Synergistic Chemotherapy and Photodynamic Therapy of Endophthalmitis Mediated by Zeolitic Imidazolate Framework-Based Drug Delivery Systems

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1. Introduction

Bacterial infections remain a significant clinical challenge during the interventional material use and after surgery. [1] The fast evolution of resistant bacteria and biofilms formation are the main problems in infectious disease treatment. [2] Commonly used strategies such as bacterial adhesion resistance, [1] bactericidal surface through contact killing, [4] antibacterial agent release, [5] and even bacterial infections self-defensive surfaces [6] and bactericidal/antifouling reversibly switchable [7] cannot achieve ideal goals as the spread of biofilms. Biofilms mainly composed of water, extracellular polymeric substances (EPS), and bacteria are related to 80% of the infectious disease. The bacteria within the biofilm matrices greatly increase the resistance as the decreased permeability of bactericides and dormancy of the bottom bacteria. [2b] Antibiofilm surfaces or nanoparticles could either inhibit biofilm development or remove the mature biofilms. The ideal antibiofilm or antibacterial surface should greatly reduce the adhesion of bacteria or long-term elimination of living bacteria through combination of different antibacterial functions to realize targeted and highly efficient treatment. [8]

In particular, endophthalmitis is mainly caused by pathogenic microorganism brought in by the surgical process which leads to uveitis, intraocular inflammation, and vitreous cavity...
and tissues damage within the eyeball. Postoperative infectious endophthalmitis may be encountered after intraocular procedure. The number of cataract cases is estimated to be 30.1 million in this decade in the United States alone, and continues growing rapidly. Although the cataract removal procedure in cataract surgery is mature and safe, severe postoperative infection will happen after intraocular lens (IOL) implantation during cataract surgery even with topical antibiotic coverage. Although the incidence of endophthalmitis in the developed countries is not high (between 0.07% and 0.13%) after IOL implantation in cataract extraction surgery, the situation is serious in the developing countries. Once endophthalmitis happens, it is commonly treated through injections of antibiotics directly into the vitreous cavity. Successful inhibition of infectious endophthalmitis greatly depends on timely diagnosis and rapid appropriate therapy, while it will be difficult to eliminate endophthalmitis once it occurs and clinically antibiotics can only be used to control the disease development. Therefore, it is urgent to search alternative approaches to enhance the efficiency of treatment.

A variety of mechanisms, such as cell development of multidrug efflux pumps, membrane permeability changing, and gene mutations, contributed to the antibiotic resistance development of pathogens. Especially, the emergence of super bacteria brings great threats to human health as the loss of bactericidal properties. Photothermal therapy (PTT) and photodynamic therapy (PDT) process great advantages in treating infections related to antibiotics resistant bacteria and biofilms due to the different antibacterial mechanisms. PDT performed great potential in diagnosing and treating infectious disease through producing reactive oxygen species (ROS) from photosensitizer under irradiations. As for endophthalmitis therapy, PDT is more suitable than PTT taking into account of patient’s eyes experience. Another important and unique advantage of phototherapy in ophthalmic disease treatment is the naturally good light transmission property comparing with skin light penetration. On the other hand, nanoparticles (NPs) such as metal–organic frameworks (MOFs) in nanoscale with a high drug-loading capacity can be designed and modified for targeting and local delivery of antibacterial agents. Furthermore, NPs need to be coated with immune evasion molecules such as polyethylene glycol (PEG) to avoid being swallowed by the body’s metabolic organs and being recognized and removed by macrophages in vivo.

In this work, zeolitic imidazolate framework-8-polyacrylic acid (ZIF-8-PAA) was first constructed to load ammonium methylbenzene blue (MB) to give ZIF-8-PAA-MB (ZPM) nanomaterial, followed by in situ formation of silver NPs (AgNPs) through dopamine reduction and then secondary modification with vancomycin (Van)/NH 2-PEG, leading to the formation of a new hybrid material, namely ZIF-8-PAA-MB@AgNPs@Van-PEG (ZPMAVP) (Scheme 1). Loading capacity, loading efficiency, and release behavior of MB in both ZIF-8 and ZIF-8-PAA were explored in phosphate buffer saline (PBS, pH 7.4) and 4-morpholineethanesulfonic acid buffer (MES, pH 5.5). Their antibacterial functions were explored using

![Scheme 1. Schematic illustration of the construction of ZPMAVP system for application in synergistic chemotherapy and photodynamic therapy of endophthalmitis.](image-url)
three kinds of bacteria *Escherichia coli* (*E. coli*, ATCC 8739), *Staphylococcus aureus* (*S. aureus*, ATCC 6538), and methicillin-resistant *S. aureus* (MRSA, ATCC 43300). Both scanning electron microscope (SEM, FEI, SIRION-100) and bacteria living/dead staining methods were used to prove the targeting and synergistic antimicrobial and antibiofilm property of PDT and AgNPs. The biocompatibility of ZPMAVP material was determined against retinal pigment epithelial cells (RPEs) and human corneal epithelial cells (HCECs). Bacterial colony counting and H&E staining methods in rabbit endophthalmitis model were used to verify the in vivo antibacterial property.

### 2. Results and Discussion

#### 2.1. Synthesis and Characterization of ZIF-8 and ZIF-8-PAA

In the present study, PAA was first incorporated into the ZIF-8 system to enhance the drug loading dose and pH-responsive release properties of the NPs. PAA rich in carboxyl groups participated in the formation and crystallization process of ZIF-8 will also increase the pH responsiveness. It is worth mentioning that the synthesis process was simple, green, and fast. As indicated in transmission electron microscopy (TEM) image (Figure 1A), the average diameter of ZIF-8 particles was ≈60 nm (Figure 1B). And the particle size greatly increased into 110 nm in diameter upon incorporating PAA into the ZIF-8 MOFs to give ZIF-8-PAA. Compared with small ions and molecules (Zn$^{2+}$ and 2,4-dimethylimidazole) that led to the formation of ZIF-8, PAA has a much higher molecular weight (10 kDa) which could also form PAA-Zn upon coordination with Zn$^{2+}$ ions. The size distribution of the obtained ZIF-8-PAA NPs centralized at 110 nm, almost twice of ZIF-8 size. It was also confirmed by dynamic light scattering (DLS) measurement that the average hydration diameters of ZIF-8 and ZIF-8-PAA were 344 and 425 nm, respectively, in the swelling state in water, indicating the higher swelling property of ZIF-8 NPs than ZIF-8-PAA NPs. It could be inferred the difference could be attributed to the much higher crosslinking degree of ZIF-8-PAA with PAA as crosslinking agent. The zeta potential (35.0 ± 0.53 mV) of ZIF-8 suggested the high positively charged surface property, which was changed into −60.4 ± 1.65 mV for ZIF-8-PAA, indicating the presence of PAA with dissociated carboxyl groups on the NPs surface. This result revealed the different structures of ZIF-8 and ZIF-8-PAA NPs including surface and size characteristics. The crystalline structures of both ZIF-8 and ZIF-8-PAA materials were verified through powder X-ray diffraction (XRD) measurement (Figure S1A, Supporting Information). Both ZIF-8-PAA and ZIF-8 showed main characteristic peaks at (011), (002), (112), (022), (013), (114), (223), (224), (134), and (044), respectively, in consistency with the published data in literature. In the Fourier transform infrared spectra (FT-IR) spectra of ZIF-8 (Figure S1B, Supporting Information), Zn–N stretching mode was found due to the absorption band at 424 cm$^{-1}$ and the absorption bands between 1100 and 1400 cm$^{-1}$ region were related to the C–N stretching. Besides, as for ZIF-8-PAA, the absorption band at 1578 cm$^{-1}$ could be associated to the shifted C=O band of −COOH groups in coordination with Zn$^{2+}$. As a result, both FT-IR and XRD measurements indicated the synthesis of highly regular ZIF-8.
and ZIF-8-PAA. Although the combination of PAA into ZIF-8 did not change the crystal structures, PAA actually participated in the framework structure with covalent bonds. The good hydrophilicity and long chains led to the exposure of PAA on the NPs surface as measured in zeta potential.

2.2. Loading of MB into ZIF-8-MB and ZPM MOFs

As a photosensitizer, MB was loaded into the ZIF-8 and ZIF-8-PAA MOFs for PDT of endophthalmitis. Two kinds of commonly used methods, drug loading during MOFs synthesis and after MOFs synthesis, were compared through measuring the loading efficiency and loading capacity.[23] After sufficient contacting and MB loading, MOFs were obtained by centrifugal precipitation. As shown in Figure 2A, the supernatant colors of ZIF-8-MB NPs both during and after synthesis were much darker than that of ZPM NPs, which indicated the higher MB loading capacity of ZPM NPs. As in Figure 2B, the color of the obtained ZPM NPs solid material was higher than the products obtained by other methods. Through deduction, the amount of MB remained in the supernatant from the total feeding amount, the loading capacity into the MOFs could be calculated. The MB loading capacity/loading efficacy were 1.5 ± 0.2 wt%/19 ± 2.5%, 5.9 ± 0.3 wt%/79 ± 4.0%, and 5.0 ± 0.3 wt%/99.5 ± 6.0% for MB-loading in ZIF-8-MB during synthesis, ZPM NPs during synthesis, and ZPM NPs after synthesis. Furthermore, to confirm the loading of MB into the MOFs, both TEM elemental mappings (Figure S4, Supporting Information) and Brunauer–Emmett–Teller (BET) surface areas (Figure S5, Supporting Information) were applied to explore the changes of specific surface area and pore size. The presence of S element in ZPM NPs indicated the successful loading of MB into the MOFs. The specific surface area changed from 1646.43 to 847.34 m² g⁻¹ and the pore size also decreased which also suggested the drug loading in MOFs. In the following experiment, MB loading of ZIF-8-MB and ZPM MOFs during synthesis was used owing to the appreciable loading capacity and preparation simplicity.

The release behaviors of MB from two kinds of MOFs were also evaluated in PBS buffer (pH 7.4) and MES buffer (pH 5.5) through dialysis method. As shown in Figure 2D, MB was released faster in MES than that in PBS for both MOFs, displaying an obvious pH-responsive property. Furthermore, the MB release speed from ZPM NPs was much faster than that from ZIF-8-MB NPs, which indicated the higher pH responsibility due to the combination of PAA. However, all of the MOFs showed burst release in the first 24 h and sustained drug release in the following 144 h. The super high specific surface area is beneficial to drug diffusion from the matrix. The combination of PAA brought extra pH-responsive groups into this system thus promoting polymer segment collapse and enhancing the swelling through carboxyl groups protonation.[24] The change of polymer segment led to the increase of MOFs pore structure permeability.

2.3. Surface Modification of ZPM NPs

In the next step, we modified the MOFs for immune cells phagocytosis escape and to reduce organ toxicity such as liver

![Figure 2. A) Photos of ZIF-8-MB dispersions before (tube 1) and after (tube 3) centrifugation, and ZPM dispersions before (tube 2) and after centrifugation (tube 4). B) Photos of freeze-dried ZPM NPs after synthesis (tube 2), ZIF-8-MB during synthesis (tube 3), and ZPM NPs during synthesis (tube 4). C) Standard curve of ammonium methylbenzene blue (MB) recorded by measuring the UV absorbance of different concentrations of samples at 630 nm. D) The release profiles of MB from ZIF-8-MB and ZPM NPs at the buffer solutions of different pH values.](image-url)
and kidney enrichment. The high content of 3,4-dihydroxyphenylalanine (DOPA), a kind of catecholic amino acid, which attributes to the excellent underwater adhesion property of marine organisms such as marine mussels on the surfaces through forming strong interactions including both covalent or noncovalent forces.[25] This greatly inspired researchers to figure out the cross-linking mechanism and apply it to surface modification of biomaterials. Dopamine can self-crosslink into a coating through complex cross-linking reactions between phenolic hydroxyl groups and amino groups.[26] In this process, functional small molecules or polymers with amino groups can be combined into polydopamine coating through participating in chemical reactions. This is the one-pot surface modification method. Also, functional molecules can be modified on the preformed polydopamine coating through secondary reactions between amino groups of the functional molecules and catechol groups on the polydopamine coating.[27] In this work, we first compared the modification reliability and functionality of two methods.

The wettability and thickness of the coatings on NPs cannot be directly measured by contact angle analyzer and spectroscopic ellipsometry.[28] As an alternative approach, the coatings were constructed on silicon wafers in the same conditions instead of that on NPs. As indicated in Figure S2A,B in the Supporting Information, the water contact angle (WCA) of the pristine silicon wafer was 75.9 ± 7.7°, which changed to 35.0 ± 3.9° after deposition of polydopamine-Ag NPs-Van-PEG composite coating. In comparison, for the two-step modification method, the WCA of polydopamine-Ag NPs was 55.8 ± 3.6° after the first step of coating, which greatly decreased into 10.3 ± 5.0° after secondary modification of Van and PEG. The result verified the much higher modification efficiency of two-step modification method than one-pot modification method. This could be due to the sufficient reactions between Van-PEG and polydopamine and the higher amount of PEG molecules on the MOFs surface.

As for the thickness changes shown in Figure S2C in the Supporting Information, the thickness coating for one-pot polymerization method increased 27.1 ± 3.3 nm, showing the rapid growth of polydopamine-Ag NPs-Van-PEG composite coating. This thickness is much higher than pure polydopamine coating as reported in the literature.[29] However, it was a surprise to find the thickness of polydopamine-AgNPs and secondary modification step was 26.4 ± 3.0 nm and 22.7 ± 7.8 nm, respectively. The step-by-step method did not promote the increase of film thickness. One way to explain the phenomenon was the coating structure of the coating reconstructed during the secondary modification process. Another reason might be the special spatiotemporal competition of dopamine self-polymerization process. It has been confirmed that the thickness of polydopamine coating cannot continuously grow over time after several hours which is different from common polymerization process.[30] It could be greatly relied on the oxidation of catechol groups that is indicated through color changes from transparent to dark brown. In this case, the two-step modification method was used to modify ZIF-8 and ZIF-8-PAA MOFs after loading of MB.

In this way, the MB-loaded MOFs were first coated with polydopamine-AgNPs coating during which AgNPs formed through in situ reduction by dopamine. Through comparison with pristine polydopamine coating that almost less than 20 nm,[31] the combination of AgNPs greatly increased the thickness of coating into 26.4 ± 3.0 nm as previously discussed (Figure S2C, Supporting Information). It was found that the particle size did not obviously increase, even slightly decreased. This might be due to the reconstruction of the MOFs by changing pH environment. And then both Van and PEG were grafted on the surface of the MOFs to increase the surface hydrophilicity as well as to enhance the targeting property toward bacterial infections. As shown in Figure S3 in the Supporting Information, the particle diameter of the finally obtained ZPMAVP NPs (round 150 nm) was larger than unmodified MOFs (around 140 nm). The amount of AgNPs in MOFs was evaluated by ICP-MS after oxidation to ionic state by HNO3. The contents of Ag in ZIF-8-PAA-MB@AgNPs NPs (ZPMA NPs) and ZPMAVP NPs were 208 and 201 mg g⁻¹, respectively. The excellent loading of Ag in MOFs could be attributed to the super high specific surface area. It proved that AgNPs not only formed on the surface of MOFs but also in the porous structure.[32]

2.4. Cellular Compatibility Evaluation

The biocompatibility of the MOFs was obtained through evaluation of the effects of concentration, laser irradiation, and the components on cell viability against two kinds of ophthalmology common cells, i.e., RPEs and HCECs. As in Figure 3A, three kinds of MOFs without laser treatment did not show any cytotoxicity against RPEs as the concentrations were higher than 40 µg mL⁻¹. Surprisingly, all of the MOFs showed high cytotoxicity against RPEs as the concentrations were higher than 40 µg mL⁻¹ after incubation for 24 h, owing to the sensitivity of RPEs to MOFs containing MB as at a certain concentration threshold. It could be inferred that during incubation with cells, MB was excited by natural light to release ROS that is toxic to cells. As for the influence of laser on cellular compatibility, Figure 3B showed that laser exactly exhibited growing cytotoxicity against both cells as the increase of ZPMAVP NPs. The rapid release of MB and ROS under laser treatment led to the generation of cytotoxicity. Specially, the cell viability was higher than 80% that of TCPS when the concentration was not higher than 10 µg mL⁻¹. The influence of MOFs concentration and laser on the number and spread of adherent cells on TCPS was also tested through FDA method (Figure 3C,D). It was found that the number of both RPEs and HCECs slightly decreased when the concentration was 20 µg mL⁻¹ and obviously decreased when the concentration increased to 40 µg mL⁻¹. The spread of both cells did not significantly change for all of the tested concentration. In the following experiments, the concentration of MOFs was selected as 10 µg mL⁻¹, and they were placed under laser 202 mW for 5 min except for special indication.

2.5. In Vitro Antibacterial Test

The targeting activity of the NPs modified with Van against S. aureus, E. coli, and MRSA was measured through SEM after incubation. Other two kinds of MOFs (ZPM and ZPMA)
were used as controls without furthermore modification with targeting molecules. As in Figure 4, the amount of ZPM NPs that integrated with three kinds of bacteria was very few. As for ZPMA NPs, the amount increased a lot for three kinds of bacteria. The oxidation of AgNPs into Ag$^+$ was beneficial to the interaction with negatively charged bacteria. Many mechanisms contributed to the bactericidal function against bacteria,\cite{33} which led to the death of bacteria, reduction of remaining amount on the surface, and the damage to bacterial structure. After further modification of the materials with Van and PEG, the number of MOFs combined with bacteria or bacterial corpse further increased. Van is not only a high efficient antibiotic but also a commonly used targeting molecule especially with Gram-positive bacteria.\cite{34}

The bactericidal and antiadhesive activities of the MOFs materials were explored through agar plate counting method, bacterial LIVE/DEAD staining method, and SEM measurement. First, the bacterial survival and bactericidal kinetics were quantitatively measured by flat counting method after shake flask contacting culture. As shown in Figure 5A–C, the number of bacteria almost did not change after incubation for 4 h. The numbers of bacteria slightly decreased under laser treatment for \textit{E. coli} and \textit{S. aureus}. As for ZPM MOFs, three kinds of bacteria did not show obvious reduction without laser treatment. On the contrary, bacteria greatly decreased under laser, indicating the high efficiency of PDT treatment. Comparing with ZPM material,

![Figure 3. A) Cell viability of retinal pigment epithelial cells (RPEs) incubated with ZPM NPs, ZPMA NPs, and ZPMAVP NPs for 24 h. B) Cell viability of HLECs and RPEs incubated with ZPMAVP NPs with or without laser treatment (202 mW cm$^{-2}$ for 5 min) for 24 h ($^*$ $p$ < 0.05, **$p$ < 0.01). Fluorescent images of live/dead staining on C) RPEs and D) HCECs after incubation with various concentrations of ZPMAVP NPs with or without laser irradiation (202 mW cm$^{-2}$) for 5 min. Scale bar: 10 µm.]

![Figure 4. SEM images showing the targeting effect of ZPM NPs, ZPMA NPs, and ZPMAVP NPs toward \textit{E. coli}, \textit{S. aureus}, and MRSA. Scale bar: 1 µm.]

![Figure 4. SEM images showing the targeting effect of ZPM NPs, ZPMA NPs, and ZPMAVP NPs toward \textit{E. coli}, \textit{S. aureus}, and MRSA. Scale bar: 1 µm.]

![Figure 4. SEM images showing the targeting effect of ZPM NPs, ZPMA NPs, and ZPMAVP NPs toward \textit{E. coli}, \textit{S. aureus}, and MRSA. Scale bar: 1 µm.]}
ZPMA NPs material showed effective bacteria killing function owing to the presence of AgNPs. This is the reason why AgNPs was combined into this system: not only combined another antibacterial approach in a simple way but also enhanced antibacterial efficiency.[35] Furthermore, the grafting of targeting and hydrophilic molecules, the bactericidal property of ZPMAVP MOFs further increased, as compared with ZPMA MOFs.

The conclusions were confirmed by bacterial LIVE/DEAD staining method as shown in Figure 5D–F. Three kinds of bacterial biofilm maintained integral structure and bacterial viability for both no treatment and laser treatment, suggesting the absence of antibiofilm property. As for the PDT effect, no obvious living E. coli and MRSA could be found after laser treated only 5 min at 202 mW, indicating the excellent bacteria killing efficacy. The number of S. aureus was also greatly decreased with negligible remaining stubborn colonies. In addition, two kinds of AgNPs-loaded MOFs also showed certain biofilm eradication activity owing to its good diffusion and biofilm penetration property. Specifically, the ZPMAVP MOFs exhibited the highest biofilm eradication activity because of the superiority to combine with bacteria. In addition, the pH-responsiveness and rapid MB release from the materials is beneficial to local high concentration enrichment around the bacterial biofilm for PDT. The short time treatment and low power of laser are highly suitable for biomedical application. After PDT, the sustained Ag\(^{+}\) release will play a long-term antibacterial function. In summary, the synergistic antimicrobial properties of PDT, fast and high efficient biofilm removal, and AgNPs-based long-acting bacteria killing will exert ideal long-lasting biofilm inhibition and therapeutic effects.

To further verify the biofilm removal function, SEM images were obtained to distinguish the remaining bacteria and bacterial corpse on silicon wafers (Figure 6). The PDT method showed excellent biofilm eradication function as the greatly
reduction of adhered bacteria on the surface. However, there was also some difference between SEM and fluorescence images especially for E. coli which could be attributed to the difference of substrates (TCPS vs silicon wafer). A large number of E. coli remained on silicon wafer after PDT treatment owing to the easily adhesion on silicon and the short time of PDT. The remaining E. coli might have been killed due to the high efficiency of PDT as shown in Figure 5B,D. As for the surface property difference of different biomaterials, the obtained MOFs can also be constructed on the surface as bacterial infections self-defensive coatings as reported in our previous works. On this basis, the surface of MOFs should be modified for charged for layer-by-layer self-assembly multilayer films or directly be combined into hydrogels on biomaterials.

2.6. Antibacterial Mechanism Exploration

The antibacterial mechanism and the generation of cytotoxicity were explored through measuring the production of ROS during PDT. As indicated in Figure 7, nearly no ROS could be detected for three kinds of bacteria that were treated by neither laser treatment without contacting with ZPMAVP MOFs nor the RPEs contacting without laser excitation. Only the bacteria contacting with NPs under laser generated apparent ROS. However, the RPEs incubated with the MOFs produced some ROS leading to a few green stained cells, which could be ascribed to the lack of cell wall structures for mammalian cells. The interactions between RPEs and Ag⁺ released from the MOFs and the enrichment inside RPEs led to the generating of ROS. Furthermore, the amount of ROS greatly increased under laser owing to the excitation of MB. The generation of ROS should be the main reason of cytotoxicity against RPEs and HCECs.

2.7. In Vivo Antibacterial Tests

As relatively closed systems, the eyes are the windows of the soul. Patients will suffer great pains once eye infection
occurs. The injection of antibiotics became the only way to control the progression of the disease but it is difficult to eliminate the infections. In this work, two efficient sterilization methods with the property to inhibit bacterial resistance development were combined to exert synergistic antibacterial effects against endophthalmitis. As discussed in the in vitro antibacterial tests, the ZPMAVP MOFs could fast eliminate bacteria in 5 min through PDT owing to the great pH-responsiveness to bacterial infections. Simultaneously, AgNPs could play a long-lasting bactericidal effect without further laser irradiation. First, the biocompatibility was verified again through injection of the MOFs into animal eyes and laser was applied. As shown in Figure S6 in the Supporting Information, the images of the anterior segment of the eye were taken by the slit lamp on the zero, 1st, 3rd, 5th, and 7th day after MOFs injection. It was found that the cornea was clear. There was no obvious glint and exudation in the anterior chamber, and the conjunctiva was not significantly hyperemic. As indicated in Figure S6C,D, HE staining sections showed clear structure of the retina, no abnormalities, and no obvious structural abnormalities and inflammatory reactions. This is consistent with the in vitro results in cell compatibility evaluation. It can be concluded that this concentration of ZPMAVP MOFs (10μg mL⁻¹) can be used to treat endophthalmitis.

After that, bacterial endophthalmitis model was successful constructed in eyes of New Zealand White Rabbit through injection of bacterial solutions (Figure S7, Supporting Information). It was observed that all eyes became turbid, endophthalmitis and anterior chamber flare after 1 d. By extracting the vitreous fluid, it was found that the bacteria had proliferated rapidly through counting the concentration of bacteria which was more than 1×10¹⁰ CFU mL⁻¹. After that, the bacterial infected eyes were used as controls treated by clinically used antibiotics Van or PBS (Figure 8). It could be found that the eyeballs in the control group were basically white. And the anterior chamber empyema, conjunctival hyperemia, and a large amount of purulent secretion were clearly observed. As for the eyes treated by Van, the development of endophthalmitis was well controlled. However, the symptoms had not been completely cured which was consistent with the actual clinical situations. In the experimental group treated by MOFs under laser, the symptoms were mild with mild conjunctival congestion and no obvious anterior chamber empyema.

After 7 d, the rabbit was euthanized and the eyeball was removed for further pathological examination. Then the vitreous humor was cultured on agar plate to count the bacteria concentration. The result in Figure 9 showed that the bacteria in the control group maintained more than 1×10¹⁰ CFU mL⁻¹, while the Van and PDT administrated group was less than 1×10⁵ and 1×10³ CFU mL⁻¹, respectively. It showed in H&E staining images that the retinal structure of the control group was almost absent, and many inflammatory cell infiltrations

![Figure 7. ROS levels of RPEs, S. aureus, E. coli, and MRS under different treatments: control, laser, ZPMAVP NPs, and ZPMAVP NPs + laser (202 mW cm⁻²). Scale bar: 10 μm.](http://www.small-journal.com)

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were observed. The retinal structure of the experimental group was still basically present. Furthermore, the infiltration of inflammatory cells in PDT treated groups was significantly less than that of the PBS and Van treated groups. In addition, inflammation degree scores of endophthalmitis upon various treatments also indicated the excellent performance in reducing of inflammation reaction (Figure S8, Supporting Information). As observed in the in vitro antibacterial experiments, PDT showed high efficient bacteria killing functions. Comparing with subcutaneous treatment, PDT in ophthalmic disease treatment processes natural unique advantages owing to the good light transmission activity. As a result, the PDT method showed superiority in ophthalmic disease treatment.

3. Conclusion

Bacterial endophthalmitis is a serious threat to the patient's vision and is one of the most important diseases leading to the blindness of eyes. In this work, a novel MOFs drug delivery
system was constructed for synergistic chemotherapy and PDT of endophthalmitis. The obtained ZPMAVP material showed enhanced pH-responsiveness and loading capacity of MB after combination of PAA. Measurements of TEM, DLS, and spectral analysis confirmed the in situ reduction of AgNO₃ into AgNPs by dopamine and the secondary modification Van/NH₂-PEG. In cellular compatibility evaluation, the MOFs exhibited low photo-cytotoxicity against both RPEs and HCECs when the concentration was lower than 10 µg mL⁻¹. In vitro antibacterial and biofilms eradication properties against S. aureus, E. coli, and MRSA indicated the high efficient chemotherapy and photodynamic synergistic therapy properties. Furthermore, in vivo biocompatibility evaluation and mice endophthalmitis model verified the physiological safety and excellent bacterial endophthalmitis treatment properties. Due to the naturally good light transmission of the eyes, the optical therapy system constructed in this study shows promising potential application in the treatment of ophthalmic diseases.

4. Experimental Section

Synthesis and Characterization of ZIF-8-MB and ZPM NPs—Synthesis of ZIF-8-MB and ZPM: According to the literature report, ZPM NPs were prepared as follows. Specifically, Zn(NO₃)₂·6H₂O (1.17 g), PAA (0.5 mL), 2,4-dimethylimidazole (22.7 g), and MB (0.088 g) were dissolved in ultrapure water (88 mL) at room temperature. Under stirring, the solution became turbid navy blue quickly. After 4 h, the nanocrystals were collected by centrifugation and washed three times with ultrapure water. In the end, the samples were dried at 40 °C in a vacuum oven. As controls, ZIF-8-PAA, ZIF-8, and ZIF-8-MB and were synthesized in the same procedure.

Synthesis and Characterization of ZIF-8-MB and ZPM NPs—Particle Size and Zeta Potential of ZIF-8-MB and ZPM: Both ZIF-8-MB and ZPM NPs were diluted with distilled water to determine the zeta potential and size distribution using the Zetasizer (ZetaPlus, Brookhaven Instruments Corporation, Holtsville, NY). FT-IR spectra were tested from 400 to 4000 cm⁻¹ using a spectrometer with KBr pellets. XRD spectra measurement was done on an X-ray diffractometer (DB Advance, Bruker AXS GmbH, Karlsruhe, Germany) using CuKα radiation ranging from 5° to 65° with scanning rate at 5° min⁻¹. The morphology of the ZIF-8-MB and ZPM NPs was investigated through TEM. For TEM observations, the samples were negatively stained with phosphotungstic acid (0.5% mass fraction) on a copper grid.

Surface Modification of ZPM NPs: The consequent of dopamine self-crosslinking assisted surface modification method through one-pot polymerization and secondary grafting of Van and mPEG-NH₂ onto AgNPs by dopamine and the secondary modification Van/NH₂-PEG. In cellular compatibility evaluation, the MOFs exhibited low photo-cytotoxicity against both RPEs and HCECs when the concentration was lower than 10 µg mL⁻¹. In vitro antibacterial and biofilms eradication properties against S. aureus, E. coli, and MRSA indicated the high efficient chemotherapy and photodynamic synergistic therapy properties. Furthermore, in vivo biocompatibility evaluation and mice endophthalmitis model verified the physiological safety and excellent bacterial endophthalmitis treatment properties. Due to the naturally good light transmission of the eyes, the optical therapy system constructed in this study shows promising potential application in the treatment of ophthalmic diseases.

Molecular Oxygen Measurement: To explore antibacterial mechanism, the intracellularly generated molecular oxygen was measured through a Cellular Reactive Oxygen Species Detection Assay Kit (2',7'-dichlorodihydrofluorescein diacetate, DCFH-DA). After DCFH-DA dissolved in ethanol (3.5 mg in 721 µL), the solution was diluted 10 times in DMEM/F12 for stock at −20 °C. For molecular oxygen measurement, the solution was diluted another 100 times in DMEM/F12 solution. After PDT treatment of ZPMAVP NPs under laser irradiation, the RPEs or bacteria were incubated with DCFH-DA working solutions in dark for 5 min. Subsequently, 2.5% glutaraldehyde was used to fix the samples overnight followed by ethanol gradient dehydration (50, 75, 85, 95, and 100 wt%, respectively) for 15 min in each step. The obtained samples were applied for SEM investigation.

Cellular Compatibility of ZPMAVP NPs—Cellular Compatibility of ZPMAVP NPs: In CCK-8 (Beyotime, China) assay, both RPEs (ATCC 8318) and HCECs (from ATCC, SRA01/04) were used to quantitatively evaluate compatibility of ZPMAVP NPs in PDT treatment under fixed laser irradiation parameter (5 min, 202 mW cm⁻²). Both RPEs and HCECs were grown in DMEM/F12 (1:1) mixture medium supplemented with 10% fetal bovine serum, 100 U mL⁻¹ penicillin, and 100 µg mL⁻¹ streptomycin in a 5% CO₂ incubator at 37 °C. After incubation with different concentration of the NPs (0–40 µg mL⁻¹), the medium of RPEs or HCECs were replaced with new medium (100 µL) containing CCK-8 (10 µL, Beyotime, China). Water-soluble formazan would form after 2 h incubation at 37 °C, which was aspirated to a new 96 well plate for absorbance measurement using microplate reader (Multiskan MK33, Thermo Electron Corporation, China) at 450 nm.

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**Conflict of Interest**

The authors declare no conflict of interest.

**Keywords**

antibacterial materials, biofilms, endophthalmitis, metal–organic frameworks, photodynamic therapy

**Supporting Information**

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

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**Animal Experiments of Antibacterial Activity**

The study was approved by the Institutional Animal Care and Use Committee of Wenzhou Medical University. The rabbits were treated according to guidelines set by the Association for Research in Vision and Ophthalmology. For the in vivo antibacterial property evaluation, 30 New Zealand white rabbits weighing between 2.5 and 3.5 kg were used to set up the bacterial endophthalmitis model before drug intervention. The rabbits were evenly separated into three groups and treated with PBS, Van or ZPMAVP + laser.

**Animal Experiments of Antibacterial Activity—Establishment and Evaluation of Bacterial Endophthalmitis Model**

Conventional surgical instruments and consumables were sterilized by high-temperature autoclave, autoclaved, and dried in an oven. The eyes of the animal were anesthetized with serratazine hydrochloride for three times every 5 min before injection of *S. aureus* or MRSA to induce the intraocular bacterial infections. In order to avoid excessive intraocular pressure, part of the aqueous solution was taken out with a syringe. Tweezers were used to hold the eyeball in the conjunctiva and fascia outside the puncture point or the ipsilateral corneal margin. A 1 mL syringe connected with a 25- to 27-gauge needle, the slope facing down, was used to puncture the anterior chamber 1 mm into the limbus above the palate. After loosening the fixation and turning the needle to the cornea, 0.1 mL of aqueous humor was slowly withdrawn and gently pulled out the needle.

The *S. aureus* or MRSA solution (0.1 mL, 10⁴ CFU mL⁻¹) was drawn into the eyes with a syringe. The needle was inserted vertically from the limbus and injected into the vitreous cavity avoiding the crystal after entering to a certain depth. After pushing, the eyeball was obviously bulging. At this time, the needle pushing should be paused a while before continuing. For the pulling, the needle was pressed with a cotton swab. On the second day, the formation of endophthalmitis was observed under slit lamp and indirect ophthalmoscopy, and the vitreous humor was drawn to count the bacteria concentration.

After successfully construction of the bacterial endophthalmitis model, 0.1 mL PBS, Van or ZPMAVP NPs (400 μg mL⁻¹) combined with laser treatment (5 min, 202 mW cm⁻²) was injected into the vitreous cavity. As for the postoperative care of experimental animals, the living environment was kept clean and the drinking water was changed regularly. The changes in the anterior segment of the eye were observed under slit lamps and indirect ophthalmoscopes at 1, 3, and 7 d, respectively. After taking pictures on the 7th day, the animals were anesthetized with serratazine hydrochloride for three times every 5 min before injection of *S. aureus* or MRSA solution (0.1 mL, 10⁴ CFU mL⁻¹) was drawn into the eyes with a syringe. The needle was inserted vertically from the limbus and injected into the vitreous cavity avoiding the crystal after entering to a certain depth. After pushing, the eyeball was obviously bulging. At this time, the needle pushing should be paused a while before continuing. For the pulling, the needle was pressed with a cotton swab. On the second day, the formation of endophthalmitis was observed under slit lamp and indirect ophthalmoscopy, and the vitreous humor was drawn to count the bacteria concentration.

**Statistical Analysis**

All experiments were conducted in triplicate, and data points were expressed as the mean. Two sample t test in origin 8.0 (Microcal, USA) were used to compare data obtained with the different samples under identical treatments. A value of *p* < 0.05 was considered significant.

**References**


