Research Article

Multiclawed SiO₂ Nano-Antibacterial Agent Based on Charge Inversed Ce6 Ionic Liquid Polymers for Combating Oral Biofilm Infection

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Photodynamic antimicrobial chemotherapy (PACT) is a promising therapy against biofilm infection. However, due to the saliva clearance and obstacle of biofilm, the photosensitizer is difficult to concentrate in the infection site; then, the PACT is less effective on oral biofilm infection. In this article, we report a special nano-antibacterial agent (SiO₂-P_{Ce6-IL}) to solve the bottleneck problem of PACT in treatment of oral biofilm infections. The SiO₂-P_{Ce6-IL} was composed of SiO₂ and poly ionic liquid photosensitizer (P_{Ce6-IL}) and had tri-fold features of eliminate biofilm infection: high binding ability, breaking biofilm barriers, and enrichment photosensitizer in infection site. In oral biofilm, the SiO₂-P_{Ce6-IL} changed to SiO₂-P_{IL}⁺ like claws of octopus that could hold tightly with biofilm. Then, the poly-dodecyl on the SiO₂-P_{IL}⁺ broke down the barrier of biofilm. The results of HR-MS and zeta potential indicated that SiO₂-P_{Ce6-IL} could change to positive (SiO₂-P_{IL}⁺) in acidic environment. The interaction forces and morphology results proved that the SiO₂-P_{IL}⁺ had a higher affinity to biofilm and could destroy the biofilm structure. Then, the photosensitizer was enriched in biofilm at sites of infection. The *in vitro* and *in vivo* experiments showed that SiO₂-P_{Ce6-IL} could effectively eradicate oral biofilm infections and control of dental caries.

1. Introduction

Oral biofilm infection is most important pathogenic factor of dental caries [1, 2]. Thus, eliminating biofilm on the tooth surface is a primary strategy to prevent and treat dental caries [3]. However, with the protection, bacteria in the biofilm is difficult to eradicate by common strategy of mechanical cleaning combined antibiotics. In this background, it is urgently to develop new treatment against oral biofilm infection. One promising therapy is PACT [4]. Compared with traditional treatment, PACT could efficiently control of bacteria and bacterial biofilm infection. However, owing to rapid clearance by saliva and barrier of biofilm, the photosensitizers are not concentrated in the infection site,

and the efficacy of PACT against oral biofilm infections is limited [5]. Faced with the dilemma, the concentration of photosensitizer in the site of oral biofilm is the key to improve the PACT efficiency.

An understanding of oral biofilms matrix could offer opportunities to exploit photosensitizer delivery systems [6]. Because oral biofilm develop when microbes accumulate and form structured communities encapsulated within an extracellular matrix such as exopolysaccharides (EPS) [7, 8], the sugars are fermented by bacteria within the EPS matrix, then created highly acidic microenvironments and negative charge. The pH values often reach pH~4.5 or even lower in oral biofilm and particularly after exposure to sucrose, starch, and other cariogenic food products [9]. So,

FIGURE 1: The mechanism of multiclawed $\text{SiO}_2\text{-P}_{\text{Ce6-IL}}$ against oral biofilm.

photosensitizer delivery system that could response to lower pH microenvironments is an effective method to improve delivery efficiency. Furthermore, many researches on cationic polymers [10], especially on the charge-reversal polymers, indicated that positively charged polymers could combine with extracellular polymeric substances (EPS) [11] and increase concentration of photosensitizer in biofilm. However, due to the dense and thick structure of biofilm, the photosensitizers difficultly penetrated into biofilm; then, photochemical reaction initiated by photosensitizer occurs mainly in the outermost layers of biofilm. The PACT efficiency was not significantly improved.

To overcome these challenges, polyionic liquid that composed of polycation and anion is introduced in previous studies [12, 13]. Inspired by octopus claws, the polyionic liquid photosensitizer nanoparticle ($\text{SiO}_2\text{-P}_{\text{Ce6-IL}}$) like arms of octopus was prepared in this work. In acid microenvironment of oral biofilm, the $\text{SiO}_2\text{-P}_{\text{Ce6-IL}}$ could change to nano-claws $\text{SiO}_2\text{-P}_{\text{IL}}^+$ and firmly combine to negatively charged EPS to overcome the saliva cleaning. Then, the dodecyl on the $\text{SiO}_2\text{-P}_{\text{IL}}^+$ could pierce through the biofilm and break down the barrier of biofilm. Finally, the photosensitizer was highly concentrated in oral biofilm, and the biofilm infection was eliminated. The high binding energy between positively charged $\text{SiO}_2\text{-P}_{\text{Ce6-IL}}$ and oral biofilm, excellent breaking ability of polydodecyl, and effective enrichment of photosensitizer were the key factors to effectively eliminate oral biofilm. The mechanism of $\text{SiO}_2\text{-P}_{\text{Ce6-IL}}$ against oral biofilm is showed in Figure 1.

2. Materials and Methods

2.1. Materials. Ce6 was purchased from Frontier Scientific. KOH, methanol, methanol, absolute ethanol, cyclohexane, ammonia solution, hexanol, trimethylamine, Triton x-100, and 1,3-diphenylisobenzofuran (DPBF) were purchased from Sinopharm Chemical Reagent Co., Ltd. Artificial saliva was purchased from Beijing Regan Biotechnology Co., Ltd.

Tetraethyl orthosilicate (TEOS), 3-aminopropyl triethoxysilane (APTES), CuCl, 2-bromoisobutryl bromide, and N,N,N',N'',N'''-pentamethyldiethylenetriamine were purchased from Aladdin. *Streptococcus mutans* (*S. mutans*, ATCC 700610) was purchased from ATCC. Brain Heart Infusion Broth (BHIB) was purchased from Beijing Land Bridge Technology Co., Ltd. The live/dead BacLight bacterial viability kit (L7012) was purchased from Invitrogen. Hydroxyapatite tablets were purchased from National Biomedical Materials Engineering Technology Research Center (China). Alexa Fluor 647 was purchased from Thermo Fisher Scientific.

2.2. The Synthesis and Characterization of Multiclawed $\text{SiO}_2\text{-P}_{\text{Ce6-IL}}$. The preparation of $\text{SiO}_2\text{-Br}$ was similar with previous research [14], except the 2.4 mL of triethylamine and 2-bromoisobutryl bromide (1.2 mL) were added to prepare $\text{SiO}_2\text{-Br}$. The multiclawed $\text{SiO}_2\text{-P}_{\text{Ce6-IL}}$ was prepared by ATRP. Briefly, 10 mg Ce6-IL and $\text{SiO}_2\text{-Br}$ dispersed into a mixture solution of ethanol and distilled water (5:1, V:V) for stirring 10 min at 800 rpm. 200 μL of PMDETA and 30 mg of CuCl were quickly transferred into the flask for reaction 8 h at 35°C. The $\text{SiO}_2\text{-P}_{\text{Ce6-IL}}$ was washed with ethanol, distilled water.

The morphology of $\text{SiO}_2\text{-P}_{\text{Ce6-IL}}$ was examined by TEM and SEM. The change of $\text{SiO}_2\text{-P}_{\text{Ce6-IL}}$ zeta potentials in pH 4.5 and 7.4 was measured by a Delsa Nano C particle analyzer (Beckman Coulter Ireland Inc.).

The UV absorption of 5.0 $\text{mg}\cdot\text{mL}^{-1}$ $\text{SiO}_2\text{-P}_{\text{Ce6-IL}}$ was measured by UV-vis (MAPADA). The Ce6 loading efficiency (*L*) was calculated as formula:

$$L = \frac{C \times V}{W} \times 100\%. \quad (1)$$

L was $\text{SiO}_2\text{-P}_{\text{Ce6-IL}}$ loading efficiency; *C* was the concentration of Ce6 ($\text{mg}\cdot\text{mL}^{-1}$); *V* was solution volume (mL); *W* was the weight of $\text{SiO}_2\text{-P}_{\text{Ce6-IL}}$ (mg).

FIGURE 2: Continued.

FIGURE 2: Characterization of $\text{SiO}_2\text{-P}_{\text{Ce6-IL}}$: (a) the SEM and TEM of $\text{SiO}_2\text{-P}_{\text{Ce6-IL}}$; (b) the distribution of Si and N of $\text{SiO}_2\text{-P}_{\text{Ce6-IL}}$; (c) the $^1\text{O}_2$ concentration of $\text{SiO}_2\text{-P}_{\text{Ce6-IL}}$; (d) the element analysis of $\text{SiO}_2\text{-Br}$ by EDS.

The Ce6 released in acidic microenvironment of oral biofilm (pH 4.5) was quantified by UV-vis. Briefly, 10 mg multiclawed $\text{SiO}_2\text{-P}_{\text{Ce6-IL}}$ was placed in pH 4.5 solution 10 min and separated by centrifugation. The concentration of Ce6 in supernatant was examined by UV-vis.

DPBF was used to measure the generation of $^1\text{O}_2$ [15]. Briefly, 2.0 mL DMSO solution containing $\text{SiO}_2\text{-P}_{\text{Ce6-IL}}$ (equalled $100\ \mu\text{M}$ Ce6) and $100\ \mu\text{M}$ DPBF was irradiated by 660 nm light with a power density of $0.5\ \text{W}\cdot\text{cm}^{-2}$. The absorbance of DPBF at about 410 nm was taken to record at 1 and 2 min.

2.3. Binding Ability of $\text{SiO}_2\text{-P}_{\text{Ce6-IL}}$ to Oral Biofilm. Hydroxyapatite tablets (6.0 mm in diameter, 1.5 mm in thickness) were washed twice with PBS and incubated with artificial saliva to obtain saliva-coated hydroxyapatite. The *S. mutans* biofilm was formed on hydroxyapatite surfaces in BHIB medium which contained *S. mutans* (10^6) and 2% sucrose (w/v) for incubated 72 h at 37°C .

The binding capacity of $\text{SiO}_2\text{-P}_{\text{Ce6-IL}}$ to the hydroxyapatite and *S. mutans* biofilm was measured by UV-vis. The $0.1\ \text{mM}$ multiclawed $\text{SiO}_2\text{-P}_{\text{Ce6-IL}}$ was incubated with hydroxyapatite and biofilm in pH 4.5 and 7.4 solutions for 10 min at 37°C , and then, the concentration of $\text{SiO}_2\text{-P}_{\text{Ce6-IL}}$ was measured before and after adsorption to calculated binding capacity.

In order to examine the binding ability of $\text{SiO}_2\text{-P}_{\text{Ce6-IL}}$ to oral biofilm, the interaction between $\text{SiO}_2\text{-P}_{\text{Ce6-IL}}$ with *S. mutans* biofilm was measured by AFM. The procedure was as follows: the AFM tip (NP-O10, Bruker) contacted with epoxy. After waiting for 10 min, $1\ \mu\text{L}$ $\text{SiO}_2\text{-P}_{\text{Ce6-IL}}$ ($0.1\ \text{mM}$) was put on the tip and heated to completely cure epoxy. Then, the AFM tip was put in ultrapure water and ultrasoni-

cated for 30 s to get rid of unattached $\text{SiO}_2\text{-P}_{\text{Ce6-IL}}$. The SEM was used to check the AFM tip modified with $\text{SiO}_2\text{-P}_{\text{Ce6-IL}}$.

2.4. Photosensitizer Concentration in Biofilm. The *S. mutans* biofilm was stained with Alexa Fluor 647. Briefly, $1\ \mu\text{M}$ Alexa Fluor 647 was added to the *S. mutans* biofilms for 6 h. $20\ \mu\text{L}$ of $\text{SiO}_2\text{-P}_{\text{Ce6-IL}}$ or Ce6 ($120\ \mu\text{M}$) was added onto the *S. mutans* biofilm interaction for 30 min, and then, wash with PBS 3 times to remove unconjugated $\text{SiO}_2\text{-P}_{\text{Ce6-IL}}$ or Ce6. The laser scanning confocal microscopy (LSCM, Leica TCS SP8 STED 3X Super-resolution Confocal Microscope, Germany) was used to examine the concentration of Ce6 in biofilm.

2.5. Antibiofilm Activity. The *S. mutans* biofilm was treated with PBS, $\text{SiO}_2\text{-P}_{\text{Ce6-IL}}$, and Ce6. The biofilm incubated with BacLight live/dead dye in the dark after illumination for 15 min to observe dead/live bacteria. The CLSM images were taken to analyze the live and dead bacteria.

2.6. In Vivo Efficacy. Animal experiments were performed on a well-established model of dental caries disease as described in previous literatures [16]. Briefly, rats with two weeks old were purchased from animal center of the Air Force Medical University and screened for infection with *S. mutans*. Any rats infected with *S. mutans* prior to inoculation were removed. The animals were infected orally using an actively growing culture of *S. mutans* by oral swabbing three times a day for 2 weeks. Infected animals were randomly placed into three treatment groups of $n = 5$, and their teeth treated topically once daily. The treatment groups included (1) PBS, (2) $\text{SiO}_2\text{-P}_{\text{Ce6-IL}}$ (amount to $0.1\ \text{mM}$ Ce6) using a 660 nm LED light ($0.5\ \text{W}/\text{cm}^2$) for 15 min, and (3)

FIGURE 3: Continued.

FIGURE 3: The SEM of AFM probe bodified $\text{SiO}_2\text{-P}_{\text{Ce6-IL}}$ (a); the interaction between *S. mutans* biofilm and Ce6 (b), $\text{SiO}_2\text{-P}_{\text{Ce6-IL}}$ (c); the surface potential of *S. mutans* biofilm (d) and treated with Ce6 (e), $\text{SiO}_2\text{-P}_{\text{Ce6-IL}}$ (f); the adhesion of *S. mutans* biofilm (g) and treated with Ce6 (h), $\text{SiO}_2\text{-P}_{\text{Ce6-IL}}$ (i).

free Ce6 (0.1 mM Ce6) using a 660 nm LED light (0.5 W/cm^2) for 15 min. Each group was provided the National Institutes of Health cariogenic diet 2000 and 5% sucrose water ad libitum. The experiment proceeded for 2 weeks; all animals were weighed weekly. At the end of the experimental period, animals were sacrificed, and the teeth were observed by SEM. Finally, the heart, liver, spleen, lung, and kidney were harvested for histological sections (H&E staining analysis on a Leica SP8 microscope).

2.7. Cell Viability Assays. The cell viability of $\text{SiO}_2\text{-P}_{\text{Ce6-IL}}$ was evaluated by CCK-8 assays. L929 fibroblast cells were used as model cells and seeded into 96-well plates (6000 cells per well) with $180 \mu\text{L}$ of DMEM culturing medium in each well for 24 h. $\text{SiO}_2\text{-P}_{\text{Ce6-IL}}$ solution with concentrations from 0.2974, 0.5587, and 1.1173 mM was added to the cells and irradiated by LED light (0.5 W/cm^2) for 15 min. After being incubated for 48 h, $200 \mu\text{L}$ of MTT solution ($0.5 \mu\text{g/mL}$) was added to each well and cultured for another 2 h. $200 \mu\text{L}$ DMSO was added and shook for 10 min to dissolve the crystal. Then, the absorbance was recorded at 490 nm by a microplate reader (model 550, BioRad).

2.8. Hemolysis Assay. The red blood cells washed with PBS until the supernatant was pellucid and was diluted to 2 vol% by PBS. The Ce6 and $\text{SiO}_2\text{-P}_{\text{Ce6-IL}}$ (0.1 mM) were

immersed into 2 vol% red blood cell solutions (5 mL for each tube) and incubated at 37°C for 3 h, respectively. Then, the samples were centrifuged at 1500 rpm for 10 min, and the OD values were recorded at 545 nm. The red blood cells treated with water as the positive control, while the red blood cells treated with PBS as negative control. The hemolysis rate was determined by the equation (2):

$$\text{Hemolysis rate\%} = \frac{\text{OD}_{\text{sample}} - \text{OD}_{\text{negative control}}}{\text{OD}_{\text{positive}} - \text{OD}_{\text{negative control}}} \times 100\%. \quad (2)$$

Besides hemolysis rate, the major organs including the hearts, livers, spleens, lungs, and kidneys were collected and stained with H&E for further evaluation of biocompatibility as previously reported [17].

3. Results and Discussion

3.1. Characterization of $\text{SiO}_2\text{-P}_{\text{Ce6-IL}}$. In order to strong interaction, the 8.92% Br of ATRP grafted on SiO_2 (Figure 2(d)). As showed in Figure 2(a), the size of $\text{SiO}_2\text{-P}_{\text{Ce6-IL}}$ was about 70 nm, and the surface was more rough after initiated by CuCl. Element analysis displayed that N elements of $\text{P}_{\text{Ce6-IL}}$ were on the SiO_2 . The location of Si

FIGURE 4: (a) (A) *S. mutans* biofilm; (B) *S. mutans* biofilm treated with Ce6; (C) *S. mutans* biofilm treated with $\text{SiO}_2\text{-P}_{\text{Ce6-IL}}$; (b) live (green) and dead (red) results; (A) control group; (B) treated with Ce6; (C) treated with $\text{SiO}_2\text{-P}_{\text{Ce6-IL}}$; (c) (A–C) the morphology of *S. mutans* biofilm; (D, E) the morphology of *S. mutans* biofilm treated with Ce6; (F, G) the morphology of *S. mutans* biofilm treated with $\text{SiO}_2\text{-P}_{\text{Ce6-IL}}$; (H) the morphology of *S. mutans* biofilm combined with $\text{SiO}_2\text{-P}_{\text{Ce6-IL}}$; (I) the pores in the EPS.

and N elements was further analyzed by the Spherical Aberration Corrected Transmission Electron Microscope (ACTEM) analysis software. The analysis results showed that the N element was on the surface of Si (Figure 2(b)).

These results indicated that the $\text{P}_{\text{Ce6-IL}}$ was successful grafted on the surface of SiO_2 .

Because $^1\text{O}_2$ was a major factor in photodynamic therapy [18], the $^1\text{O}_2$ production of $\text{SiO}_2\text{-P}_{\text{Ce6-IL}}$ was examined

FIGURE 5: Tooth surface morphology with the treatment of Ce6 and $\text{SiO}_2\text{-P}_{\text{Ce6-IL}}$.

by DPBF. For the reaction of DPBF with $^1\text{O}_2$ decreased the absorption of DPBF at 410 nm, the consumption rate of DPBF corresponds to the $^1\text{O}_2$ production. Compared to the reduced absorbance of DPBF treated by Ce6 (from 3.15 to 1.75), ¹⁴ the absorbance of DPBF treated by $\text{SiO}_2\text{-P}_{\text{Ce6-IL}}$ reduced from 2.69 to 0.24, and the consumption of DPBF was 2.45 (Figure 2(c)). The $\text{SiO}_2\text{-P}_{\text{Ce6-IL}}$ had higher $^1\text{O}_2$. More $^1\text{O}_2$ production indicated that $\text{SiO}_2\text{-P}_{\text{Ce6-IL}}$ had strong oxidation capacity and high PACT efficacy.

3.2. Binding Ability of $\text{SiO}_2\text{-P}_{\text{Ce6-IL}}$ with Oral Biofilm. The *Streptococcus mutans* (*S. mutans*) is one of the primary pathogenic bacteria that cause caries. In this work, *S. mutans* biofilm as model was used to study antibacterial ability of $\text{SiO}_2\text{-P}_{\text{Ce6-IL}}$. To mimic the bacterial biofilm on the teeth, bacteria were grown for 72 h on hydroxyapatite disks. Due to acid microenvironment of *S. mutans* biofilm, the Ce6 was protonated and released; then, $\text{SiO}_2\text{-P}_{\text{Ce6-IL}}$ changed to positive charged $\text{SiO}_2\text{-P}_{\text{IL}}^+$. The biofilm had anions such as carboxyl, phosphoryl, and glycerate at the proteins or polysaccharides would produce negatively charge [19]. The electrostatic interactions would occur between $\text{SiO}_2\text{-P}_{\text{IL}}^+$ and *S. mutans* biofilm. In order to detect interaction strength, the AFM was used to detect the interaction between $\text{SiO}_2\text{-P}_{\text{IL}}^+$ and *S. mutans* biofilm. As showed in Figure 3(a), the $\text{SiO}_2\text{-P}_{\text{Ce6-IL}}$ has been successfully modified on the AFM probe. The interaction between $\text{SiO}_2\text{-P}_{\text{Ce6-IL}}$ with *S. mutans* biofilm was 954.02 nN which contributed by imidazolium cation and dodecyl in the $\text{SiO}_2\text{-P}_{\text{IL}}^+$. However, the interaction of Ce6 with *S. mutans* biofilm was only 23.35 nN, and this weak force was difficult to resist saliva cleaning. This strong interaction was also proved by SEM. As showed in Figure 4(c), the $\text{SiO}_2\text{-P}_{\text{IL}}^+$ like multiclawed octopus and could firmly

adsorb on the *S. mutans* biofilm (EPS). Along with massive positive charges of $\text{SiO}_2\text{-P}_{\text{IL}}^+$, the surface potential of *S. mutans* biofilm changed from -117 to 35 mV, and the adhesion of *S. mutans* biofilm was decreased from -76 nN to -25 nN. However, the adhesion of *S. mutans* biofilm treated by Ce6 alone was -45 nN. Due to the strong adhesion, the oral biofilm infection was difficult to eradicate. So, the decreased adhesion of $\text{SiO}_2\text{-P}_{\text{Ce6-IL}}$ will be beneficial to eliminating biofilm infection.

3.3. Antibacterial Activity In Vitro. As a result of strong interaction, the $\text{SiO}_2\text{-P}_{\text{IL}}^+$ combined with biofilm firmly and the problem of saliva cleaning was solved. But the barrier of biofilm also severely restricted the PACT efficacy against oral biofilm. As showed in Figure 4(a), the blue was *S. mutans* biofilm, and the red Ce6 was difficult to enter the biofilm (Figure 4(a)). Limited by lifetime and diffusion distance of $^1\text{O}_2$ [20], the Ce6 was difficult to damage protected bacteria outside biofilm. As showed in Figure 4(b), green represented live bacteria, and red represented dead bacteria. Most bacteria were alive after treated by Ce6 alone. So it is very important to destroy the dense structure of biofilm to improve the Ce6 concentration in biofilm. Compared to Ce6, $\text{SiO}_2\text{-P}_{\text{Ce6-IL}}$ had hydrophobic dodecyl chain which like suckers on the arm of octopus and could destroy the structure of biofilm. In order to examine the membrane breaking effect of $\text{SiO}_2\text{-P}_{\text{Ce6-IL}}$, the morphology of *S. mutans* biofilm was characterized by SEM. As showed in Figure 4(c), the pores were created in the *S. mutans* biofilm. Once the biofilm was broken, the Ce6 was highly concentrated in biofilm (Figure 4(a)). After illumination for 15 min, most of *S. mutans* in the biofilm were killed by $\text{SiO}_2\text{-P}_{\text{Ce6-IL}}$ (Figure 4(c)). The $\text{SiO}_2\text{-P}_{\text{Ce6-IL}}$ greatly

FIGURE 6: The H&E staining of the heart, liver, spleen, lung, and kidney and cytotoxicity.

improved the PACT efficacy and could effectively eliminate of *S. mutans* biofilm.

3.4. In Vivo Antibiofilm Assessment. The in vitro results revealed that multiclaved SiO₂-P_{Ce6-IL} had excellent efficacy against *S. mutans* biofilms. To further verify these conclusions, the in vivo experiments were designed. The infected mice were divided into three groups: treated with PBS, Ce6 with illumination for 15 min, and SiO₂-P_{Ce6-IL} with illumination for 15 min. The therapeutic effects of SiO₂-P_{Ce6-IL} were evaluated by SEM. As showed in Figure 5, the treatment with SiO₂-P_{Ce6-IL} was very effective in preventing the development of dental caries and completely blocked extensive enamel damage. In contrast, treatment with Ce6 alone was no significant effect and resulted in enamel loss and damage, then led to dental caries.

3.5. Biocompatibility Evaluation. Although SiO₂-P_{Ce6-IL} could effective combine with oral biofilm and almost impossible to transport into the stomach from the mouth, it was important to evaluate their biocompatibility. So the cytotoxicity, hemolysis rate, and the histological section of main organs including the heart, liver, spleen and lung, and kidney were examined. The results show that no abnormal effects or damages were observed in these organs (Figure 6), and the body weights of rats treated with SiO₂-P_{Ce6-IL} were similarly to healthy rats. The hemolysis rate and cytotoxicity were 4.2% (limit of clinical hemolysis rate < 5%) [21] and above 90% within therapeutic concentration (Figure 6). The SiO₂-P_{Ce6-IL} was a potential safe material for clinical applications in oral biofilm infection.

4. Conclusions

In this work, the SiO₂-P_{Ce6-IL} with charge reversal and high binding ability was prepared. The potential measurements showed that SiO₂-P_{Ce6-IL} could turn into positive and combine with EPS firmly in the acidic microenvironment of oral

biofilm. The morphology of *S. mutans* biofilm results showed that SiO₂-P_{Ce6-IL} could combine with EFS and destroy the structure of biofilm. The laser scanning confocal microscopy results found that the concentration of Ce6 in *S. mutans* biofilm was greatly improved, and most of bacteria in biofilm were killed. In vivo experiments showed that the SiO₂-P_{Ce6-IL} was a highly desirable property for oral biofilm infection and had high quality in the prevention of dental caries. Most importantly, SiO₂-P_{Ce6-IL} had good biocompatibility in therapeutic effective concentrations.

Data Availability

The experimental data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Authors' Contributions

Ziyi Jiao and Yonggang Teng contributed equally to this work.

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