A Transparent, Wearable Fluorescent Mouthguard for High-Sensitive Visualization and Accurate Localization of Hidden Dental Lesion Sites

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Accurate detection and early diagnosis of oral diseases such as dental caries and periodontitis, can be potentially achieved by detecting the secretion of volatile sulfur compounds (VSCs) in oral cavities. Current diagnostic approaches for VSCs can detect the existence and concentrations, yet are not capable of locating the dental lesion sites. Herein, the development of a unique approach for accurately locating dental lesion sites using a fluorescent mouthguard consisting of the zinc oxide–poly(dimethylsiloxane) (ZnO-PDMS) nanocomposite to detect the local release of VSCs is reported. The ZnO-PDMS mouthguard displays a highly sensitive and selective response to VSCs, and exhibits high fluorescent stability, good biocompatibility, and low biological toxicity in normal physiological environments. Then, the wearable ZnO-PDMS mouthguard is demonstrated to be able to identify the precise locations of lesion sites in human subjects. Combined with image analysis, the mouthguards successfully uncover the precise locations of dental caries, allowing convenient screening of hidden dental lesion sites that are oftentimes omitted by dentists. Due to low cost, long-term stability, and good patient compliance, the proposed wearable mouthguard is suitable for large-scale production and enables widely applicable, preliminary yet accurate screening of dental lesions prior to dental clinics and routine physical examinations.

Dental caries and periodontitis are common diseases of oral cavities, and bring significant economic burden to the healthcare system.[1–4] Nowadays, the incidence and prevalence of dental caries and periodontitis are high worldwide. Untreated caries have a global prevalence of 35% for all ages, with 2.4 billion of the population affected.[5,6] In particular, the cavitated or dentine carious lesions commonly exist in children (4–6 years old) and older people (50–70 years old) with prevalences of 38–56% and 22–26%, respectively.[6,7] According to the Global Health Expenditure Database, the economic burden caused by dental diseases (mainly dental caries, periodontitis, and tooth loss) totaled $413 billion globally in 2018, corresponding to 5% of the global health expenditure and 20% of the out-of-pocket health expenditure.[8,9]

Dental caries and periodontitis without timely treatment will seriously affect oral health, cause tooth loss, and require high expenditure for restoration. Advanced stages of dental caries and severe periodontitis will cause permanent damages to teeth and gums, which then require nerve extraction or replacement with porcelain teeth. In contrast, early stages of dental caries and periodontitis could be easily treated with professional topical fluoride and therapeutic sealants as well as scaling.[6,10] Thus, early diagnosis and screening of dental caries and periodontitis, especially the hidden ones, will significantly reduce the risks of further symptoms and eventual tooth loss. Yet, early stages of dental lesions are frequently sly
and sometimes deeply concealed, and thus the discovery of hidden dental lesion sites often relies on very careful examinations by dentists. However, unequal distribution of oral health professional dentists and lack of appropriate health facilities in most countries mean that access to primary oral health services is often low. For instance, per capita dentists in China is only 0.41‰, accounting for merely 17% of per capita practicing physicians (2.43‰).[11] Due to the limited resources, dentists are primarily concerned about severe lesions and painful teeth, and easily ignore teeth with slight or mild lesions. In addition, according to per capita estimates of dental expenditures due to dental diseases in 2018, the expenditure for dental diagnosis and treatment is as high as $199.7–365.85 per capita in most developed countries.[9,12] Those dental care costs are barely or partially borne by government schemes or compulsory insurance, which hinders the routine dental examinations for populations with low incomes.[12] Therefore, developing a simple, convenient, and low-cost assay for early diagnosis and precise localization of dental lesion sites is in urgent demand.

The occurrence of dental caries and periodontitis is usually related to food impaction and residues, which will breed anaerobic bacteria in the oral cavities and induce oral malodor.[13,14] When proteins, especially those having sulfur-containing amino acids (e.g., methionine, cysteine, and cystine)[15,16] are degraded by anaerobic bacteria, volatile sulfur compounds (VSCs) are generated as byproducts of metabolisms of these bacteria.[16,17] Therefore, dental caries and periodontitis are often accompanied by the local release of VSCs, such as hydrogen sulfide (H2S), methyl mercaptan (CH3SH), and dimethyl sulfide (CH3SCH3). Consequently, preliminary diagnosis of dental caries and periodontitis can be achieved by detecting the release and concentrations of VSCs at the lesion sites.[13,18]

The existing diagnostic approaches of VSCs mainly include gas chromatography[19,20] and fluorescent/colorimetric sensors based on nanomaterials.[21–25] Portable gas chromatography such as the commercially available OralChromam has been developed in recent years to detect VSCs,[20] which has high sensitivity but requires expensive and bulky instruments. Fluorescent/colorimetric sensors based on semiconductor metal-oxide nanoparticles,[26,27] carbon nanotubes,[28] and gold nanoparticles[29] have also been reported as tools for the detection of volatile compounds with advantages of high sensitivity and fast responsiveness. However, these diagnostic approaches usually focus on volatile compounds in exhaled breath and are unable to offer sufficient disease information to dentists, such as the particular locations and lesion severities of diseased teeth. Therefore, it will be highly appreciated to develop an enabling approach for screening the precise locations of the sick teeth and even hidden lesion sites that are oftentimes omitted by dentists.

Herein, we report the development of a unique wearable dental mouthguard (also known as occlusal splint) based on a fluorescent poly(dimethylsiloxane) (PDMS) material and offer an approach to accurately locate both obvious and hidden dental lesion sites, by detecting the localized release of VSCs. The fluorescent mouthguard was fabricated with the ZnO-PDMS nanocomposite material, which was the mixture of PDMS and fluorescent zinc oxide quantum dots (ZnO QDs). This transparent and elastomeric ZnO-PDMS mouthguard with visible fluorescence could specifically respond to VSCs (mainly H2S), resulting in the quenching of fluorescence. The mouthguard showed extremely high fluorescence stability under various physiological conditions, as well as exhibited good biocompatibility and low biological toxicity to human epidermal cells. On the ZnO-PDMS mouthguard, the fluorescence at the specific lesion sites could be selectively quenched by the locally released VSCs at high sensitivity (Figure 1). ZnO-PDMS mouthguards were further applied to screening the obvious and hidden dental lesion sites in human subjects, through a 7-h monitoring on volunteers. The results indicated that our wearable ZnO-PDMS mouthguards hold the promise to carry out large-scale preliminary yet accurate screening of potential dental diseases before more time-consuming and expensive hospital examinations.

To fabricate the fluorescent mouthguard, ZnO QDs were chosen as the fluorescent probe, since ZnO QDs are a biocompatible, environmentally friendly semiconductor material,[30] which exhibit high quantum yield, photostability in dispersions, and size-dependent photoluminescence.[31] Meanwhile, PDMS was used as the matrix due to its chemical inertness, optical transparency, and elasticity. It can be solvent-cast to form flexible shapes with controllable thicknesses and mechanics.[32,33] The schematic diagram of the fabrication process for a ZnO-PDMS mouthguard is shown in Figure 2A, and the corresponding photographs are illustrated in Figure S1, Supporting Information. The ZnO-PDMS mouthguard was fabricated as follows. First, based on upper/lower dentition of the patient’s teeth (I), a plaster replication (II) was prepared as mold A. Then, a wax rim (III) was used to occupy the space of the mouthguard. Afterward, another plaster mold (IV) was prepared by pouring the plaster onto the wax rim. The complete stamper mold composed of mold A (II) and mold B (IV) was achieved by removing the wax rim (III). Finally, the ZnO-PDMS dental mouthguard (VI) was obtained by filling the uncured polymer into the stamper mold. The complete stamper mold (II + IV) was then covered by PDMS (V) to form a complete mouthguard (VI). To detect VSCs at the lesion sites, the mouthguard showed extremely high fluorescence stability under various physiological conditions, as well as exhibited good biocompatibility and low biological toxicity to human epidermal cells. On the ZnO-PDMS mouthguard, the fluorescence at the specific lesion sites could be selectively quenched by the locally released VSCs at high sensitivity (Figure 1).

![Figure 1. A) Schematic diagram of a ZnO-PDMS mouthguard and its response to VSCs, produced by the occurrence of: a) dental caries or b) periodontitis, in comparison with c) a healthy tooth. B) Fluorescence images of a ZnO-PDMS mouthguard applied for visualizing dental lesion sites in vivo: i) before and ii) after being worn by a patient with dental caries, for locating the lesion sites.](image-url)
ZnO-PDMS nanocomposite (V) into the gap of the stamper between molds A and B, and solidified at 80 °C for 30 min.

The ZnO QDs with fluorescence emission wavelength (λem) of 565 nm were chosen for the mouthguards, since they possess a high quantum yield of 3.5%[34] with λem located in the sensitive region of the cone cells of human eyes (i.e., 550–570 nm).[35] The ZnO-PDMS nanocomposite with the mass-to-volume ratio of 1:20 was selected due to the low consumption of ZnO QDs yet sufficiently high fluorescence intensity (Figures S2 and S3, Supporting Information). In Figure 2B, the λem of the ZnO QD solution and the corresponding ZnO-PDMS nanocomposite were almost the same at ≈560 nm (Figure 2B). These results indicated that the ZnO-PDMS nanocomposite successfully preserved the behaviors of ZnO QDs and exhibited the same fluorescence characteristics with ZnO QDs. The average size of ZnO QDs measured by transmission electron microscopy (TEM) was 4.3 nm (Figure 2C).

Figure 2D shows that the uncured ZnO-PDMS nanocomposite was transparent and viscous with good fluidity, enabling convenient preparation of various shapes. The nanoporous structures of the cured ZnO-PDMS nanocomposite would allow ZnO QDs to be exposed to and respond to gas molecules. The as-prepared ZnO-PDMS mouthguards remained almost transparent under daylight (Figure 2E) and showed strong yellow fluorescence under ultraviolet (UV) light (Figure 2F). As revealed in Figure 2G,H, the ZnO-PDMS mouthguard exhibited good flexibility and high stretchability that withstood continued tensing and twisting (Movie S1, Supporting Information), and maintained consistent fluorescence properties under UV light (Movie S2, Supporting Information). As further illustrated in Movie S3, Supporting Information, the ZnO-PDMS mouthguard was convenient to wear and perfectly fitted to each tooth position, indicating its patient comfortability.

The responses of the ZnO-PDMS mouthguards to VSCs and the stability of ZnO-PDMS in different environments are shown in Figure 3. The ZnO-PDMS mouthguards were exposed to respective VSC gases (e.g., H2S, CH3SH, and CH3SCH3) for 3 min (Figure 3A), and it was observed that the fluorescence emissions were quenched by CH3SCH3 and CH3SH, and dramatically quenched by H2S to almost zero (Figure 3B). Figure 3C also indicates that the fluorescence of the ZnO-PDMS mouthguards was rapidly quenched by the VSC gases and then remained relatively stable for an extended period, in which it was especially sensitive to the H2S gas. As a major component of VSCs, H2S is partially dissolved in saliva and partially volatilized to the oral cavity. Therefore, the response sensitivities of the ZnO-PDMS mouthguards to both H2S gas and solution were further assessed. During a 60-min exposure to different concentrations of H2S gas, the fluorescence of the ZnO-PDMS mouthguards at each concentration was quenched in a time-dependent manner, and the quenching rate became faster as the concentration of H2S gas was increased from 9.43 to 121.67 ppm (Figure 3D). In comparison with those to H2S gas, the responses of ZnO-PDMS to H2S solution were much quicker, in which the fluorescence emissions all became invisible in 5 min when exposed to H2S solutions of different concentrations (11.6–116 mm; Figure 3E).

The ZnO-PDMS mouthguards showed a high selectivity to H2S, comparing to the other ones in Figure 3F. The fluorescence intensity of ZnO-PDMS was significantly decreased after
its exposure to the H\(_2\)S solution for 15 min, while only slight fluctuations were observed after exposure to the atmosphere, H\(_2\)O, CO\(_2\) (5 vol%), and urea solution (5 wt%) for as long as 3 h (Figure S4, Supporting Information). The reason for the fluorescence of ZnO-PDMS being selectively quenched by sulfide molecules lies in that the visible fluorescence of ZnO QDs is related to their surface states,[36,37] and the reaction with sulfide molecules will cause the disappearance of cavities.
of surface defects on the surfaces of ZnO QDs, leading to the quenching of fluorescence. Collectively, ZnO-PDMS has displayed highly sensitive and selective responses to both aqueous and gaseous VSCs including H₂S, CH₃SH, and CH₃SCH₃, which paves the way for further detection and localization of VSCs in the oral cavity.

Wearable dental devices contacting with the human body require the sensing material to have physiological stability and good biocompatibility. The ZnO-PDMS mouthguard exhibited excellent mechanical stability, revealed by its stable fluorescence intensity under repeated and continuous compressions (Figure 3G). The physiological stability of the ZnO-PDMS mouthguard was proven by consistent fluorescence in artificial saliva at body temperature for 72 h (Figure 3H), with three reversible pH cycles between 7.0 and 3.0 (Figure 3I), or with pH decreasing from 7.0 to 2.0 (Figure S5, Supporting Information). It is worth mentioning that the fluorescence of the ZnO-PDMS nanocomposite material could maintain its stability during storage at room temperature for up to 3 years evaluated (Figure S6, Supporting Information).

The cytocompatibility of ZnO-PDMS was demonstrated by incubating HaCaT human epidermal cells with slices of the ZnO-PDMS mouthguard (1.0 cm²) for 72 h. Fluorescence staining results showed that the HaCaT cells well-attached and even migrated onto the ZnO-PDMS mouthguard in 72 h (Figure 3J). Furthermore, CCK8 analysis quantitatively displayed that the proliferation of HaCaT cells had no statistically significant difference between the control and the ZnO-PDMS groups during the 72-h monitoring (Figure 3K). Furthermore, the biological safety of the ZnO-PDMS mouthguard was demonstrated by the low release dose of Zn⁺⁺ of 0.028 mg after 72 h, which is only 0.034% of the minimum toxic dose of Zn⁺⁺ for humans (Table S1, Supporting Information). Therefore, our ZnO-PDMS mouthguard exhibited appropriate chemical inertness, good physiological stability, and low biological toxicity, which is deemed to be safe for human usage.

The localization capability of the ZnO-PDMS mouthguards to reveal the hidden lesion sites of oral diseases (e.g., periodontitis or dental caries) is illustrated in Figure 4. The schematic diagram of the tooth positions (TPs) 11–48 of permanent dentition is shown in Figure 4A. To demonstrate the locations of lesion sites at different TPs, artificial saliva (as control) or artificial saliva containing H₂S as disease-mimics were dropped onto the as-fabricated ZnO-PDMS mouthguards at different TPs. Specifically, drops of the solution containing H₂S were imposed onto the top of TP 27 at 0 min, the site of palatal cusp of TP 16 at 7 min, and the interdental space of TPs 23/24 at 14 min (Figure 4B). Fluorescence intensities of the sites covered with the control artificial saliva on the ZnO-PDMS mouthguard were stable during the entire experimental period at all three TPs (Figure 4C). In contrast, the fluorescence intensities for all three TPs covered with the artificial saliva containing H₂S on the ZnO-PDMS mouthguard rapidly declined within the subsequent 7 min, and lost 40–50% of the original intensities eventually (Figure 4D). It could be concluded that the locations of fluorescence quenching all precisely matched the sites where artificial saliva containing H₂S was dropped, with no exception.

To further mimic the complex environment in real human patients, simulated saliva with oral malodor was prepared by adding certain concentrations of VSCs into the artificial saliva. The as-prepared artificial saliva with VSCs was dropped onto the above-mentioned three sites of TPs on the ZnO-PDMS mouthguard at 0 min. The fluorescence images in Figure 4E indicated the decrease of fluorescence intensities at the specified locations where artificial saliva containing VSCs was dropped. By analyzing the fluorescence images, the average fluorescence intensities of the areas with obvious fluorescence quenching, revealed the approximate trend of fluorescence changes, which reached plateaus in 60 min for all three TP sites (Figure 4F). The rates and degrees of the fluorescence quenching upon treatment with artificial saliva containing the same concentration of VSCs were nearly identical at the different locations. These results suggested that the ZnO-PDMS mouthguard was able to accurately detect the precise tooth locations releasing VSCs with good accuracy, which would guarantee the potential application for screening the status of each tooth for oral cavity in human subjects.

The ZnO-PDMS mouthguards were further applied to screening the locations of dental caries in situ and in vivo on human subjects. Eight volunteers were randomly recruited from Sun Yat-sen University to wear the ZnO-PDMS mouthguards for a 7-h clinical monitoring, among whom three were identified as healthy and five were patients having different degrees of dental lesions. The photograph of the ZnO-PDMS mouthguard after worn by one of the healthy volunteers (Control 1) is shown in Figure 5A(i). Upon wearing of the mouthguard, the teeth could be clearly seen through the mouthguard, suggesting that ZnO-PDMS was transparent without affecting the aesthetic appearance, and the soft texture endowed it with good patient compliance (from patient feedback). During the 7-h monitoring, the fluorescence images of ZnO-PDMS mouthguard worn on each volunteer was captured at each hour, among whom five of them had obvious fluorescence quenching on the mouthguards. Figure 5A shows the fluorescence images of the healthy volunteer without fluorescence quenching (Control 1) at: i) 0 and iii) 7 h, where Figure 5B–F display the corresponding fluorescence images of the five patients with different levels of fluorescence quenching (Patient 1–5, Pa. 1–5) at: i) 0 and ii) 7 h. 3D image analyses of the fluorescence intensities of the ZnO-PDMS mouthguards at 7 h are further displayed in Figure 5A–F(iv), where the sites with evident fluorescence quenching are shown as blue topography and marked by corresponding TPs. For the control, no visible change was observed in the fluorescence images of the ZnO-PDMS mouthguard, and the fluorescence intensity retained at high levels (>95%) according to the 3D image analysis during the 7 h. In contrast, the fluorescence intensities were obviously quenched for Pa. 1–5 with suspected dental diseases. The dental lesion site was marked when the relative fluorescence intensity (F/F₀) of ten adjacent pixels surrounding the TP had declined for more than 20%. Taking Pa. 1 as an example, 3D image analysis suggested several dental lesion sites at TPs 13, 24, and 25. To confirm the results revealed by our mouthguards, all volunteers were further very carefully diagnosed by a professional dentist to determine their dental status (Table 1), and the photographs of Pa.1–5 with different degrees of dental caries are exhibited in Figure 5B–F(iii), respectively. Compared to the clinical diagnosis of the dental status of Pa.1, our detection and analysis results not only accurately located the sites...
of dental caries (TP 25), but also revealed other hidden dental lesion sites (TPs 13 and 24) that were difficult to be found out without very careful and time-consuming examinations.

To further verify the consistency between the test results by our mouthguards and clinical diagnosis, fluorescence intensities of the corresponding sites at the precise TPs were further extracted and quantitatively analyzed for decay along with time (Figure 5G). While the fluorescence intensities of the mouthguard worn by the control group displayed slight fluctuation (below \( \approx 5\% \)) during the 7 h (Figure 5G(a)), obvious fluorescence quenching was clear at the precise TPs with decay for Pa. 1–5. After 7 h, the fluorescence intensities at TPs with shallow caries remained at 77.5% (Pa. 1, Figure 5D(b)), while the fluorescence intensities at TPs with deep caries of residual crown decreased to 62.2% (Pa. 5, Figure 5D(f)). The quantitative analyses of the ZnO-PDMS mouthguard fluorescence indicated good agreement with the results of clinical diagnosis and the dental status of the Pa. 1–5 illustrated in Table 1. The results suggested that the ZnO-PDMS mouthguards not only successfully displayed the precise locations of dental caries, but also revealed hidden dental lesions offering more information for clinical diagnosis.

In conclusion, a convenient and sensitive wearable device based on the fluorescent ZnO-PDMS nanocomposite has been applied to detecting and screening the accurate locations of hidden dental lesion sites, which is potentially conducive to the prevention and early treatment of periodontitis and dental caries. Compared with the existing strategies, our ZnO-PDMS material and the mouthguard feature the following unique advantages: i) simple preparation, low cost, and long-term...
Figure 5. In vivo screening of the locations of hidden dental lesion sites of human teeth by the ZnO-PDMS mouthguards. A) The ZnO-PDMS mouthguard worn on a healthy volunteer (Control 1) for 7 h: i) photograph of the transparent and soft ZnO-PDMS mouthguard; fluorescence images: ii) before and iii) after wearing for 7 h; iv) 3D image analysis of the fluorescence intensities exacted from (iii). B–F) The ZnO-PDMS mouthguards worn by Pa. 1–5 for 7 h: fluorescence images: i) before and ii) after wearing; iii) photographs of the dental status for Pa. 1–5, respectively, where the TPs of dental caries or residual teeth are marked in numbers; iv) 3D image analysis of the fluorescence intensities exacted from corresponding photographs. The TPs of dental caries found in (iii) are presented as numbers in red, and other TPs of hidden dental lesion sites are noted by numbers in black. Color bars in (A–F) represent $F/F_0$ (%). G) Fluorescence intensity changes of TPs on the ZnO-PDMS mouthguards worn by (a) the control and (b–f) Pa. 1–5 with the sites of decay from 0 to 7 h.
stable performance; ii) accurate localization ability to reveal the hidden lesion sites at each tooth; and iii) good patient compliance due to the transparency without affecting aesthetic appearance, and the softness of the ZnO-PDMS mouthguard to maintain conformability and comfortability. Importantly, these favorable characteristics of our wearable mouthguard make it possible for large-scale production, which will enable the massive preliminary yet accurate screening and localization of dental lesions prior to dental clinics and routine physical examinations, significantly lowering the cost associated with diagnosis and subsequent treatment.

However, it is worth mentioning that certain conditions may possibly affect the capacities of the mouthguard in the screening of dental caries and periodontitis. For example, the volatile compounds including VSCs released from the breath into the mouth might have an influence; yet, this condition will likely end up with uniform fluorescence decrease on the entire mouthguard, rather than at any specific tooth position. In the case of food leftovers on the teeth, they typically occur at locations that have the most likelihood to cause periodontitis, which therefore, are also the tooth positions that we are interested about. Even so, such an influence exerted by food leftovers on the teeth can still be, in practice, avoided by proper cleaning (e.g., brushing, flossing, and mouth-washing) before wearing the mouthguard.

Moreover, ZnO-PDMS as a porous inert material, holds the ability to serve as a drug carrier, which will endow it with the capability of localized drug delivery. As such, the simultaneous diagnosis and treatment will be quite feasible on the same device serving as an enabling theranostic platform. In the future, this platform based on the mouthguard could also expand its applications from dental healthcare to the other areas such as oral biochemical tests, diagnosis of respiratory and gastrointestinal diseases, and additional wearable monitoring for public health.

**Experimental Section**

**Chemicals: Zinc acetate (Zn(CH₃COO)₂•2H₂O, 99.5 %) was purchased from Fu Chen Chemical Company (China). PDMS (components A and B) was purchased from Dow Corning (USA). Iron sulfide (FeS) and lithium hydroxide (LiOH•H₂O) were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. (China) for obtaining H₂S. HCl, NaOH solution, and concentrated H₂SO₄ were purchased from Damao Chemical Co., Ltd. (China). The H₂S saturated solution was generated from the reaction between FeS and diluted H₂SO₄ as reported.[39] Plaster was purchased from Guangzhou Bosheng Gypsum products Co., Ltd. (China), and dental model wax was purchased from Shanghai New Century Co., Ltd. (China). Sodium alginate was purchased from Shanghai Medical Instruments Co., Ltd. (China), and separation agent for gypsum was from Shanghai New Century Dental Materials Co., Ltd. (China). AS was purchased from Dongguan Xingheng Technology Co., Ltd. (China).

**Synthesis of ZnO-PDMS:** ZnO nanoparticles were synthesized following previous reports with minor modification.[40] Zn(CH₃COO)₂•2H₂O was dissolved in boiling ethanol and then cooled to 0 °C. LiOH•H₂O was added into the Zn(CH₃COO)₂ ethanol solution and the mixture was stirred violently under ultrasonication for 3 h. The reaction stopped when the mixture became transparent. Hexane was added in the mixture to precipitate ZnO nanoparticles and the precipitate was purified through centrifugation and ethanol wash. Finally, the purified ZnO nanoparticles were re-dispersed in ethanol for further use. PDMS was first prepared by mixing the component A thoroughly with the component B (at a mass ratio of A:B = 10:1), and then the purified ZnO ethanol solution was added into the PDMS mixture to prepare the uncured ZnO-PDMS nanocomposite. The ZnO-PDMS nanocomposite was cured at 80 °C for at least 30 min.

**Locating the Disease-Mimicking/Lesion Sites at Different TPs:** The concave surface of the ZnO-PDMS mouthguard was placed upward, while the maxillofacial face was downward. i) Drops of the solution containing H₂S were imposed on the top of the TP 27 at 0 min, the position of the palatal cusp of TP 16 at 7 min, and the interdental space of TPs 23 and 24 at 14 min. The fluorescence images of the ZnO-PDMS mouthguard were captured at 0, 7, 14, and 21 min, respectively. ii) The simulated saliva including VSCs with oral malodor was prepared by adding 17.4 mm of H₂S, 0.009 mm of CH₃SH, and 0.205 mm of CH₃SCH₃ in artificial saliva, according to literature.[13] The ZnO-PDMS mouthguard was dripped with 50 μL of the as-prepared simulated saliva on TP 27, TP 16, and TPs 23 and 24 at 0 min. The fluorescence images of the ZnO-PDMS mouthguard were captured for up to 60 min. The fluorescence intensities were extracted, and calculated for the average values and standard deviations.

**In Vivo Screening Using ZnO-PDMS Mouthguards:** Volunteers were recruited from Sun Yat-sen University. For each volunteer, one pair of the ZnO-PDMS mouthguards were customized. After gargling, the ZnO-PDMS dental mouthguards were worn by the eight volunteers for 7 h. The fluorescence images of the ZnO-PDMS mouthguards were captured after washing them using distill water at each hour. Fluorescence images were preprocessed to remove the background noise using Photoshop CC 2019 (Adobe, USA). Matrix data of each fluorescence image were extracted by Python 3.7. The F/F₀ (%) values were obtained by dividing the

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**Table 1.** Dental status of all the eight volunteers diagnosed by the mouthguards and then careful examination by the dentist.

<table>
<thead>
<tr>
<th>No. of volunteer</th>
<th>Sex</th>
<th>Age</th>
<th>TP</th>
<th>Dental status detected by a dentist</th>
<th>Degree of decay[‡]</th>
<th>Fluorescence decrease detected by mouthguard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 1</td>
<td>Male</td>
<td>26</td>
<td>–</td>
<td>Healthy</td>
<td>–</td>
<td>0.30%</td>
</tr>
<tr>
<td>Control 2</td>
<td>Female</td>
<td>21</td>
<td>–</td>
<td>Healthy</td>
<td>–</td>
<td>1.30%</td>
</tr>
<tr>
<td>Control 3</td>
<td>Male</td>
<td>28</td>
<td>–</td>
<td>Healthy</td>
<td>–</td>
<td>0.55%</td>
</tr>
<tr>
<td>Pa. 1</td>
<td>Female</td>
<td>19</td>
<td>25</td>
<td>Dental caries</td>
<td>+</td>
<td>22.50%</td>
</tr>
<tr>
<td>Pa. 2</td>
<td>Male</td>
<td>28</td>
<td>28</td>
<td>Dental caries</td>
<td>+</td>
<td>27.60%</td>
</tr>
<tr>
<td>Pa. 3</td>
<td>Female</td>
<td>24</td>
<td>14</td>
<td>Dental caries</td>
<td>++</td>
<td>28.10%</td>
</tr>
<tr>
<td>Pa. 4</td>
<td>Female</td>
<td>26</td>
<td>14</td>
<td>Residual root</td>
<td>+++</td>
<td>34.50%</td>
</tr>
<tr>
<td>Pa. 5</td>
<td>Female</td>
<td>26</td>
<td>36</td>
<td>Residual crown</td>
<td>+++</td>
<td>37.80%</td>
</tr>
</tbody>
</table>

‡“+” represents shallow caries; “++” represents moderate caries; “+++” represents deep caries.
fluorescence intensities of the images to the corresponding intensities at 0 min. 3D color map surfaces could be further derived by Origin 9.0 using the matrix data of the fluorescence quenching percentages. The lesion sites with obvious fluorescence quenching were also quantified for average values and standard deviations with time. After detection using the mouthguards, each volunteer was further clinically diagnosed for dental caries by a dentist in the Department of Stomatology at Guangzhou Women and Children’s Medical Center. Research ethical reviews for clinical studies on human subjects were approved by the ethics committee of Guangzhou Women and Children’s Medical Center and informed written consent was obtained for all human volunteers.

Supporting Information
Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest
The authors declare no conflict of interest.

Keywords
dental caries, dental mouthguards, fluorescent nanocomposites, periodontitis, volatile sulfur compounds

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