causes inflammatory changes on the periodontium which can lead to the destruction of tissue and loss of attachment. Traditionally, dental plaque is often detected by clinicians either by screening the plaque directly from the tooth surface or changing its colour with a disclosing solution. But these assessment methods have the limitation of being subjective. Therefore, the results may vary from clinician to clinician. Recent methods of plaque detection takes advantage of the ability of natural teeth to fluoresce, when the light is absorbed at a specific wavelength and then emitted in a higher wavelength. The difference between natural fluorescence of sound tooth and that with dental plaque and calculus can be quantified using light-emitting devices, such as laser, xenon or LED, etc.,. The review article focuses on the recent techniques for detecting plaque and calculus using the fluorescence phenomenon.

KEYWORDS: Dental plaque, Calculus, Detection, Recent advances, Fluorescence, Quantitative Light Induced Fluorescence, Laser Fluorescence, LED Fluorescence.

INTRODUCTION

Oral health is an integral part of general health. It contributes to determine the **overall health** of an individual. The World Health Organization (WHO) defines oral health as "a state of being free from mouth and facial pain, oral and throat cancer, oral infection and sores, periodontal (gum) disease, tooth decay, tooth loss, and other diseases and disorders that limit an individual's capacity in biting, chewing, smiling, speaking, and psychosocial wellbeing.[1] The oral cavity harbors natural bacteria, biofilm, plaque and other deposits. The bacterial composition of plaque remains relatively stable despite regular exposure to minor environment changes. This **microbial homeostasis** is due to dynamic balance of both synergistic and antagonistic microbial interactions. However, the homeostasis can breakdown, leading to shifts in the balance of micro flora, thereby predisposing to diseases such as dental caries and periodontal diseases, which are a few major global health burden.[2] These diseases which share a common source of origin, are the result of the interaction between the bacteria in the dental plaque and the host over a period of time. Attempts should be made at finding out the most effective and easiest ways to diagnose dental plaque, and to educate patients on oral hygiene compliance for a better life. However, if the patient has to effectively clean their mouth, they must be able to identify areas that require more attention and those which are clean. As plaque is generally colourless it is usually stained prior to assessment. Plaque identification may be done either by screening the plaque directly from the tooth surface,[3] changing its colour with a disclosing solution,[4] or using the ability of natural teeth to fluoresce under blue light.[5] By these methods, every patient can be made aware about their plaque in situ. It generates curiosity in the patients, and an obvious and apparent concern for its prompt removal. The article elaborates on the major drawbacks of the conventional disclosing agents and focuses on the recent advances in detecting plaque by quantification using various light emitting devices and their advantage over the conventional methods to detect plaque.

Disclosing agents and their limitations

Dental plaque has the ability to retain a large number of dye substances which can be used for disclosing purposes. Disclosing dyes work by changing the colour of dental plaque so that it contrasts with the white tooth surface. This property is related to interaction because of the polarity difference between the components of the plaque and the dyes. Particles are bound to the surface by electrostatic interaction (proteins) and hydrogen bonds (polysaccharides).[6] However these agents have certain limitations:

1. Tooth-colored restorative materials are susceptible to color change as plaque disclosing agents have an effect on the colour stability of restorative materials
2. They causes staining of esthetic brackets
3. The implementation of motivational techniques using disclosing agent shall be effective only when periodic reinforcement of oral hygiene is done.
4. Reproductive toxicity and mutagenic effects of erythrosine in male mice.[7,8]

Newer methods of plaque detection

Newer imaging techniques that overcome the various limitations of conventional disclosing methods have emerged to detect the presence of dental plaque and caries. Benedict in 1928 observed the phenomenon of Fluorescence where the light is absorbed in a specific wavelength and then emitted in a higher wavelength. During this transition, certain molecules (fluorophores) de-excite electronically from higher energy level to a lower energy level. Similar characteristic has been observed in the dental tissues, since the pattern of light absorption and re-emission (spectrum of fluorescence) of the dental tissues varies according to the excitation light wavelength. The difference between natural fluorescence of sound and carious dental tissues can be quantified using light-emitting devices, such as laser, xenon or LED.[9]

The development of numerous alternative non-invasive detection methods based on the phenomenon of fluorescence, such as laser fluorescence devices (DIAGNOdent and DIAGNOdent pen), quantitative light-induced fluorescence (QLF), fluorescence camera (Vistacam), LED technology, can offer objective assessments, where traditional methods could be supplemented by quantitative measurements.

Quantitative light induced fluorescence

Quantitative light-induced fluorescence or QLF is a method in which visible and harmless light excites the teeth to produce fluorescent images, which can be stored on disk and computer analyzed. Bacterial metabolites in and on the teeth are detected and quantified using this method. The concept was first introduced by Bjelkhagen et al. in 1995 a prototype clinical device, based on the original concept, called QLF™ was introduced.[10] The principle of QLF™ is based on fluorescence where blue light with peak intensity of 405 nm illuminates and excites tooth tissue. The re-emitted fluorescent light is collected via a modified dental mirror by a video camera. The collection and analysis of the images are performed using custom made software. To enable the calculation of fluorescence loss in the tooth structure, the fluorescent radiation of sound tissue at the lesion site is reconstructed by interpolation from the fluorescent radiance of sound tissue surrounding the lesion. The difference between the measured values and the reconstructed ones gives the resulting fluorescent loss in the lesion. The digitized live images are stored on a personal computer and displayed real time on a screen.[11]

Red fluorescence (RF) and presence of bacteria:

Excitation of red extrinsic fluorophores from bacterial metabolites with blue light causes red or orange fluorescence. These bacterial metabolites, porphyrins and also possibly extrinsic and intrinsic polysaccharides are present in old anaerobic plaque as well as in calculus. So far the bacteria identified as producing red fluorescing metabolites are mainly related to periodontitis, and these bacteria are mostly haemin dependent. The presence of RF is considered a property of matured biofilm or plaque, which is associated with poor oral hygiene and as such with both caries and periodontitis.

Laser fluorescence

The detection of dental plaque can be outperformed very accurately using laser fluorescence method. The application is easy and very safe as laser-induced fluorescence can avoid high risk ionising radiations. When the laser irradiates the tooth, the light is absorbed by organic and inorganic substances present in the dental tissues, as well as by metabolites from oral bacteria.[12] These metabolites may be porphyrins that are produced by several types of oral bacteria that produces red fluorescence. Laser-induced fluorescence can accurately and sensitively diagnose subgingival calculus compared to the conventional periodontal probing. Several in vitro studies have shown that oral microorganisms and their metabolites like porphyrins, metalloporphyrins and other chromatophores contain the **fluorophores** that are emitted from the dental calculus and carious lesions. This ability of calculus to emit fluorescent light following irradiation with light of a certain wavelength enables the detection of calculus. Based on the auto fluorescent property of calculus, the newer diagnostic instrument has been developed, the Diagnodent (KaVo, Biberach, Germany). The device was initially launched for caries diagnosis. Later, the device was modified to enable calculus detection. Diagnodent is able to measure wide range of fluorescence intensities, transformed and shown on a digital display as relative calculus-detection values from 0-99 (Table 1).[13] Readings corresponding to the calculus are indicated by a beep with an increasing sound frequency as the display value increases.

Table 1: Diagnodent readings corresponding to clinical conditions.

S. no	Diagnodent Readings	Clinical Finding
1	Greater than 40	Mineralized deposits
2	5-40	Small calcified plaque sites
3	Lesser than 5	Clean root surfaces

Keylaser 3 (Kavo, Biberach, Germany) is the only commercially available device, which combines calculus detection as well as removal in a feedback-controlled manner. The automated device contains a 655-nm

InGaAs diode laser for calculus detection and a 2940-nm Er:YAG laser for calculus removal. The Er:YAG laser is activated only when the threshold value for the diode laser exceeds 0-99. As soon as the reading falls below the threshold value, the treatment laser turns off. This mechanism of Keylaser 3 helps to optimize the calculus removal and reduces the side effects associated with Er:YAG laser.[14]

Laser-induced autofluorescence (LIAF) spectroscopy is evolving as a powerful tool to detect and characterize biochemical and morphological changes occurring in the human body based on the changes in the fluorescence signatures. The system consisted of a 404 nm diode laser for excitation of fluorescence and a miniature fiber-optic spectrometer (Ocean Optics, Model: USB 2000FL VIS-NIR) connected to the USB port of a computer to record the spectrum from gingival plaque. One leg of the bifurcated optical fiber (400-μm diameter) guides the light from the laser to the plaque surface through a handpiece made of stainless steel while another fiber of the same diameter collects the fluorescence signal from plaque to the spectrometer through a long wavelength pass filter (Schott GG420). An experienced periodontist identified the site for measurement and places probe tip on the plaque at the gingival margin without disturbing the plaque or gingiva while a physicist, well trained in optical spectroscopy, recorded the spectral data from each patient. Fluorescence intensity (FI) ratios were calculated using the intensity values of peaks observed at 510 and 630 nm in the LIAF spectra. The ratio was correlated with the clinical plaque score of the study population for discriminating different grades of plaque from grade 0 (absence of plaque) to grades 1, 2, and 3 (presence of plaque).[15]

Led fluorescence

One of the developed diagnostic procedures that employs fluorescence diagnostics with low-intensity lasers and LED light sources is LED Fluorescence device. Considering the need of a robust quantitative detection method for dental plaque and calculus, and the possibility of improvement by utilizing more favorable excitation wavelength and cheaper light source, LED lights has been used for dental fluorescence detection and intraoral camera with fluorescence function. With advances in LED technology, UV emitting LEDs have now been developed. The ultraviolet (UV) spectrum is traditionally divided into three UV bands: UVA (315-400 nm, also termed black light, long wave or near UV), UVB (280-315 nm, also termed middle UV - responsible for the sunburn response), and UVC (200-280 nm, also termed short wave UV). UVA is the important band in terms of diagnostic applications in dentistry. Common UVA wavelengths in commercially available LEDs are: 375, 385, 395 and 405 nm. UVA LEDs are small, long lasting and very reliable on comparison with other light sources. UVA light emits visible red fluorescence from deposits of mature dental plaque on the surface of teeth, restorations, or dental appliances. The fluorescence arises because of the presence of porphyrin (Protoporphyrin-IX (PP9)), in bacteria. PP9 is found in high amounts in Gram negative oral bacteria, and since the levels of Gram negative bacteria increase as the dental plaque biofilm matures, red fluorescence is associated with mature dental plaque. This has been studied since the seminal work of Bommer and Benedict in the mid-1920's, who first reported the characteristic red fluorescence from dental plaque and dental calculus respectively, when using UVA light sources. The Durr Vistaproof System uses six LEDs emitting at 405 nm and considered to be an ideal wavelength in the UVA waveband for revealing PP9 fluorescence. The captured images from the camera unit are ported back to a computer via a USB interface for analysis. The DBSWIN analysis software highlights the intensity of the red signal from PP9, providing a numerical score that can assist treatment decisions, in much the same way as has been done with QLF.[16]

The SOPROCARE® Camera is a device that uses the properties of tissue autofluorescence as well as those of selective chromatic amplification in order to reveal calcified or non-calcified dental plaque and gingival inflammation. The camera illuminates the dental tissues with a wavelength between 440 and 680 nm. The excited material absorbs the energy and reconstitutes it as fluorescent light. This means the image obtained can be superimposed over the natural anatomical image and the nuances that are invisible in white light can be revealed in real time. It is also the only camera that uses autofluorescence and selective amplification color to highlight inflammation gingival and to distinguish between old and new dental plaque (PERIO mode).[17]

At- home method for detecting dental plaque

Many individuals lack good technique and dexterity, but most of the problems relate to the nature of plaque itself. Plaque is basically tooth-coloured, making it effectively camouflaged. In the 1970s Prof. Barrie Gillings published research and popularized fluorescein dye which has an exclusive property that – under normal light, plaque stained with Fluorescein appeared barely discernable but, when exposed to a low energy ultra-violet light, would fluoresce bright yellow. Many ADA members recognized the system as a useful educational and preventive tool for the general community. The Dental Health and Research Foundation embraced the concept and for many years sponsored a portable Plaque Disclosing Tunnel which toured multiple locations, including Royal Agricultural Shows, showing thousands of families, where their tooth brushing needed to improve. It was also used by the Foundation's Dental Health Educators, who visited schools in purpose built caravans. Invariably children enjoyed seeing their teeth from a different perspective.[18]

Recently Gillings refined the system for home use by modifying electric toothbrushes and connecting their batteries to UV-emitting LEDs in the handles. At the

same time it was suggested that Fluorescein shall be incorporated into ordinary toothpaste. Teeth can be cleaned in the usual manner, the mouth rinsed and the brush needs to be turned around and the quality of cleaning is assessed using the UV light. Those areas where plaque remains are identified and the brushing is repeated with particular attention to the 'Trouble Spots'. Finally the teeth is viewed again to confirm whether all the residual plaque has been removed.

CONCLUSION

It is believed that "One of the biggest challenges to medicine is the incorporation of technology in clinical practice". Visual examination, by observing clinical characteristics and appearance of the lesions, provides most of necessary information to the clinician. However, by the time deep lesions are detected by radiographic examination, the disease progression becomes more severe. To enhance treatment planning and efficacy, several systems for plaque and calculus detection and/or elimination have been developed, based on different technologies. Such auxiliary methods can contribute positively in the process of detection of plaque and calculus at the early stages. However, the clinician should be aware of their correct use and follow the manufacturer instructions. Besides, their disadvantages and affecting factors should also be kept in mind as it could play a major role in the decision-making process of both diagnosis and treatment. Nevertheless, these methods have their own limitations in terms of cost, technique sensitivity, elaborative set up, etc.,. The Clinicians may have to eventually rely on their ability to reproduce the results given by the detecting device, which may even lead to a manual error. Future research should focus on these limitations and requirements for forming newer devices and techniques for early detection and removal of plaque and calculus.

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