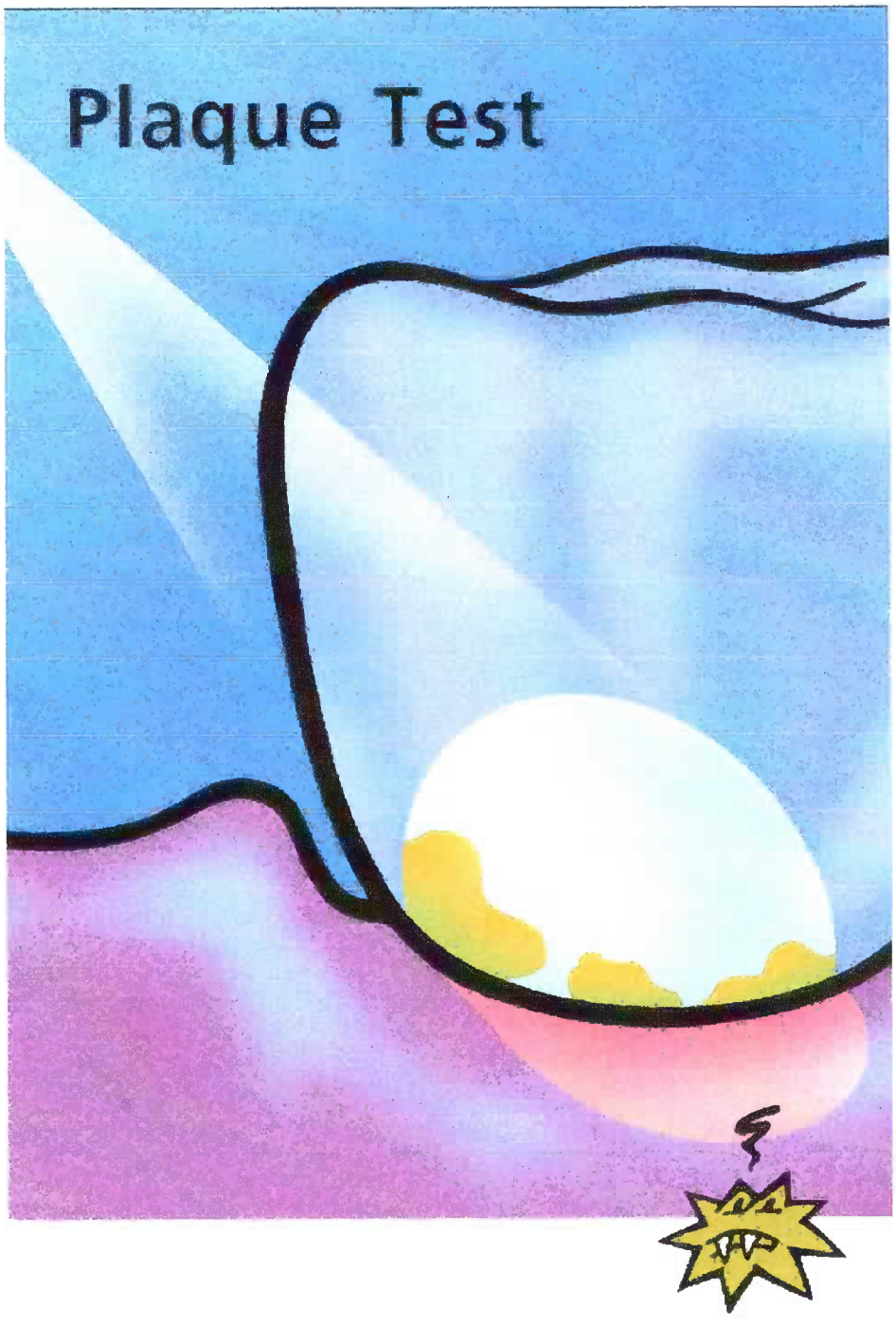

Scientific Documentation

ivoclar vivadent
passion vision innovation

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1. Introduction

1.1 Plaque

The human oral cavity is generally colonized with a host of different microorganisms (bacteria, yeasts). On the tooth surfaces, the bacteria may form a biofilm, also known as plaque (see Fig. 1). In addition to bacteria, plaque comprises carbohydrates and proteins which promote the adherence to the enamel and act as a "protective cover" and reservoir of nutrients, where the bacteria supply each other with metabolites.

If plaque is not removed, it "matures" – macromolecules strengthen the plaque and increase its adherence to the dental enamel. Via deposition of minerals, plaque may develop into calculus.

The metabolic processes of the plaque bacteria cause a drop in the pH value in the plaque. As a result, the underlying enamel may begin to dissolve and, eventually, caries may develop. In addition, a build-up of plaque may lead to inflamed gums, known as gingivitis. If left untreated, gingivitis may progress to periodontitis or peri-implantitis in patients with implant-supported restorations. This may severely endanger the health of teeth and gums and the survival of restorations.

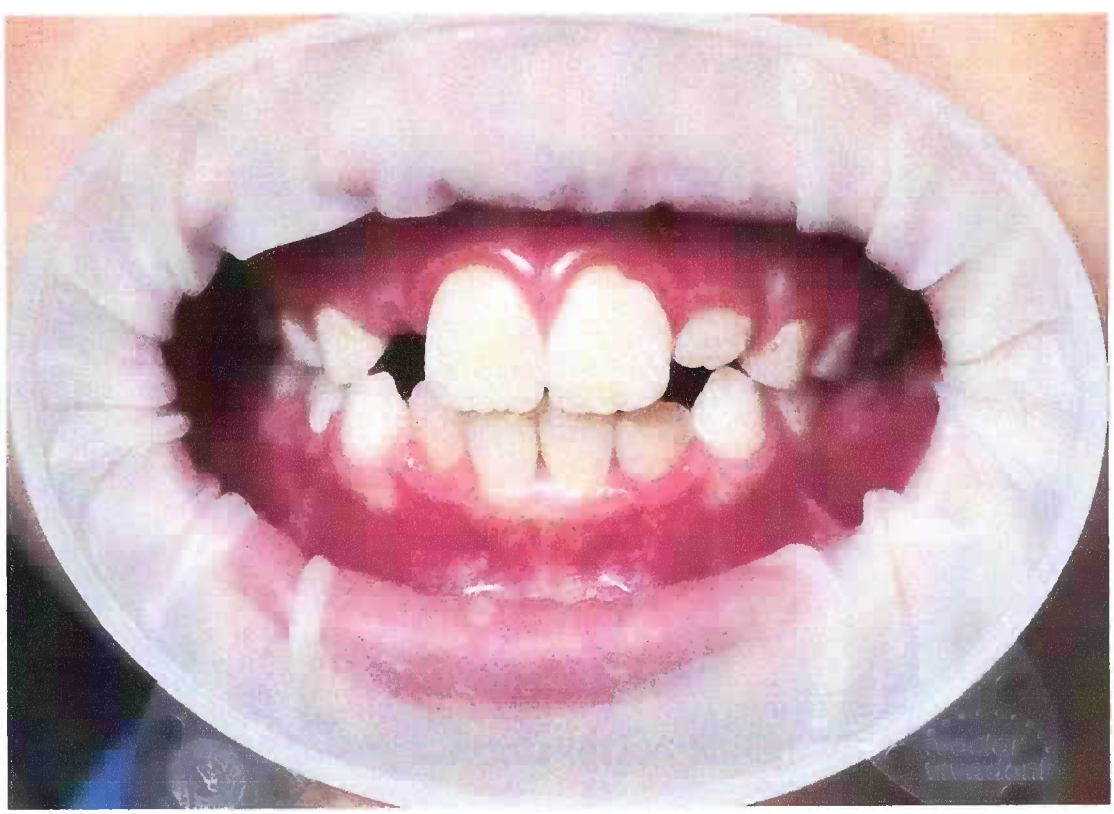

Fig. 1: Plaque on the teeth and in particular, along the gingival margins in a young patient.

1.2 Plaque disclosing agents

Removing plaque at regular intervals is of central importance to oral health and the maintenance of restorations. Unfortunately, plaque is not easy to detect for the untrained eye. Plaque disclosing agents, which colour plaque to make it visible, provide a useful aid. They can be utilized to show patients where plaque has settled on their teeth and which sites need to be cleaned more thoroughly. The patients' motivation for improving oral hygiene may increase as a result.

Furthermore, plaque disclosing agents help dentists in the initial examination of the patient, e.g. to determine the plaque index. The plaque index is correlated with the caries risk, as studies in children [1] and elderly people have shown. [2]. Various measures can be taken to prevent tooth decay in patients with a high plaque index. These measures include advice on how to clean teeth more effectively, regular professional teeth cleaning and the use of chlorhexidine-containing products to reduce the bacterial count in the oral cavity.

Patients with tooth replacements or implants are not able to clean all sites, e.g. of an implant superstructure, sufficiently even if they practice exemplary oral hygiene. Colouring the plaque at these difficult-to-clean sites assists dentists in explaining to patients why certain measures, e.g. regular professional teeth cleaning (PTC), are important for the maintenance of restorations and why they do not represent "just another way of making money". The effectiveness of these measures can be assessed after they have been implemented by again determining the plaque index with a plaque disclosing agent – the patients will notice that fewer areas than before show up in the plaque test and therefore will be able to see for themselves that the effort and expenditure have been worthwhile.

Various dyes are utilized to detect plaque:

- Erythrosine (red-purple)
- Phloxine B plus patent blue or E133 (stains older plaque blue and newer plaque pink)
- Fluorescein (fluoresces yellow-green under UV light)

The Plaque Test of Ivoclar Vivadent contains the sodium salts of fluorescein as colouring agent.

1.3 Fluorescein

Fluorescein is a colouring agent that, when excited with blue light, fluoresces yellow-green in the range of 500 nm [3]. The molecular structure of fluorescein is shown in Fig. 2. A patent for the use of this dye in dental diagnostic methods was filed in the USA in 1967 by Herbert Brilliant (US Patent 3-309-274; 1967). The fluorescence of fluorescein depends on the pH value; the fluorescence is more intense at a low pH value. The optimal fluorescence intensity is achieved at a pH of approx. 5.5 [4]. As a result, areas that are susceptible to tooth decay show up particularly well because the lactic acid production of the cariogenic bacteria causes a persistent drop in the pH value.

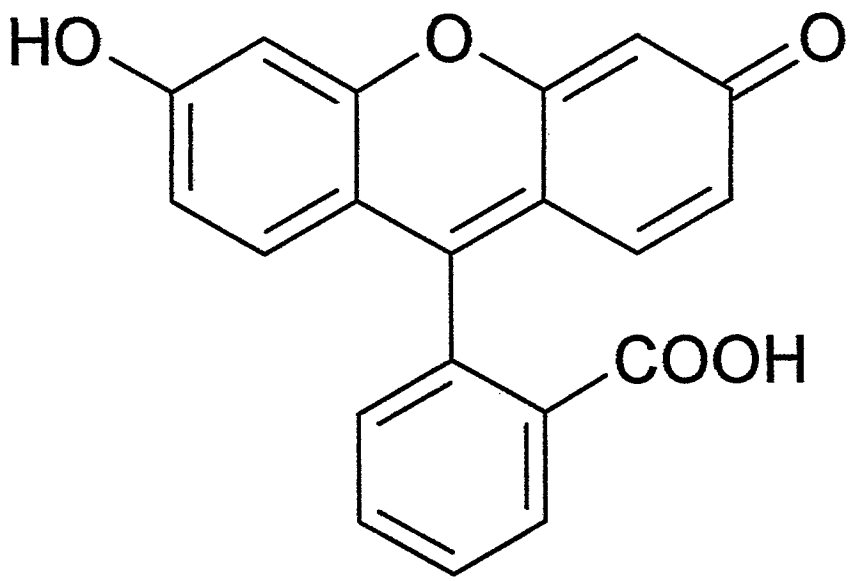

Fig. 2: Strucural formula of fluorescein

1.4 Plaque Test

Fig. 3: Plaque Test of Ivoclar Vivadent

The Plaque Test of Ivoclar Vivadent comprises the fluorescent colouring agent fluorescein. It exposes plaque on teeth as a yellow colour and on gingiva as a green colour. The teeth appear blue and the gingival tissues dark blue under a blue light source [4]. As a result, plaque can be clearly distinguished from the surrounding tissues (see Fig. 4).

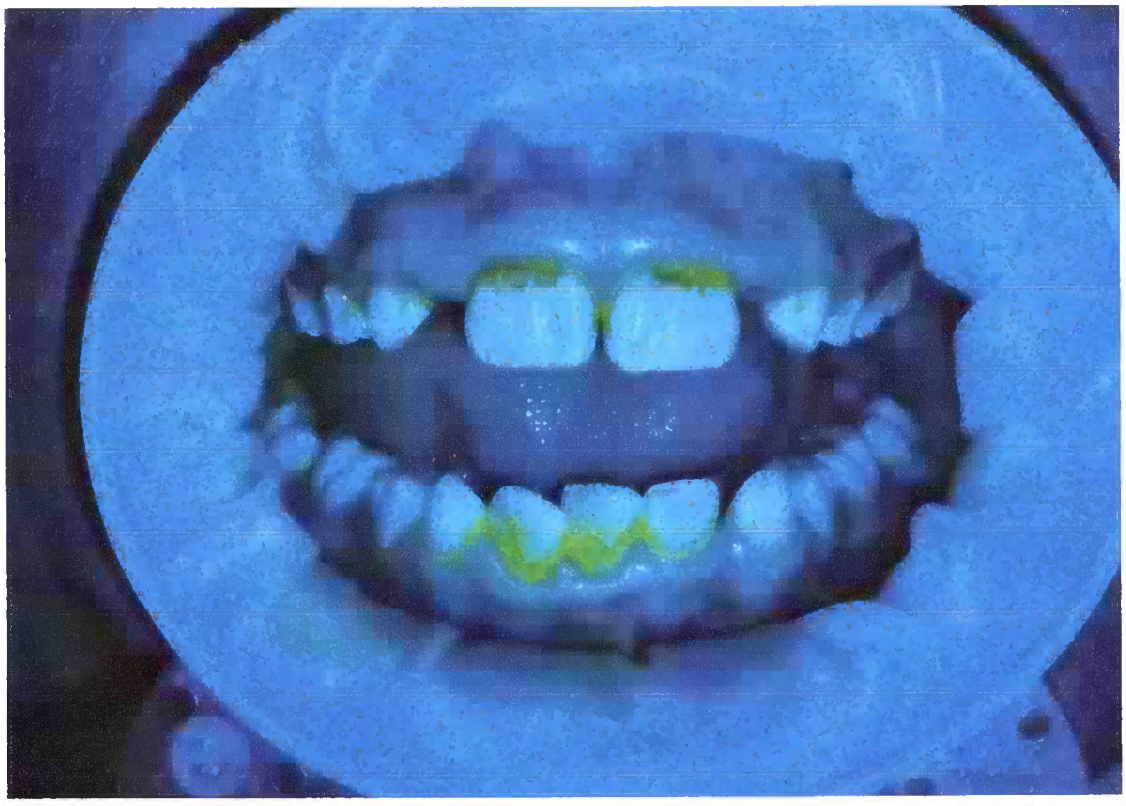

Fig. 4: Plaque stained with the Plaque Test disclosing agent in the patient's mouth. On exposure to blue light, plaque shows up with a yellow-green colour whilst the teeth appear blue and the gums dark blue.

The fluorescein-containing Plaque Tests offers several advantages over other plaque disclosing agents:

- Fluorescein only colours plaque; the gums, tongue natural teeth and restorations keep their own colour. In addition, fluorescein is not visible in daylight and, as a result, the use of the Plaque Test does not entail any esthetic impairment [5].
- The taste of fluorescein is generally described as acceptable.
- Fluorescein is better suited for digital quantification methods than erythrosine because the latter colours plaque in a red-purple colour and consequently does not create sufficient contrast to the red colour of the gingiva [4]. Therefore, the Plaque Test is also suitable for the systematic computer-based evaluation of plaque build-up, e.g. in the course of a clinical study.

2. Composition

Plaque Test

Disclosing agent to make the otherwise invisible biofilm (plaque) on teeth visible

Standard composition (in % by weight)	
Glycerol	55.0
Distilled water	44.0
Fluorescein sodium, potassium dihydrogen phosphate, 4-hydroxybenzoic acid ethyl ester	1.0

3. Investigations and clinical experience

3.1 What exactly shows up in the Plaque Test?

Plaque consists of a host of components, such as microorganisms, polysaccharides, glycoproteins and proteins. Which of these components are stained by fluorescein? This question was already the subject of researchers in the 1970-ies.

3.1.1 Study by Salkin et al

Salkin *et al* carried out a microscopic examination of heat-fixed plaque samples dyed with fluorescein. At baseline, the patients participating in the study were free of plaque. The plaque samples collected after 2 hours demonstrated only a low level of fluorescence. Plaque samples collected after 8 hours revealed staining cocci and rod-shaped bacteria. After 12 hours, a large number of stained microorganisms and epithelial cells were found in the plaque samples. After 36 hours, a large number of microorganisms were again found, but hardly any epithelial cells. Based on these findings, the authors concluded that most plaque components are fluorescein-stained with the possible exception of the pellicle. In addition, the study showed that samples taken from mature plaque were staining more intensely than samples collected from fresh biofilm that was only a few hours old [6].

3.1.2 Study of Ivoclar Vivadent

Researchers at the R&D department of Ivoclar Vivadent also investigated this issue. For this purpose, they prepared cultures of *S. mutans* (cariogenic bacteria) and plaque samples and stained them with different staining solutions:

1. SYTO® 13 / propidium iodide (SYTO® 13 stains the nucleic acids of living cells with a green colour; propidium iodide stains dead cells with a red colour)
2. Plaque Test
3. Sodium fluorescein in water

Figure 5a shows that living *S. mutans* bacteria fluoresce green and dead bacteria fluoresce red after having been stained with SYTO 13 and propidium iodide. A similar picture can be seen in Figures 5b and 5c, except that these images contain no fluorescent red parts: the bacteria appear bright green. These samples were stained with the Plaque Test and a fluorescein dying solution respectively.

- This means that the fluorescent colouring agent of the Plaque Test is able to stain living plaque bacteria such as cariogenic *S. mutans*.

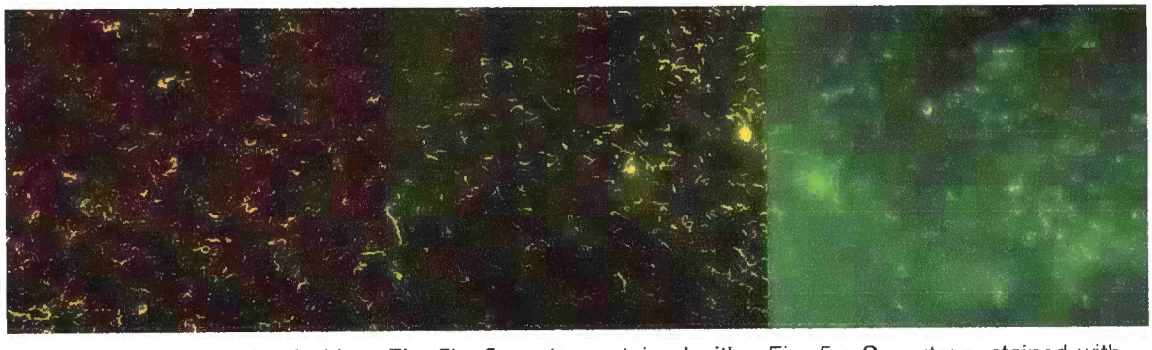

Fig. 5a: *S. mutans*, stained with SYTO 13 and propidium iodide

Fig. 5b: *S. mutans*, stained with the Plaque Test

Fig. 5c: *S. mutans*, stained with sodium fluorescein

In addition, plaque samples were also stained. The samples processed with SYTO 13 and propidium iodide clearly showed that plaque consists, to a large extent, of dead cells (reddish staining in Fig. 6a). The samples, however, also contained areas that appeared yellow as a result of overlapping red and green fluorescence. These are the areas where mainly living bacteria can be found. A similar picture can be seen in Fig. 6b: fluorescent green clusters of mainly living bacteria against a largely unstained background of dead matter.

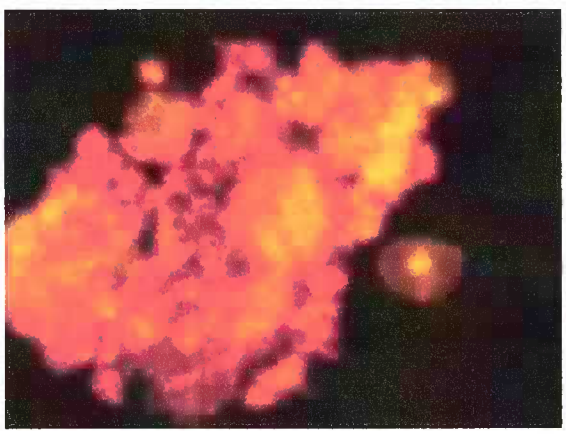

Fig. 6a: Plaque, stained with SYTO 13 and propidium iodide

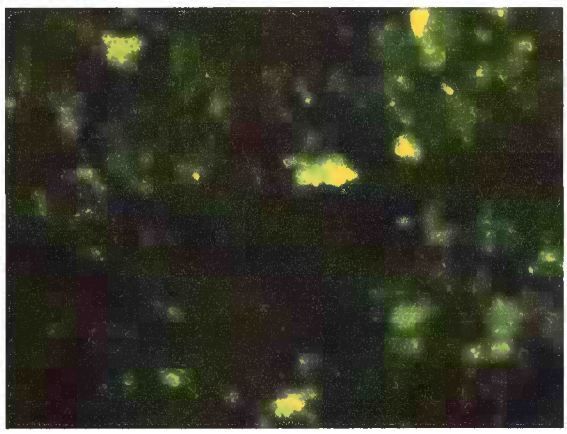

Fig. 6b: Plaque, stained with the Plaque Test

- The Plaque Test stains mainly living bacteria in the plaque. This characteristic shows the benefit of the product: It can be used to detect living plaque bacteria that may potentially be harmful (e.g. cariogenic).

3.2 What is the clinical benefit?

Plaque disclosing tests enable dentists to determine the plaque index and to provide patients with a clear picture of their oral health needs. The motivation to clean the teeth more thoroughly may increase as a result. The Plaque Test can also be used to teach patients effective cleaning techniques [5] – the teeth can be easily checked for plaque that has not been removed by illuminating them with a polymerization lamp.

Clinical studies showed that the plaque scores decreased in patients who were informed on how to improve their oral health by means of a plaque disclosing agent [7] – for instance, the plaque score dropped by 69.9% in a study by Edwards *et al.*, which used three different disclosing liquids [5]. The author pointed out that fluorescein is the ideal staining solution because, unlike erythrosine and Fast Green, it does not discolour the teeth and mucous membrane.

4. Biocompatibility

All ingredients of the Plaque Test are approved for use in cosmetics (see Cosmetics Directive 76/768/EEC). If the product is used correctly, it does not pose any health risks to users or patients.

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Ivoclar Vivadent AG
Research and Development
Scientific Services
Bendererstrasse 2
FL - 9494 Schaan
Liechtenstein

Contents: Dr Kathrin Fischer
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