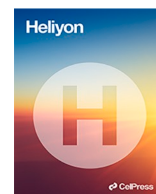
Heliyon 10 (2024) e31788

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Gallium-based metal–organic frameworks with antibacterial and anti-inflammatory properties for oral health protection

Gongyuan Song [a], Min Li [b], Bing Zhou [c], Hongguang Qi [d], Jie Guo [e,*]

[a] *Shijiazhuang Stomatology Hospital, Shijiazhuang, 050000, China*
[b] *Handan Stomatology Hospital, Handan, 056000, China*
[c] *Cangzhou People's Hospital, Cangzhou, 061000, China*
[d] *Gucheng County Hospital of Hebei Provence, 253800, China*
[e] *Department of Stomatology, The Fourth Hospital of Hebei Medical University, Shijiazhuang, 050000, China*

ARTICLE INFO

Keywords:
Gallium
Oral infection
Metal-organic frameworks
Antibacterial
Anti-Inflammatory

ABSTRACT

The fascial space of the oral and maxillofacial region contains loose connective tissues, which possess weak anti-infection ability and are often prone to infection, leading to acute suppurative inflammation and sepsis through blood. Although antibiotic use can reduce the probability of bacterial infections, owing to the emergence of antibiotic-resistant bacteria, the search for new antimicrobial drugs is imminent. Herein, we report a metal–organic framework (MOF) antibacterial material designed and synthesized with gallium (Ga) as the central atom, which possesses significant antibacterial, anti-inflammatory, and antioxidant effects. Our data suggested that GA-based MOFs (Ga–MOFs; 1 μg/mL) could sufficiently kill *Porphyromonas gingivalis*, *Streptococcus pyogenes*, and *Staphylococcus aureus*. Ga–MOFs exhibited a bactericidal effect against these three pathogens by disrupting biofilm formation, exopolysaccharide production, and bacterial membrane integrity. In addition, we found that 1 μg/mL of Ga–MOFs was not cytotoxic to human oral epithelial cell (HOEC) lines and it significantly reduced the adhesion of the three pathogens to HOEC. Ga–MOFs protect macrophages from excessive oxidative stress by scavenging excess intracellular reactive oxygen species and upregulating antioxidant gene levels, thereby enhancing cellular antioxidant defense. In addition, Ga–MOFs can promote the transformation of macrophages from the proinflammatory phenotype to the anti-inflammatory phenotype, thereby protecting oral health. Herein, novel Ga–MOF materials were chemically synthesized for therapeutic applications in oral infections, which provides new ideas for the development of novel nonantibiotic drugs to accelerate patient recovery.

1. Introduction

Oral and maxillofacial space infections are common and frequent infectious diseases in oral and maxillofacial surgery, with multiple sites of infection including the mouth, throat, face, and neck. Owing to multiple potential communication gaps in the oral and maxillofacial regions that can be connected, inflammation can easily spread to adjacent tissue gaps. In severe cases, it can cause multigap infections, leading to rapid disease progression. Multiple complications are often fatal [1]. Although the use of antibiotics can effectively reduce the probability of bacterial infection, with the and irrational drug use in recent years, the treatment of oral and

* Corresponding author.
E-mail address: 48901970@hebmu.edu.cn (J. Guo).

https://doi.org/10.1016/j.heliyon.2024.e31788
Received 22 February 2024; Received in revised form 21 May 2024; Accepted 22 May 2024
Available online 22 May 2024
2405-8440/© 2024 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC license (http://creativecommons.org/licenses/by-nc/4.0/).

maxillofacial space infections is becoming increasingly difficult [2]. Many traditional treatment methods have been proven to be unsuccessful. To reduce the risk of oral infections, we should seek new solutions, including new antibiotics and the development of alternative treatment strategies. Furthermore, we need to deepen our understanding regarding the complex interactions between host and microbial factors.

In recent years, the antimicrobial activity of novel nanomaterials such as carbon nanotubes, graphene, hydrogels, and metal––organic framework (MOF) has attracted extensive attention from researchers [3–6]. Among them, MOF has received particular attention, mainly owing to its good biodegradability. MOF materials have antimicrobial activities, and *in vivo*, they effectively inhibit the toxicity of high-concentration metal ions by gradually slowing down their release. Meanwhile, the selection of suitable ligands can make MOF released under specific conditions, achieving targeted delivery effects [7]. Therefore, it is of great significance to synthesize antimicrobial MOF materials and develop new nanomedicines for treating oral interstitial infections.

Metal antibacterial agents (such as copper, zinc, and silver) may cause metal toxicity and accumulate in the vital organs of the human body, threatening health. Gallium (Ga)-based antimicrobials can effectively treat infections caused by *Pseudomonas aeruginosa*, and their primary mode of action involves interference with bacterial iron metabolism [8,9]. This demonstrates the strong potential of Ga to address the crisis of antibiotic resistance. In addition, in a recent report involving a preliminary Phase I clinical trial, Ga nitrate exhibited a therapeutic effect on chronic *P. aeruginosa* lung infection and improved lung function in patients with cystic fibrosis, indicating that Ga is a safe and effective treatment for infectious diseases [10]. However, owing to the low delivery efficiency of Ga compounds to infected tissues, *in vivo* targeted therapy is not effective. In addition, current anti-infective therapies based on MOF materials tend to focus on the clearance of bacteria while ignoring the excessive inflammatory response caused by bacteria, which leads to worsened tissue damage. Therefore, the development of MOF antibacterial materials with strong antibacterial action and inflammation regulation ability is of great significance for healing tissue damage caused by bacterial infection.

Herein, Ga was used as the central atom to design and synthesize MOF materials and the related characterization was conducted. Transmission electron microscopy (TEM), scanning electron microscopy (SEM), X-ray powder diffraction (XRD), and X-ray single crystal diffraction were applied to analyze the morphology and structure of GA-based MOFs (Ga–MOFs). Bacteria that are common pathogens of oral infections, including *Porphyromonas gingivalis*, *Streptococcus mutans*, and *Staphylococcus aureus*, were utilized for the *in vitro* evaluation of the antimicrobial efficacy of Ga–MOF materials. The effect of Ga–MOFs on the proliferation and cell activity of oral cells cultured *in vitro* was examined via cell viability assay. We further investigated whether Ga–MOFs could exhibit antioxidant and anti-inflammatory effects after the infection of macrophages by *P. gingivalis*, *S. mutans*, and *S. aureus*.

As an anti-infection material, Ga–MOFs gradually release Ga^{3+} through biodegradation, effectively avoiding the toxicity of high-concentration metal ions. At the same time, Ga–MOFs exhibit good antioxidant and anti-inflammatory effects, which is of great significance for recovering tissue damage caused by infection. Herein, we utilized chemical methods to synthesize novel Ga–MOF materials and screened the ones that were the most effective. To the best of our knowledge, this is the first study on MOF materials using Ga ion as the central atom to address oral infection, providing a new idea for developing novel antibacterial drugs and speeding up the rehabilitation of patients.

2. Materials and methods

2.1. Synthesis of Ga–MOFs

Ga nitrate hydrate (50 mg) and 1,3,5-benzenetricarboxylic acid (20 mg) were added to a mixed solvent of 5 mL N, *N*-dimethylformamide and trifluoroacetic acid (100 μL) in a 20-mL scintillation vial. The bottle was placed in an oven preset at 120 °C for 3 days for complete reaction. The white powder was collected via centrifugation and dried for use before washing several times with water and ethanol. In order to enhance the applicability and stability of Ga MOF materials in biological research, we dissolved them in PBS pH = 7.4 buffer for use. Ready to use and ready to use (use now and match now).

2.2. Bacterial strains and cell lines

P. gingivalis (ATCC 33277), *S. mutans* (UA159), and *S. aureus* (ATCC 6538) strains were maintained in our laboratory. *P. gingivalis* strains were cultivated in BHI broth with 0.0005 % hemin and 0.0001 % menadione. *S. aureus* strains were cultivated in Luria–Bertani broth. *S. mutans* strains were cultured in Todd Hewitt broth. All the strains were incubated at 37 °C and 5 % CO_2. The human oral epithelial cells (HOECs, BNCC340217, purchased from BeNa Culture Collection, China) and THP-1 cells (ATCC, TIB-202, preserved in our laboratory) were cultured in DMEM (Gibco, USA) with 10 % FBS (Gibco, USA). The humidity and atmosphere were maintained at 37 °C and 5 % CO_2, respectively.

2.3. Antibacterial capacity

To evaluate the antimicrobial capacity of Ga–MOF, a coculture of Ga–MOFs with bacteria was performed. Briefly, each of the three bacteria was activated, centrifuged, and diluted with PBS buffer. Next, the bacteria were inoculated into a culture medium containing different concentrations of Ga–MOFs and cultured separately (37 °C, shaking). Finally, 100 mL of bacterial culture was diluted on agar plates and incubated at 37 °C for 24 h to measure the number of colonies on the plates.

2.4. Biofilm assay

The culture medium (100 μl) was added to a 96-well plate, and 10 μl of the overnight bacterial culture medium was inoculated and incubated at 37 °C for 36 h. Then, the culture solution was sucked out, 200 μl sterile PBS buffer was added to each hole, and the plate hole was washed thrice. Methanol (100 μl) was added to each well and then aspirated after 15 min and dried naturally. Crystal violet solution (1 %, 100 μL) was added to each well and stained for 5 min at room temperature. After complete drying, 100 μL of 33 % glacial acetic acid solution was added to each hole and acted in a thermostat at 37 °C for 30 min to dissolve the crystal violet. The OD values of the solutions in the culture wells were measured using an enzyme-labeled instrument.

2.5. Exopolysaccharide measurement

Bacterial culture (50 mL, with or without Ga–MOF incubation) was obtained and centrifuged (2000×*g* for 15 min). The supernatant was subsequently filtered through a 0.22-μm filter. Precooled 100 % ethanol was added and stored at 2 °C overnight. The precipitate was collected, centrifuged at 10000×*g* for 20 min, and the ethanol was repeated three times. The resulting precipitates were dissolved in H_2O_2 and dialyzed using a dialysis tube (2000–3500 Da) at 2 °C for 48 h. The obtained dialysate was an exopolysaccharide (EPS) extract, which was quantified using a colorimetric phenol–sulfuric acid method.

2.6. Lactate dehydrogenase release

Lactate dehydrogenase (LDH) release was measured using a cytotoxicity measurement kit (Solarbio, China). Briefly, each of the three bacteria was activated and then cultured in Ga–MOFs or DMSO. After 4 h of growth, the three bacterial supernatants were separated via centrifugation. The obtained supernatant was determined according to the instructions of the LDH reagent kit. For cell measurements, the cells were pretreated with Ga–MOFs for 3 h. Then, the cell culture supernatants were obtained via centrifugation to detect LDH activity.

2.7. Cell viability

HOECs (Source: BLUEFBIO, Catalog Number: BFN607212669) were inoculated in 96-well plates with 5×10^3 cells per well. After 24 h, the cells were treated with different concentrations of Ga–MOFs. Cell viability was measured using the CCK-8 kit (Solarbio, China), and the results were standardized with those of the control group.

2.8. Evaluation of reactive oxygen Species–Scavenging activities of Ga–MOFs

The antioxidant capacity of Ga–MOFs was determined using the reactive oxygen species (ROS) kit (Solarbio, China). According to the standard method, Ga–MOFs were added into the culture medium of the experimental group, which was incubated at 37 °C in a 5 % CO_2 cell culture incubator. The supernatant was removed via centrifugation. Then, a 2,7-dichlorodihydrofluorescein diacetate (DCFH-DA) probe at a concentration of 10 μM was added to cover the cells. The cells were washed thrice using serum-free cell medium to fully remove the DCFH-DA that did not enter the cells. Finally, the cells were collected and examined using a fluorescence spectrophotometer.

2.9. Q-PCR

The RNA was reverse-transcribed using the PrimeScriptTM RT kit (Takara Bio, Japan) following the manufacturer's instructions. Target RNA was amplified and quantified using a 2x Super SYBR Green q-PCR Master mix (Takara Bio, Japan). Finally, the changes in gene expression were quantified using the $2^{-\Delta\Delta CT}$ method.

2.10. Cytokine analysis

The amounts of IL-1β, TNF-α, IL-10, and TGF-β released into the culture medium after treatment with Ga–MOFs were analyzed using an enzyme-linked immunosorbent assay kit (Takara Bio, Japan) following the manufacturer's instructions.

2.11. Statistical analysis

The results of the study were analyzed using GraphPad Prism 9.0. Comparisons between the two groups were analyzed using Student's t-test test. All quantitative data were expressed as mean ± SD, and each experiment was repeated at least thrice. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Preparation and characterization of Ga–MOFs

The Ga–MOF morphology was investigated using SEM, which revealed a size of ~400 nm with a spherical shape and a spike-like outer surface (Fig. 1a). The same was also reflected in the TEM image (Fig. 1b). The XRD spectrum (Fig. 1c) evidenced the successful preparation of Ga–MOFs as a pristine type of MIL-96. The slight shift of the first sharp peak proved the deformation of MIL-96, as previously reported. Fig. 1d shows the FT-IR analysis of Ga–MOFs, and the strong absorption peaks at 1350 and 1593 cm^{-1} suggested the Vsym and Vasym vibrations of the C–O group, respectively, revealing the introduction of trimesic acid into Ga–MOFs. The carboxylic groups of Ga–MOFs allowed immeasurable potentials in biodelivery media areas, such as drug delivery. The composition of Ga–MOFs was further confirmed via energy-dispersive X-ray elemental mapping, and the uniform element distribution of Ga, C, and O also indicated the successful construction of Ga–MOFs (Fig. 1e–h).

3.2. Antibacterial activity of Ga–MOFs against P. gingivalis, S. mutans, and S. aureus

The antimicrobial effect of Ga–MOFs on *P. gingivalis*, *S. mutans*, and *S. aureus* was closely related to its concentration. At concentrations of 0–0.25 μg/mL, the viability of *P. gingivalis*, *S. mutans*, and *S. aureus* was hardly affected (Fig. 2a–c). When the concentration was increased to 0.5 μg/mL, the viability of *P. gingivalis*, *S. mutans*, and *S. aureus* were affected to varying degrees (Fig. 2a–c). Notably, the survival rates of *P. gingivalis*, *S. mutans*, and *S. aureus* were significantly reduced when Ga–MOF concentrations exceeded 1 μg/mL, killing >90 % of bacteria (Fig. 2a–c). In addition, the effectiveness of the Ga–MOF sterilization was time-dependent. Therefore, at a concentration of 1 μg/mL, Ga–MOFs almost completely killed *P. gingivalis*, *S. mutans*, and *S. aureus* after >10 h (Fig. 2d–f). These results show that Ga–MOFs exhibited excellent antimicrobial activity against P. *gingivalis*, *S. mutans*, and *S. aureus*.

3.3. Influence of Ga–MOFs on the toxicity factor of P. gingivalis, S. mutans, and S. aureus

For the bactericidal mechanism of Ga–MOF, we investigated its effects on the virulence factors of *P. gingivalis*, *S. mutans*, and *S. aureus*, including biofilm formation, EPS production, and bacterial membrane integrity. Ga–MOFs significantly reduced the ability of *P. gingivalis*, *S. mutans*, and *S. aureus* to form biofilms (Fig. 3a). EPS measurements revealed that *P. gingivalis* (~58 %), *S. mutans* (~65 %), and *S. aureus* (~70 %) exhibited reduced EPS when Ga–MOFs were used (Fig. 3b). In addition, we performed L-LDH membrane leakage assays to examine the effect of Ga–MOF on the membrane integrity of *P. gingivalis*, *S. mutans*, and *S. aureus*. Compared with the DMSO group, Ga–MOFs exhibited stronger disruptions to *P. gingivalis*, *S. mutans*, and *S. aureus* bacterial membranes and increased LDH release (Fig. 3c). These results further suggest that Ga–MOFs demonstrate good antimicrobial activity.

3.4. Evaluation of the toxicity of Ga–MOF to the HOEC line

To further evaluate the cellular toxicity of Ga–MOFs, we used different concentrations of Ga–MOFs to stimulate HOEC cells. The

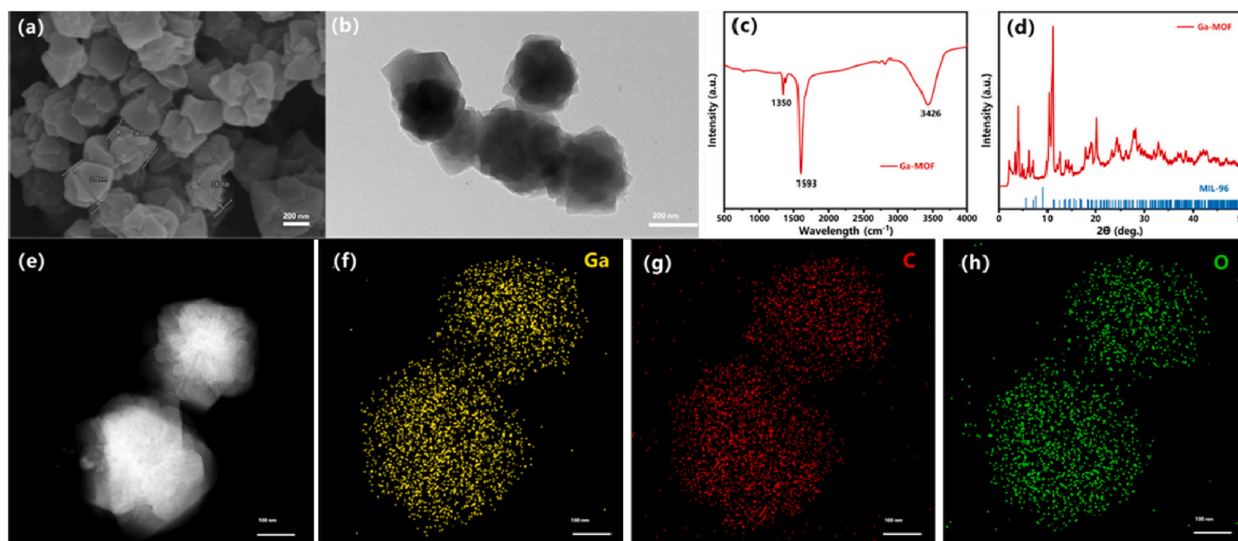


Fig. 1. Characterization of a nanoscale Ga–MOF. (a) Typical SEM and (b) TEM images of Ga–MOFs. (c) XRD spectrum of Ga–MOFs. (d) FT-IR analysis of Ga–MOFs. (e)–(h) High-angle annular dark-field scanning transmission electron microscopy (HAADF–STEM) and energy-dispersive X-ray spectroscopy (EDS) elemental mapping of Ga–MOF.

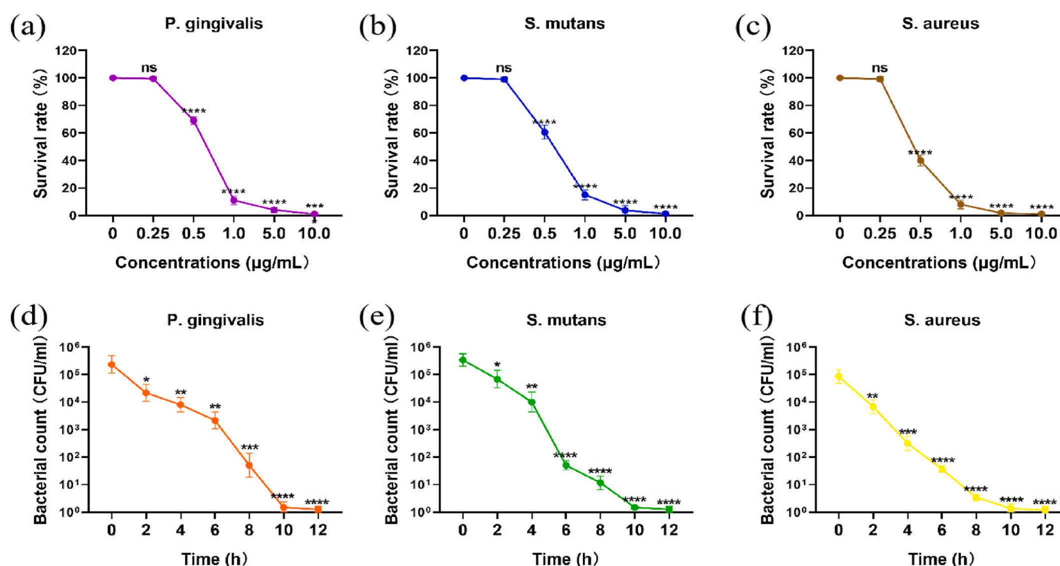


Fig. 2. Ga–MOFs showing excellent antibacterial activity. (a) Survival of *P. gingivalis* was examined via coincubation with different concentrations of Ga–MOFs. (b) Survival of *S. mutans* was examined via coincubation with different concentrations of Ga–MOFs. (c) Survival of *S. aureus* was examined via coincubation with different concentrations of Ga–MOFs. (d) Ability of Ga–MOFs to kill *P. gingivalis* at different incubation times. (e) Ability of Ga–MOF to kill *S. mutans* at different incubation times. (f) Ability of Ga–MOF to kill *S. aureus* at different incubation times. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$, ns: no statistical significance.

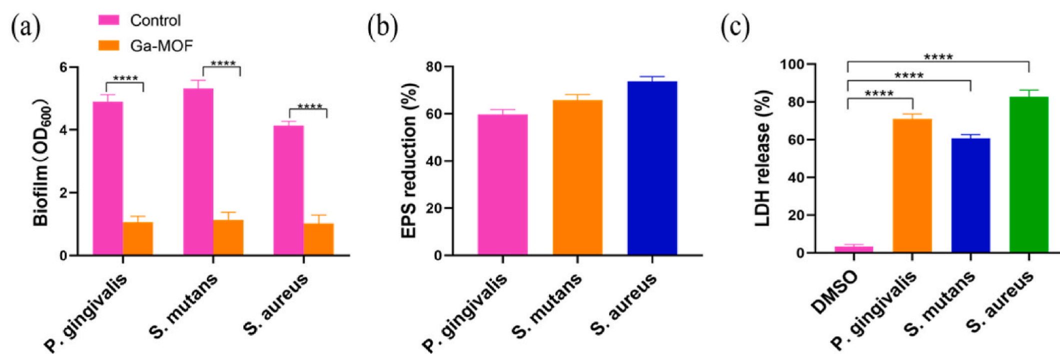


Fig. 3. Influence of Ga–MOFs on bacterial toxicity factors. (a) Influence of Ga–MOFs on the biofilms of *P. gingivalis*, *S. mutans*, and *S. aureus*. (b) Influence of Ga–MOF on the EPS generation of *P. gingivalis*, *S. mutans*, and *S. aureus*. (c) Ga–MOF effect on *P. gingivalis*, *S. mutans*, and *S. aureus* membrane integrity. ****$p < 0.0001$.

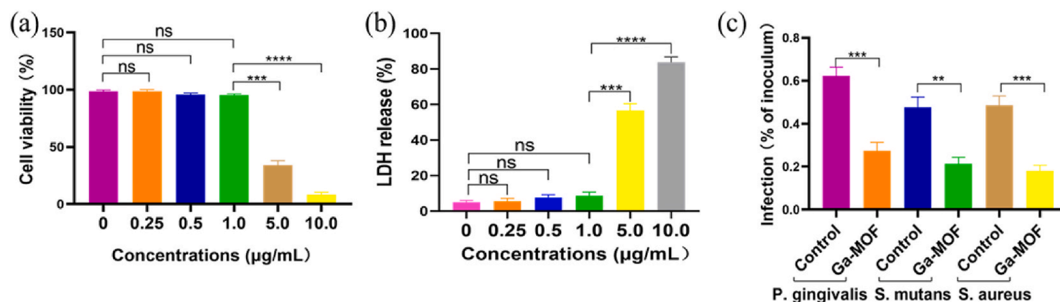


Fig. 4. Evaluation of the cytotoxicity of Ga–MOFs. (a) HOEC cell viability with different Ga–MOF concentrations. (b) Effect of different concentrations of Ga–MOF on LDH release from HOEC cells. (c) Effect of Ga–MOFs on the adhesion of HOEC cells pretreated with *P. gingivalis*, *S. mutans*, and *S. aureus*. **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$, ns: no statistical significance.

results revealed that Ga–MOFs were not toxic to HOEC cells at a concentration range of 0–1.0 μg/mL (Fig. 4a). However, they was significantly cytotoxic to HOEC cells when the concentration exceeded 5.0 μg/mL (Fig. 4a). Similarly, LDH experiments also suggested that Ga–MOFs hardly affected LDH release from HOEC cells when their concentration did not exceed 1.0 μg/mL (Fig. 4b). In addition, we found that Ga–MOFs significantly reduced the adhesion ability of the three pathogens to HOEC cells during *P. gingivalis*, *S. mutans*, and *S. aureus* infections (Fig. 4c). These results suggest that Ga–MOFs exhibit little toxicity toward cells within a certain range while still exhibiting bactericidal activity.

3.5. Antioxidant Effect of Ga–MOFs in vitro

Using the DCFH-DA method, we found that Ga–MOFs significantly reduced the ROS content of THP-1 cells, which was induced by *P. gingivalis*, *S. mutans*, and *S. aureus*, compared with that in the control group (Fig. 5a). To explore the potential molecular mechanisms of the antioxidant activity of Ga–MOFs, we examined the gene levels of antioxidation-related enzymes, including superoxide dismutase-1 (SOD-1) and heme oxygenase-1 (HO-1). q-PCR results revealed that Ga–MOFs significantly promoted the upregulation of SOD-1 and HO-1 in THP-1 cells (Fig. 5b and c). These results suggest that Ga–MOFs protect macrophages from excessive oxidative stress by scavenging excess ROS within THP-1 cells and upregulating the levels of antioxidant genes, thereby enhancing macrophage antioxidant defense.

3.6. Effect of Ga–MOF on inflammation induced by P. gingivalis, S. mutans, and S. aureus

Using THP-1 cells as the infection model, we found that *P. gingivalis*, *S. mutans*, and *S. aureus* induced inflammation. Specifically, the levels of the classical inflammatory cytokines IL-1β, IL-6, and TNF-α were increased and those of IL-10, TGF-β, and Arg-1 were decreased (Fig. 6a–f). However, after infection with *P. gingivalis*, *S. mutans*, and *S. aureus*, Ga–MOFs significantly decreased mRNA levels of IL-1β, IL-6, and TNF-α and increased the mRNA levels of IL-10, TGF-β, and Arg-1 (Fig. 6a–f). In addition, the extracellular levels of IL-1β, TNF-α, IL-10, and TGF-β were measured, and as expected, Ga–MOFs decreased the levels of proinflammatory factors IL-1β and TNF-α (Fig. 7a and b) and increased the levels of anti-inflammatory factors IL-10 and TGF-β (Fig. 7c and d). These results suggest that Ga–MOFs promoted the transformation of macrophages from the proinflammatory phenotype (M1) to the anti-inflammatory phenotype (M2) to protect oral health.

4. Discussion

Oral and maxillofacial infections are common diseases in the field of dentistry, manifested by common symptoms such as redness, swelling, heat, pain, and dysfunction. However, owing to the anatomical and physiological characteristics of the oral and maxillofacial region, the occurrence, development, and prognosis of these infections have their peculiarities. The maxillofacial region has a rich blood supply, which transports the infection to the bloodstream, leading to sepsis or septicemia. Although the use of antibiotics is the traditional approach to fighting these infections, this practice presents potential drawbacks, including low concentrations of target drugs, the need for hospitalization for monitoring, and antibiotic resistance. In recent years, the unreasonable use of antibiotics in clinics has led to the emergence of drug-resistant strains, which will increase the difficulty of clinical treatment. Antibiotic resistance is one of the major challenges facing humanity in recent years [11,12]. Therefore, the search for novel antimicrobial drugs (not only antibiotics) is of considerable importance and has become a worldwide challenge.

Ga is well known in the medical field for its anticancer activity. The mechanism of its therapeutic action is inactivating the enzyme ribonucleotide reductase (which is beneficial for the rapid proliferation of cancer cells) by substituting the homologous iron ions, leading to cell apoptosis through the mitochondrial pathway. As the amount of Ga taken up by cancer cells is greater than that by normal cells, normal cells are not negatively affected, but the viability of cancer cells is reduced [13,14]. In addition to its anticancer activity, Ga exhibits bacteriostatic and bactericidal effects on different opportunistic bacteria (e.g., *P. aeruginosa*, *Mycobacterium tuberculosis*, and *Klebsiella pneumoniae*). Ga is a key element in bacterial metabolism and signaling function as it participates in major

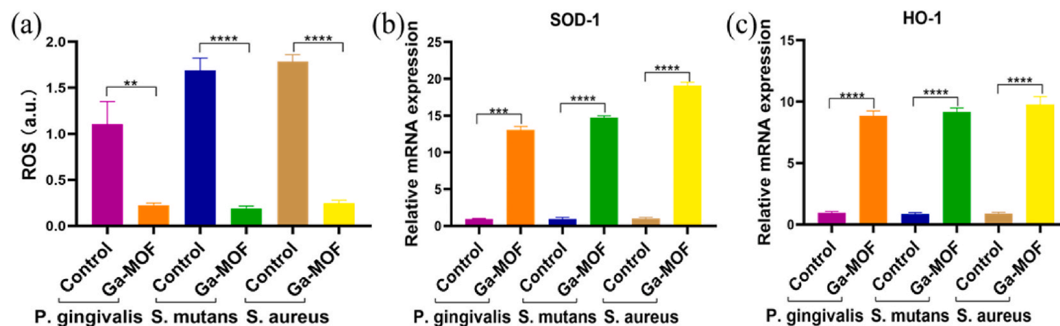


Fig. 5. Evaluation of the antioxidant activity of Ga–MOFs. (a) Effect of Ga–MOFs on ROS induced by *P. gingivalis*, *S. mutans*, and *S. aureus*. SOD-1 (b) and HO-1(c) mRNA expression levels in infected THP-1 cells in the presence of Ga–MOF. $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

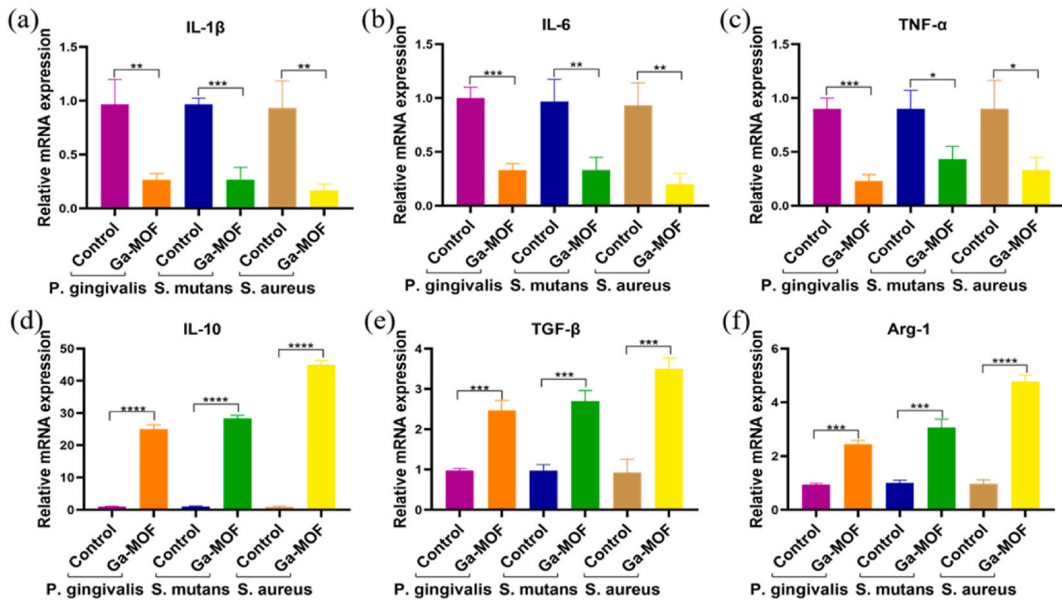


Fig. 6. Evaluation of the anti-inflammatory properties of Ga–MOFs. (a) IL-1β, (b) IL-6, (c) TNF-α, (d) IL-10, (e) TGF-β, and (f) Arg-1 mRNA expression levels in infected THP-1 cells in the presence of Ga–MOFs. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$.

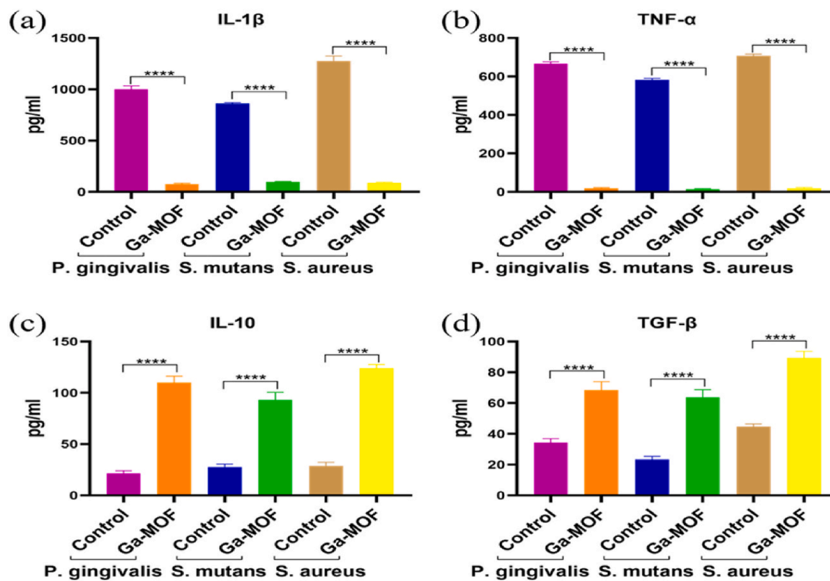


Fig. 7. Evaluation of the anti-inflammatory properties of Ga–MOF. (a) IL-1β, (b) TNF-α, (c) IL-10, and (d) TGF-β protein expression levels in infected THP-1 cells in the presence of Ga–MOFs. $***P < 0.001$, $****P < 0.0001$.

biological processes, including cellular respiration, DNA synthesis, and other mechanisms [15]. During an infection, bacteria face an iron shortage because the host reduces the availability of iron to prevent the bacteria from multiplying. As a result, bacteria have developed high-affinity mechanisms for iron uptake. Microbes cannot easily distinguish between the two ions, which allows Ga to be taken up by cells that need iron. Ga bypasses bacterial defenses and enters the cell as an iron carrier, thus providing the host with redox inactive metal cofactors instead of the natural iron ion, subsequently impairing bacterial biochemical processes [16,17]. Ga-based antimicrobial therapies are still in their infancy despite some recently published insights into the bacteriostatic and bactericidal properties of Ga. Based on this, Ga may be a potential means of controlling persistent infections with untreatable pan-resistant bacteria.

The addition of metal ions to bioactive materials has been a subject of interest over the past decades. MOFs are a class of compounds formed by the coordination of metal ions or clusters with organic ligands. MOFs have a porous structure capable of encapsulating various drugs and therapeutic agents, and these active ingredients can be immobilized within the cavities of MOFs through covalent or

noncovalent interactions. MOFs are considered potential candidates for overcoming bacterial resistance as drug-delivery vehicles for antimicrobial therapy and can be tailored for specific clinical applications, such as oral infections [18,19].

Herein, we demonstrated that Ga–MOFs exhibited excellent antibacterial activity against *P. gingivalis*, *S. mutans*, and *S. aureus*. The antibacterial effect of Ga–MOF on *P. gingivalis*, *S. mutans*, and *S. aureus* was closely related to its concentration and increased with increasing Ga–MOF content. At Ga–MOF concentrations of 0–0.25 μg/mL, the activity of *P. gingivalis*, *S. mutans*, and *S. aureus* was almost unaffected. When Ga–MOF concentration exceeded 1 μg/mL, the survival rate of *P. gingivalis*, *S. mutans*, and *S. aureus* significantly reduced and >90 % of the bacteria were killed. Most pathogenic bacterial infections are associated with bacterial biofilm formation. Biofilms have high drug resistance, antiphagocytosis abilities, and strong adhesion, which can lead to a wide range of bacterial infectious diseases and are one of the most important sources of bacterial infections [20,21]. Biofilms are difficult to remove completely, resulting in repeated infections, treatment failure, and even death. Therefore, it is essential to develop alternative and effective strategies for eradicating bacterial biofilms. Herein, Ga–MOFs significantly reduced the ability of *P. gingivalis*, *S. mutans*, and *S. aureus* to form biofilms. EPS is more resistant to environmental stress and is a key molecule involved in biofilm formation, which can increase resistance to antibiotics and host immune responses [22]. EPS measurements revealed that when Ga–MOFs were used, EPS was reduced by ~58 % for *P. gingivalis*, ~65 % for *S. mutans*, and ~70 % for *S. aureus*. In addition, the LDH experiment indicated that Ga–MOFs exhibited strong destructive effects on the bacterial membranes of *P. gingivalis*, *S. mutans*, and *S. aureus*, resulting in increased LDH release. Nevertheless, Ga–MOF demonstrates excellent bactericidal ability at doses not exceeding 1.0 μg/mL without affecting cell activity.

Oxidative stress refers to the failure of the cellular endogenous antioxidant system to effectively clear a large number of free radicals accumulated in the body, leading to an imbalance in the oxidative and antioxidant systems in the body and participating in cell apoptosis and inflammatory or cell function changes by activating a series of signal pathways [23,24]. Bacterial infection can cause oxidative stress injury in the oral and maxillofacial regions. The mechanism of excessive ROS on oral tissue cell damage includes accelerating cell senescence, apoptosis, amplifying inflammatory response, and tissue destruction [25]. Therefore, clearing ROS would be an effective route for anti-inflammatory therapy to block the worsening of inflammation and protect oral tissue from oxidative damage. We evaluated the antioxidant effect of Ga–MOFs on THP-1 cells using the DCFH-DA assay. Ga–MOFs significantly decreased the ROS levels induced by *P. gingivalis*, *S. mutans*, and *S. aureus*. In addition, Ga–MOFs significantly promoted the upregulation of antioxidant genes *SOD-1* and *HO-1* in THP-1 cells. These results suggest that Ga–MOFs protect macrophages from excessive oxidative stress by clearing excess ROS in THP-1 cells and upregulating antioxidant gene levels, thereby enhancing the antioxidant defense capacity of macrophages. Inflammatory cytokines significantly affect the polarization of macrophages [26]. To examine the effect of Ga–MOFs on the production of inflammatory cytokines in activated macrophages, we examined the gene and protein levels of classical proinflammatory markers (IL-1β, IL-6, and TNF-α) and anti-inflammatory markers (IL-10, TGF-β, and Arg-1). q-PCR results revealed that compared with the control group, Ga–MOFs significantly inhibited the gene expression levels of IL-1β, IL-6, and TNF-α and promoted the gene expression levels of IL-10, TGF-β, and Arg-1. The same results were confirmed at the protein level. Thus, Ga–MOFs exhibits excellent antioxidant and anti-inflammatory potential.

In conclusion, we may develop an effective antimicrobial strategy to treat oral bacterial infections using nonantibiotic Ga–MOFs. Their excellent antibacterial effect is attributed to the ability of Ga–MOFs to inhibit biofilm formation (*P. gingivalis*~ 4.5 times, *S. mutans*~ 4.7 times and *S. aureus*~ 4.0 times), EPS production (*P. gingivalis*~ 58 %, *S. mutans*~ 65 % and *S. aureus*~ 70 %), and bacterial membrane integrity (*P. gingivalis*~ 21.3 times, *S. mutans*~ 18.2 times and *S. aureus*~ 24.8 times). Further studies have shown that Ga–MOF can remove excess ROS content in cells (*P. gingivalis*~ 5.0 times, *S. mutans*~ 8.9 times and *S. aureus*~ 7.2 times) and promote the transformation of macrophages from M1 to M2, thus protecting oral health. Therefore, the study of Ga–MOFs in oral infection provides a new way to develop novel antibacterial drugs and accelerate patient recovery.

In future research, animal models can be considered to evaluate whether Ga–MOF causes organ toxicity *in vivo* and obtain more information regarding the antibacterial effects of Ga–MOFs *in vivo*. In addition, future studies exploring whether combination with antibiotics can exert a synergistic effect will increase the possibility of the clinical application of Ga–MOFs. These research results can further enhance the potential of Ga–MOFs as an antimicrobial agent.

Ethics approval and consent to participate

Our study was approved by the Ethics Committees of The Fourth Hospital of Hebei Medical University (Shijiazhuang, China) (approval number: 2021KY197).

Funding

This study was funded by the Hebei Provincial Health Commission Youth Science and Technology project (Grant NO.20221329).

Data availability

The data used and analyzed in the current study are available from the corresponding author upon a reasonable request.

CRediT authorship contribution statement

Gongyuan Song: Writing – original draft, Data curation, Conceptualization. **Min Li:** Validation, Methodology. **Bing Zhou:**

Methodology, Conceptualization. **Hongguang Qi:** Methodology. **Jie Guo:** Writing – review & editing, Supervision, Project administration, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] S.R. Caruso, E. Yamaguchi, J.E. Portnof, Update on antimicrobial therapy in management of acute odontogenic infection in oral and maxillofacial surgery, Oral Maxillofac. Surg. Clin. 34 (1) (2022) 169–177.
[2] S. Bigus, G. Russmüller, P. Starzengruber, H. Reitter, C.L. Sacher, Antibiotic resistance of the bacterial spectrum of deep space head and neck infections in oral and maxillofacial surgery - a retrospective study, Clin Oral Investig 27 (8) (2023) 4687–4693.
[3] Y. Liu, H. Liu, S. Guo, Y. Zhao, J. Qi, R. Zhang, J. Ren, H. Cheng, M. Zong, X. Wu, et al., A review of carbon nanomaterials/bacterial cellulose composites for nanomedicine applications, Carbohydr. Polym. 323 (2024) 121445.
[4] K. Shankar, S. Agarwal, S. Mishra, P. Bhatnagar, S. Siddiqui, I. Abrar, A review on antimicrobial mechanism and applications of graphene-based materials, Biomater. Adv. 150 (2023) 213440.
[5] G. Li, Z. Lai, A. Shan, Advances of antimicrobial peptide-based biomaterials for the treatment of bacterial infections, Adv. Sci. 10 (11) (2023) e2206602.
[6] D. Han, X. Liu, S. Wu, Metal organic framework-based antibacterial agents and their underlying mechanisms, Chem. Soc. Rev. 51 (16) (2022) 7138–7169.
[7] L. Guo, W. Kong, Y. Che, C. Liu, S. Zhang, H. Liu, Y. Tang, X. Yang, J. Zhang, C. Xu, Research progress on antibacterial applications of metal-organic frameworks and their biomacromolecule composites, Int. J. Biol. Macromol. 261 (Pt 2) (2024) 129799.
[8] Z. Wang, J. Li, B.M. Benin, B. Yu, S.D. Bunge, N. Abeydeera, S.D. Huang, M.H. Kim, Lipophilic Ga complex with broad-spectrum antimicrobial activity and the ability to overcome gallium resistance in both Pseudomonas aeruginosa and Staphylococcus aureus, J. Med. Chem. 64 (13) (2021) 9381–9388.
[9] S.P. Valappil, E.A. Abou Neel, K.M. Zakir Hossain, W. Paul, D. Cherukaraveedu, B. Wade, T.I. Ansari, C.K. Hope, S.M. Higham, C.P. Sharma, Novel lactoferrin-conjugated gallium complex to treat Pseudomonas aeruginosa wound infection, Int. J. Biol. Macromol. 258 (Pt 1) (2023) 128838.
[10] C.H. Goss, Y. Kaneko, L. Khuu, G.D. Anderson, S. Ravishankar, M.L. Aitken, N. Lechtzin, G. Zhou, D.M. Czyz, K. McLean, et al., Gallium disrupts bacterial iron metabolism and has therapeutic effects in mice and humans with lung infections, Sci. Transl. Med. 10 (460) (2018) eaat7520.
[11] S. Walesch, J. Birkelbach, G. Jézéquel, F.P.J. Haeckl, J.D. Hegemann, T. Hesterkamp, A.K.H. Hirsch, P. Hammann, R. Müller, Fighting antibiotic resistance-strategies and (pre)clinical developments to find new antibacterials, EMBO Rep. 24 (1) (2023) e56033.
[12] E.M. Darby, E. Trampari, P. Siasat, M.S. Gaya, I. Alav, M.A. Webber, J.M.A. Blair, Molecular mechanisms of antibiotic resistance revisited, Nat. Rev. Microbiol. 21 (5) (2023) 280–295.
[13] W. Lee, C.E. Lee, H.J. Kim, K. Kim, Current progress in gallium-based liquid metals for combinatory phototherapeutic anticancer applications, Colloids Surf. B Biointerfaces 226 (2023) 113294.
[14] X.X. Peng, H. Zhang, R. Zhang, Z.H. Li, Z.S. Yang, J. Zhang, S. Gao, J.L. Zhang, Gallium triggers ferroptosis through a synergistic mechanism, Angew Chem Int Ed Engl. 62 (36) (2023) e202307838.
[15] N. Kircheva, T. Dudev, Competition between abiogenic and biogenic metal cations in biological systems: mechanisms of gallium's anticancer and antibacterial effect, J. Inorg. Biochem. 214 (2021) 111309.
[16] V. Vinuesa, M.J. McConnell, Recent advances in iron chelation and gallium-based therapies for antibiotic resistant bacterial infections, Int. J. Mol. Sci. 22 (6) (2021) 2876.
[17] G. Centola, F. Xue, A. Wilks, Metallotherapeutics development in the age of iron-clad bacteria, Metallomics 12 (12) (2020) 1863–1877.
[18] M. Shen, F. Forghani, X. Kong, D. Liu, X. Ye, S. Chen, T. Ding, Antibacterial applications of metal-organic frameworks and their composites, Compr. Rev. Food Sci. Food Saf. 19 (4) (2020) 1397–1419.
[19] R. Li, T. Chen, X. Pan, Metal-organic-framework-based materials for antimicrobial applications, ACS Nano 15 (3) (2021) 3808–3848.
[20] O. Ciofu, C. Moser, P.Ø. Jensen, N. Høiby, Tolerance and resistance of microbial biofilms, Nat. Rev. Microbiol. 20 (10) (2022) 621–635.
[21] B. Trubenová, D. Roizman, A. Moter, J. Rolff, R.R. Regoes, Population genetics, biofilm recalcitrance, and antibiotic resistance evolution, Trends Microbiol. 30 (9) (2022) 841–852.
[22] L.Z. Ma, D. Wang, Y. Liu, Z. Zhang, D.J. Wozniak, Regulation of biofilm exopolysaccharide biosynthesis and degradation in Pseudomonas aeruginosa, Annu. Rev. Microbiol. 76 (2022) 413–433.
[23] A. Tikhomirova, M.M. Rahman, S.P. Kidd, R.L. Ferrero, A. Roujeinikova, Cysteine and resistance to oxidative stress: implications for virulence and antibiotic resistance, Trends Microbiol. 32 (1) (2024) 93–104.
[24] J.O. Aribisala, S. Sabiu, Redox impact on bacterial macromolecule: a promising avenue for discovery and development of novel antibacterials, Biomolecules 12 (11) (2022) 1545.
[25] J.M. Oh, H. Kim, The effect of oral bacterial infection on DNA damage response in host cells, Am. J. Cancer Res. 13 (7) (2023) 3157–3168.
[26] X. Sun, J. Gao, X. Meng, X. Lu, L. Zhang, R. Chen, Polarized macrophages in periodontitis: characteristics, function, and molecular signaling, Front. Immunol. 12 (2021) 763334.