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Advances in the use of fluorescent probes in oral medicine diseases

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Abstract: *The oral cavity is the place where the human body and the outside world communicate, and there are many microorganisms. Its health is closely related to human health, so the monitoring and research of oral diseases is of great significance to the world's public health cause. At the same time, fluorescent probes are also popular in research in recent years, and significant progress has been made in the field of medicine. In this review, we will introduce the application of fluorescent probes in the oral medicine diseases.*

Keywords: *Fluorescent probes, Caries, Periodontal disease, Oral mucositis, Oral candidiasis*

1. Introduction

The human body exists in nature as a complex individual consisting of many parts, and the proper functioning of each part is of vital importance in maintaining human health. The oral cavity is one of the channels through which the human body communicates with the outside world, it is one of the places where the largest number of microorganisms are present in the human body, it is the place where the digestive tract begins[1]. Although with the continuous improvement of people's living standards, we have a deeper and deeper understanding of the oral cavity as a part of the human body, and attach more and more importance to it. However, oral diseases are still one of the major public health responsibilities in the world, which requires people in the whole society to study its disease mechanism, clinical manifestations, differential diagnosis, prevention and treatment[2]. It has been associated with many other diseases in the human body. For example, there is a significant correlation between oral diseases and cardiovascular diseases[3], diabetes mellitus[4], and digestive diseases[5]. Therefore, it is important for researchers to increase the monitoring and treatment of oral cavities and oral diseases for human medicine and health. Over the years, with the continuous development of medical technology, the identification of some biomarkers of disease is the basis of many analytical methods. Compared with the traditional methods, the fluorescence imaging technology shows its unique superiority, and its commonly used chemical tools are fluorescent probes, which are a popular research topic in recent years[6].

Fluorescent probes use fluorescent substances as indicators and make them emit fluorescence under the excitation of certain wavelengths of light, and then achieve quantitative and qualitative analysis of the detected substances by detecting their fluorescence. The fluorescent probe usually consists of three parts: recognition, linker, and fluorescence, which determine the roles of selectivity and specificity, linkage, and sensitivity of the probe, respectively[7]. Fluorescent probes can be classified according to different attributes, such as they can be divided into organic and inorganic probes according to material properties. They can also be divided into molecular probes and nanoprobes according to the probe size, and can also be divided into metal ion fluorescent probes and biomolecular fluorescent probes according to the species of substances to be measured. Fluorescent probe technology has been widely noticed and applied to the medical field because of its simple operation, low cost, and fast detection speed[8]. In dentistry, fluorescent probes are mainly used in cancer surveillance and diagnosis, but are slowly expanding their applications such as oral microbiological detection.

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2. Oral medicine diseases

Oral medicine diseases mainly include diseases of the dental pulp, periodontal and mucosal tissues, such as caries, periodontal disease, endodontic and periapical diseases, oral mucosal diseases and so on. The use of fluorescent probes in dental diseases mainly focuses on caries and periodontal diseases, as shown in Table 1.

Table 1: Applications of fluorescent probes in oral diseases

fluorescent probes	Detection Objects	applications	reference
Naphthylimide Ratio Fluorescent Probes	Bacterial glucosyltransferases	Caries prevention and treatment	[10]
FISH oligonucleotide probes	streptococcus mutans	Caries prevention and treatment	[11]
ZnO-PDMS	VSCs	Caries screening	[13]
FISH oligonucleotide probes	viruses	Caries microbiology research	[14]
FISH oligonucleotide probes	streptococcus mutans, streptococcus haemoglobinus, streptococcus gordonii	Dental composites properties research	[15]
FISH oligonucleotide probes	streptococcus mutans	Biomaterials research and development	[16]
Laurdan hydrophobic fluorescent probes	streptococcus mutans	Effect of CBG on bacteria	[18]
FISH oligonucleotide probes	streptococcus mutans	Caries drug development	[19]
C_2A	Porphyromonas gingivalis	Disease correlation	[22]
JC-1 probes, DCFH-DA/MitoSOX probes	rats 、 Human THP-1 monocytes	Periodontitis mechanisms	[23]
DHE probes, DCFH-DA probes	Obese patients with periodontitis	Periodontitis mechanism and treatment	[25]
DCFH-DA	Periodontal stem cells	Periodontitis treatment	[26]
H2DCFDA	Periodontal stem cells	Periodontitis mechanisms	[27]
DPBF	L929 cells	Periodontitis treatment	[28]
DCFH-DA	rats	Periodontitis treatment materials	[29]
ICG	oral mucosa	Oral mucositis Treatment	[30]
redox probes	HaCaT cells	Oral mucositis Imaging	[31]
Caal probes	candida albicans	Disease mechanisms	[32]

2.1 Caries

Caries is one of the most common oral diseases, Streptococcus pyogenes is the main cariogenic bacteria[9], and glucosyltransferases (GTFs) are the main virulence factors, and its level is closely related to the cariogenicity of the bacteria, so the real-time detection and analysis of GTFs is very meaningful for the diagnosis, prevention and treatment of caries.Lei Feng et al[10] successfully designed a naphthylimide derivative (PENA) ratiometric fluorescent probe for the detection of endogenous GTFs in Streptococcus pyogenes and established a system for high-throughput, screening of GTFs inhibitors. In addition, they have identified several potential inhibitors of GTFs in green tea, especially galloyl gallate. Amy Lynn Melok et al[11] found that the green tea polyphenol epigallocatechin-3-gallate was effective in inhibiting Streptococcus pyogenes growth using a molecular probe kit labelled with a fluorescent dye.

Early manifestations of dental caries are hidden, and there are also some lesions that cannot be directly visualized and probed, sometimes relying on doctors to make an early diagnosis of them, while the detection of volatile sulphide gases (VSCs) in the oral cavity may enable early diagnosis of caries[12]. Xuemeng Li et al[13] reported a fluorescent mouthguard based on a dimethicone material fabricated by using zinc oxide quantum dots to react specifically to VSCs resulting in a fluorescence

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burst, thus enabling the screening of dental caries lesions. However, this mouthguard can be affected by other factors such as interfering gases during breathing, food debris, etc., and comfort and safety during wear are two important considerations.

Dongyeop Kim et al[14] used a FISH oligonucleotide fluorescent probe to label caries-associated bacteria in order to study the biogeography of human caries-associated microorganisms. The results showed that Streptococcus pyogenes was distributed in the center, while Streptococcus non-morphicus and other bacteria wrapped around the center in a highly ordered form. This extracellular scaffolding forms a protective shell, and because of this Streptococcus pyogenes displays significant antimicrobial and acid resistance. This framework offers a possibility for the study of other human microbial diseases.

Haohao Wang et al[15] demonstrated that the incorporation of dimethylamino hexadecyl methacrylate (DMAHDM) into oral biomaterials effectively inhibited plaque biofilm formation by fluorescence in situ hybridization using oligonucleotide probes of Streptococcus mutants, Streptococcus haematobium, and Streptococcus gordonii, and Iris Xiaoxue Yin et al[16] also used the corresponding oligonucleotide probes to develop silver nanoparticles AgNPs for the inhibition of caries biofilm growth, which are biocompatible but still have low toxicity.

Streptococcus mutans is a Gram-positive bacterium and some cannabinoids have been found to have significant antimicrobial activity against Gram-positive bacteria[17].Muna Aqawi et al[18] investigated the effect of cannabidiol (CBG) on Streptococcus mutans using Laurdan hydrophobic fluorescent probes, and found that CBG exerted a pronounced antimicrobial effect, affected the membrane structure and reduced membrane fluidity, inhibiting its proliferation. CBG was also found to prevent the pH drop of the bacteria.

Yuan Liu et al[19] used FISH oligonucleotide fluorescent probe to investigate the efficacy of ferumoxytol iron oxide nanoparticles (FerIONP) based on the study of severe caries associated with iron deficiency anaemia. The results showed that FerIONP preferentially binds to the biofilm of Streptococcus mutans and then generates free radicals to kill the bacteria and that it has significant specificity. They provide clinical evidence for the efficacy of FerIONP and demonstrate the therapeutic potential of nanoparticles as targeted anti-infective nanomedicines.

2.2 Periodontal disease

Periodontal disease is a chronic inflammatory disease that develops in association with many microorganisms, and if left untreated, it can lead to oral pain, loosening and loss of teeth, affecting mastication, and complicating other systemic diseases[20]. Porphyromonas gingivalis is the main causative agent of periodontal disease[21], which secretes ginkgolides with trypsin activity, so Porphyromonas gingivalis and ginkgolides (RgpB) have attracted the attention of research scholars as a target for diagnostic and therapeutic treatments of periodontal disease.Colman Moore et al[22] reported a dual-mode fluorescent and photoacoustic molecular probe, C2A, for the detection of Porphyromonas gingivalis and Porphyromonas gingivalis. Aeromonas gingivalis, which was used to induce fluorescence and photoacoustic off states using intramolecular dimerization of peptide-conjugated elastin dyes. A 5-fold photoacoustic enhancement and >100-fold fluorescence enhancement was achieved after hydrolytic cleavage by arginine-specific RgpB protein. The limit of detection for RgpB was 1.1 nM, and the limit of detection for Porphyromonas gingivalis was 4.4E4 CFU/mL. This study demonstrates the relevance and potential utility of a disease model of generalised Porphyromonas gingivalis infection.

Chunlan Jiang et al[23] used a JC-1 fluorescent probe to detect mitochondrial membrane potential as well as DCFH-DA/MitoSOX for the detection of reactive oxygen species ROS within human THP-1 monocytes and mitochondria in the study of periodontitis mechanisms. The results showed that methylene blue-mediated photodynamic therapy (MB-PDT) induced macrophage apoptosis in vitro and in vivo in rats with periodontitis, suggesting a mechanism that may be an alternative way to alleviate periodontitis in addition to the antimicrobial effect of MB-PDT. Meanwhile, MB-PDT induced apoptosis in human THP-1 macrophages via the mitochondrial cystatinase pathway.

Obesity and periodontitis share a common inflammatory component ROS in their pathophysiology[24], Mayte Martínez-Herrera et al[25] in order to study the effect of non-surgical periodontal treatment as well as to evaluate the effect of dietary cure on parameters of leukocyte oxidative stress and leukocyte-endothelial cell interactions in an obese population. In the study, DHE fluorescent probe and DCFH-DA fluorescent probe were used to assess the parameters of oxidative stress in polymorphonuclear leukocytes (PMNs) from obese patients with periodontitis. The

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experimental data showed that non-surgical periodontal treatment reduced ROS in subjects' leukocytes and could improve leukocyte homeostasis, whereas dietary treatment as an adjuvant reduced systemic inflammation and increased the antioxidant status, which in turn modulated leukocyte endothelial dynamics.

Tetrahedral framework nucleic acid (tFNA) is composed of four single-stranded DNAs and is widely used in biological applications such as drug delivery, biosensing, imaging, etc. Mi Zhou et al[26] based tFNA on its antioxidant and anti-inflammatory effects to investigate whether it can protect periodontal tissues in periodontitis by inhibiting the release of inflammatory factors. They used a DCFH-DA fluorescent probe in their experiments to detect the level of ROS in periodontal membrane stem cells (PDLSCs). tFNA was added after PDLSCs induced inflammation by lipopolysaccharide and ligated filaments. tFNAs were shown to reduce the release of pro-inflammatory cytokines and the level of cellular reactive oxygen species in PDLSCs, and protect periodontal tissues. This study provides an experimental basis for the future use of tFNAs in the regenerative treatment of periodontitis.

Guya D. Marconi et al[27], in their study of the role of ascorbic acid (AA) in primary cultures of human periodontal stem cells (hPDLSCs) exposed to Porphyromonas gingivalis lipopolysaccharide (LPS-G), used the H2DCFDA fluorescent probe to assess the level of oxidative stress in periodontal stem cells, concluding that AA could exert an enhanced protective effect on periodontitis models by reducing ROS production. However, this study was only conducted in vitro and needs to be supported by further in vivo experiments.

Manlin Qi et al[28] used a DPBF fluorescent probe to measure ROS produced by L929 cells in vitro, because the DPBF fluorescent probe can irreversibly react with ROS, when they investigated the effect of a core-shell structure, UCNPs@TiO2 nanoparticles, which was designed and developed by themselves on periodontally related pathogens. They found that the structure can produce ROS and kill bacteria, which is very effective in the protection of periodontal tissues.

Yue Sun et al[29] prepared a simple multifunctional nanocomposite CeO2@Ce6NPs, by coating red light-excited photosensitizer hydrogen chloride e6 (Ce6) on cerium dioxide nanoparticles, which in turn bi-directionally modulates the effect to achieve simultaneous bactericidal and inflammation elimination. In their experiments, they injected rats with DCFH-DA fluorescent probes and found that the regenerative potential of the tissue around the inflammation was enhanced by the injection of CeO2@Ce6NPs.

2.3 Oral mucositis

Oral mucositis (OM) is an inflammatory, erosive and ulcerative process of the oral mucosa with an increased risk of bacterial infection.

Weifeng Shao et al[30] successfully prepared an in situ mucosal adhesion hydrogel (PPP_E) based on PLGA-PEG-PLGA (PPP) and epigallocatechin-3-gallate (EGCG). The hydrogel would form spontaneously in response to body temperature and adhere to the wound surface when applied to the ulcerated oral mucosa, achieving rapid repair of oral mucositis. In the study, ICG was used as a fluorescent probe to track whether the PPP_E hydrogel adhered to the gingival ulcer wound. The results of the study showed that EGCG incorporation significantly improved the tissue adhesion properties of PPP hydrogel at 37°C.

Katia Rupel et al[31] in order to evaluate the effect of different wavelengths of laser light on oxidative stress in vivo and in vitro in oncology patients suffering from OM on two cell types abundantly present in inflamed oral mucosa. In the course of their experiments, they examined ROS levels in HaCaT cells using a fluorescent protein-based redox probe. Their findings suggest that combined multi-wavelength irradiation protocols could be considered to reach tissues located at different depths.

2.4 Oral candidiasis

Candida albicans is a commensal organism in the human microbiota and is at homeostasis with the microbiota and epithelial tissues in healthy individuals Increasing evidence suggests that faecal Escherichia coli and Candida albicans coexist in several samples of human diseases including tongue mucosal sensation, sputum, septicemia and root canal infections. Here, Akshaya Lakshmi Krishnamoorthy et al[32] used Candida albicans and faecal E. coli to interact with each other to form a strong mucosal biofilm, which leads to tissue destruction. They used Caal fluorescent probe to detect

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Candida albicans in their experiment along with EUB 338 fluorescent probe to detect faecal E. coli. The results revealed that faecal E. coli up-regulated several Candida albicans genes responsible for tissue adhesion, biofilm and hyphae formation, and invasion, while genes controlling faecal E. coli biofilm formation, virulence and tissue invasion were down-regulated.

3. Conclusions

Currently, fluorescent probes are gradually used to study the etiological mechanism, diagnosis and treatment of dental diseases such as caries, periodontal disease and mucositis. This paper summarizes the main use of fluorescent probes in oral medicine diseases which is focusing on caries and periodontal diseases. They used related bacterial specific fluorescent probes to the mechanism and drug material development of caries. In the study of the mechanism and treatment of periodontal diseases, they more used redox probes to detect the level of cellular or bacterial oxidative stress to support the conclusion. In addition, most of the research used clinical real oral disease patients are rarely available, and is currently limited to in vitro cell or animal experiments. Therefore, developing more clinically practical fluorescent probes is a top priority.

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