ZEOLITIC IMIDAZOLATE FRAMEWORK COATED ZINC COMPOSITE AS ANTIMICROBIAL MATERIAL

CROSS-REFERENCE TO RELATED APPLICATION

This application claims the benefit of priority of Singapore Patent Application No. 10202250146M, filed on 13 June 2022, the content of which being hereby incorporated by reference in its entirety for all purposes.

TECHNICAL FIELD

The invention generally relates to a zeolitic imidazolate framework (ZIF) coated zinc composite and its use as antimicrobial material.

BACKGROUND

The application of metal organic framework (MOF) as antimicrobial materials has attracted much attention. Mechanistic models on how MOFs eliminate bacteria include: (1) the release of biocidal metal ions, ligands or clusters. The design of the MOF can be tuned to allow controlled release of antimicrobial agents to the targets; (2) MOFs can be utilized as carriers of antimicrobial agents or other drugs within their pores and can be modified for targeted delivery; and (3) MOFs of smaller dimension can kill bacteria due to the general toxicity of nanoparticles.

A zeolitic imidazolate framework (ZIF), a specific subclass of MOF, was recently reported to exert antibacterial activity by the generation of reactive oxygen species (ROS) via transfer of photoelectrons trapped at Zn^{+} centers within the ZIF, through ligand to metal charge transfer (LMCT). However, this framework requires energy input to excite the electrons to cause generation of reactive oxygen species (ROS).

It is therefore desirable to provide a composite, material and a method that seek to address at least one of the existing problems, or at least to provide an alternative.

SUMMARY

According to a first aspect of the invention, an antimicrobial composite comprising a zinc core with a zeolitic imidazolate framework (ZIF) shell is provided.

In some embodiments, the zeolitic imidazolate framework shell comprises imidazole or imidazole derivative linking moiety.

According to a second aspect of the invention, a ZIF-based antimicrobial material comprising a substrate and an antimicrobial composite comprising a zinc core with a zeolitic imidazolate framework (ZIF) shell is provided.

According to a third aspect of the invention, a method of preparing an antimicrobial composite comprising a zinc core with a zeolitic imidazolate framework (ZIF) shell is provided. The method comprises mixing zinc powder with an imidazole selected from the group consisting of 2H-imidazole (HIm), 2-methylimidazole (MeIm) and benzimidazole (BnIm) at a temperature ranging from 80°C to 160°C for a time duration in presence of a solvent to obtain an antimicrobial zeolitic imidazolate framework (ZIF) coated zinc composite.

BRIEF DESCRIPTION OF THE DRAWINGS

Various embodiments of the invention are described hereinbelow in the detailed description with reference to the following drawings:

Figure 1 shows the synthesis reaction of the zeolitic imidazolate framework (ZIF) coated zinc composite according to various embodiments of the invention.

Figure 2 illustrates SEM images of a Zn/ZIF(MeIm) core/shell composite according to one of the embodiments of the invention and corresponding SEM-EDX (Zn, O, N) analysis. The images clearly show the presence of zeolitic imidazolate framework (ZIF) layer coating on zinc.

Figure 3 illustrates a powder XRD spectrum of Zn/ZIF(MeIm) core/shell composite according to one of the embodiments of the invention. It contains both patterns from zinc (30-90^{o}) and ZIF(MeIm) (7-20^{o}).

Figure 4 shows a chart of antiviral efficacy of Zn/ZIF(MeIm) core/shell composite according to one of the embodiments of the invention against influenza A virus. (*) denotes full elimination of influenza A titer after specified timepoints.

Figure 5 shows an exemplary assembly as referred to in Example 3, of a ZIF-coated zinc composite (500) deposited on a glass slide (510), with a layer of microbes (520) and a plastic film placed over the microbes (530).

DETAILED DESCRIPTION

The following detailed description is made with reference to the accompanying drawings, showing details and embodiments of the present disclosure for the purposes of illustration. Features that are described in the context of an embodiment may correspondingly be applicable to the same or similar features in the other embodiments, even if not explicitly described in these other embodiments. Additions and/or combinations and/or alternatives as described for a feature in the context of an embodiment may correspondingly be applicable to the same or similar feature in the other embodiments.

In the context of various embodiments, the articles “a”, “an” and “the” as used with regard to a feature or element include a reference to one or more of the features or elements.

In the context of various embodiments, the term “about” or “approximately” as applied to a numeric value encompasses the exact value and a reasonable variance as generally understood in the relevant technical field, e.g., within 10% of the specified value.

As used herein, the term “and/or” includes any and all combinations of one or more of the associated listed items.

As used herein, “comprising” means including, but not limited to, whatever follows the word “comprising”. Thus, use of the term “comprising” indicates that the listed elements are required or mandatory, but that other elements are optional and may or may not be present.

As used herein, “consisting of” means including, and limited to, whatever follows the phrase “consisting of”. Thus, use of the phrase “consisting of” indicates that the listed elements are required or mandatory, and that no other elements may be present.

In a first aspect, an antimicrobial composite comprising a zinc core with a zeolitic imidazolate framework (ZIF) shell is provided.

The term “composite” as used herein refers to a substantially solid material that is composed of two or more discrete elements with different properties. Each of the elements retains its identity, while when put together, contributes unique and desirable properties to the composite.

A "zeolitic imidazolate framework" or "ZIF" refers to a zeolitic framework comprising a zeolitic structure having an imidazole, an imidazolate derivative, or an imidazolate linking group.

In various embodiments, the zeolitic imidazolate framework shell comprises an imidazole or an imidazole derivative linking moiety. The imidazole or imidazole derivative linking moiety is derived from one imidazole or imidazole derivative selected from the group consisting of 2H-imidazole (HIm), 2-methylimidazole (MeIm) and benzimidazole (BnIm).

In one embodiment, the antimicrobial composite is a Zn/ZIF (HIm) core/shell composite comprising a zinc core and a zeolitic imidazolate framework (ZIF) shell with an imidazole moiety derived from 2H-imidazole (HIm).

In another embodiment, the antimicrobial composite is a Zn/ZIF (MeIm) core/shell composite comprising a zinc core and a zeolitic imidazolate framework (ZIF) shell with an imidazole moiety derived from 2-methylimidazole (MeIm).

In yet another embodiment, the antimicrobial composite is a Zn/ZIF (BnIm) core/shell composite comprising a zinc core and a zeolitic imidazolate framework (ZIF) shell with an imidazole moiety derived from benzimidazole (BnIm).

The antimicrobial composite of the invention has an antimicrobial mechanism that involves a spontaneous reactive oxygen species (ROS) generation based on electron donation from the zinc core to the zeolitic imidazolate framework (ZIF) shell through self-corrosion of the zinc core. This mechanism is unique and different from other ZIF-based composites. For other ZIF-based composites, the generation of reactive oxygen species (ROS) occurs via transfer of photoelectrons trapped at the Zn^{+} centers within the ZIF, through ligand to metal charge transfer (LMCT). This mechanism uses light to excite the electron to induce ROS generation.

The antimicrobial composite of the invention is in powder form. The composite can be used as an additive in a formulation for use as a coating or for forming a thin-film powder coating. Any suitable type of ZIF-based antimicrobial material may be formed using the antimicrobial composite of the invention. The antimicrobial composite may be deposited or coated onto a surface of a substrate using suitable methods known in the art.

In a second aspect, a ZIF-based antimicrobial material comprising a substrate and an antimicrobial composite comprising a zinc core with a zeolitic imidazolate framework (ZIF) shell is provided. Examples of suitable substrates include, but are not limited to, substrate comprising material such as glass, plastic, metal, silicon, polymer, etc.

In a third aspect, a method of preparing an antimicrobial composite comprising a zinc core with a zeolitic imidazolate framework shell is provided. The method comprises mixing zinc powder with an imidazole selected from the group consisting of 2H-imidazole (HIm), 2-methylimidazole (MeIm) and benzimidazole (BnIm) at a temperature ranging from 80°C to 160°C for a time duration in presence of a solvent to obtain an antimicrobial zeolitic imidazolate framework (ZIF) coated zinc composite.

The zinc powder can be any suitable type of zinc powder obtained directly commercially. The powder should contain a high zinc content, such as at least 90% zinc by weight. The average particle size of the zinc powder is in the range from 10 nm to 1 mm. In various embodiments, the zinc powder is present in the antimicrobial composite in an amount of 10 to 99 wt % based on the total weight of the antimicrobial composite.

In one embodiment, the solvent is water or a mixture comprising water and N,N'-dimethylformamide (DMF). One skilled in the art will appreciate that other suitable type of solvent can be used without departing from the scope of the invention.

The mixing of the zinc powder with the imidazole can be carried out for any suitable time duration. In some embodiments, the mixing is carried out for 1 to 20 hours.

In various embodiments, the imidazole is present in an amount of from 0.01 to 20 wt% or less than 5 wt% based on the total weight of the antimicrobial composite.

The method may further include drying the zeolitic imidazolate framework (ZIF) coated zinc composite. Drying may be carried out for any suitable period of time, for example, from 6 to 24 hours or 8 to 12 hrs. The period of time will depend on factors such as for example, the moisture present in the ZIF-coated zinc composite and the temperature of drying. In certain embodiments, drying is conducted at a temperature from 55°C to 65°C or at 60°C. In an exemplary embodiment, the ZIF-coated zinc composite is dried in an oven. One skilled in the art will appreciate that other suitable type of drying method can be employed without departing from the scope of the invention.

The antimicrobial composite of the invention is relatively easy to synthesize as it is a one pot synthesis, and the method is easy to scale-up. The composite possesses high antimicrobial activity and long-lasting efficacy. The synthesized ZIF-coated zinc composite (Zn/ZIF) has an antimicrobial mechanism that involves spontaneous ROS generation through the self-corrosion of the zinc core. This phenomenon allows transfer of electrons from the zinc core to the zeolitic imidazolate framework (ZIF) shell, consequently reducing dissolved oxygen to ROS. The ZIF-coated zinc composite (Zn/ZIF) is able to release ROS spontaneously without energy input.

To facilitate a better understanding of the invention, the following examples of specific embodiments are given. In no way should the following examples be read to limit or define the entire scope of the invention. One skilled in the art will recognize that the examples set out hereinbelow are not an exhaustive list of the embodiments of this invention.

EXAMPLES

Example 1 - Preparation of ZIF-coated zinc composite

The composite comprising a zinc core with a zeolitic imidazolate framework (ZIF) shell (Zn/ZIF core/shell composite) was prepared by reacting stabilized zinc powder directly with an imidazole. In this example, three types of imidazole were used including 2H-imidazole (HIm), 2-Methylimidazole (MeIm) and Benzimidazole (BnIm). Commercially available stabilized zinc powder (purchased from Sigma Aldrich), protected with a layer of zinc oxide shell, was used as received.

The stabilized zinc powder was mixed with imidazole (2H-imidazole (HIm)), a base in an amount of 0.01 to 20 wt% in water. The mixture was subsequently heated at a temperature between 80°C and 160°C, for about 1 to 20 hours. ZIF-coated zinc composite (Zn/ZIF) was obtained after cooling to room temperature. The powders were then washed with ethanol to remove excess imidazole and allowed to dry at 60°C in the oven overnight. These steps were repeated using 2-Methylimidazole (MeIm), and Benzimidazole (BnIm).

As shown in the SEM and TEM images in Figure 2, a deposited layer of crystalline ZIF nanoparticles was clearly observed. The ZIF layer was thin, and the presence of the layer was confirmed using X-ray diffraction (XRD) technique. Figure 3 illustrates a powder XRD spectrum of Zn/ZIF(MeIm). It contains both patterns from zinc (30-90°) and ZIF(MeIm) (7-20°). Peaks observed in the range of 7-20° are the patterns of ZIF-8 (ZIF(MeIm)).

Example 2 - Release of Reactive Oxygen Species (ROS) as active antimicrobial species

The term "Reactive Oxygen Species (ROS)" refers to a collective of highly reactive oxygen species, with positive redox potential values. It is often used in photocatalytic disinfection processes involving TiO_2, MoS_2, etc., which requires energy input for the ROS generation.

The four main species of ROS are superoxide radicals ($\cdot O_2^-$), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$) and hydroxyl radicals ($\cdot OH$).

The concentration of superoxide radicals ($\cdot O_2^-$) was determined by adding 10 mg of ZIF-coated zinc particles (Zn/ZIF) into a solution of 100 uM XTT (2,3-Bis-(2-Methoxy-4-Nitro-5-Sulfophenyl)-2H-Tetrazolium-5-Carboxanilide) as probe and measuring the absorbance at 470 nm. The absolute concentration of the superoxide released by the particles was calculated by comparing the measured absorbance against a calibration curve. Each measurement was performed in triplicates and repeated for at least 3 independent experiments using the three samples (Zn/ZIF (MeIm), Zn/ZIF (HIm) and Zn/ZIF (BnIm).

Hydrogen peroxide (H_2O_2) concentration released was determined by means of a xylenol orange assay. 100 mg of particles were suspended in 10 mL of deionised water. 100 uL aliquots was drawn from the suspension at predetermined timepoints and 20 uL of the solution was added to a xylenol orange indicator solution. The absorbance of the resultant solution was read at 595 nm, and the absolute concentration of the hydrogen peroxide present was determined by calculation against a calibration curve. Each measurement was performed in triplicates and repeated for at least 3 independent experiments using the three samples (Zn/ZIF (MeIm), Zn/ZIF (HIm) and Zn/ZIF (BnIm).

The concentration of hydroxyl radicals ($\cdot OH$) was determined by adding 10 mg of ZIF-coated zinc particles (Zn/ZIF) in a solution of 500 uM sodium terephthalate solution as an analyte and measuring fluorescence at λ_{ex} 310 nm and λ_{em} 424 nm. The absolute concentration of the hydroxyl radicals released by the particles was calculated by comparing the measured fluorescence against a calibration curve. Each measurement was performed in triplicates and repeated for at least 3 independent experiments using the three samples (Zn/ZIF (MeIm), Zn/ZIF (HIm) and Zn/ZIF (BnIm).

The absolute concentrations of singlet oxygen ($^1O_2$) could not be determined due to the unavailability of quantifiable oxygen. Instead, the reaction of the generated oxygen from the ZIF-coated zinc particles with 1,3-Diphenylisobenzofuran was monitored over a specified period, where the conversion to diketone product was used to estimate the relative rate of release of oxygen from the ZIF-coated zinc (Zn/ZIF) particles.

Table 1: ROS Release Profile of ZIF-Coated Zinc (Zn/ZIF) Particles

Sample	$\cdot O_2^-$ [uM]	H_2O_2 [uM]				$^1O_2$ (conversion)			$\cdot OH$
	18h	1h	4h	8h	24h	2h	6h	24h	[uM]
Zn/ZIF (MeIm)	703	21	51	98	155	11%	30%	50%	Neg
Zn/ZIF (HIm)	645	19	55		105				
Zn/ZIF (BnIm)	950	17	70		104				
Zn/ZnO	560	23	55	118	160				

The results in Table 1 show that ZIF-coated zinc (Zn/ZIF) composite of the present invention are able to release appreciable amounts of superoxide ($\cdot O_2^-$) and hydrogen peroxide (H_2O_2) species spontaneously in aqueous solutions without energy input in the form of incident light. The results also show that ZIF-coated zinc (Zn/ZIF) materials are also able to release higher amount of superoxide ($\cdot O_2^-$) as compared to Zn/ZnO core/shell material.

Example 3 - Evaluation of Antibacterial Efficacy

The antimicrobial activity of the ZIF-coated zinc (Zn/ZIF) particles was assessed with a modified protocol based on ISO 22196/ JIS Z 2801.

Referring to Figure 5, 20 mg of the ZIF-coated zinc (Zn/ZIF) particles (500) were dispersed homogenously in ethanol and deposited on a glass slide (510) measuring 2.5 cm by 2.5 cm. Ethanol was allowed to evaporate completely, and blank glass slides were included as a control.

A 100 uL aliquot of 10^6 CFU mL^{-1} of microbes (520) was introduced onto the glass slides (some coated with ZIF-coated zinc (Zn/ZIF) particles, while some is a blank as a control). A plastic film (530) was placed over the aliquot to ensure even coverage, and the slides were allowed to incubate at 35ºC for 18 hours. The surfaces were then washed with a 10 mL aliquot of TSB or PBS solution, and subsequently diluted serially. Log reduction of viable bacteria was then assessed by standard plate count techniques.

The antibacterial properties of the surfaces were assessed against *E. coli* (G(-), ATCC 8739) and *S. aureus* (G(+), ATCC 6538P) and *C. albicans* (fungi, ATCC 10231). As shown in Table 1, the ZIF-coated zinc (Zn/ZIF) particles demonstrated excellent

antimicrobial properties. The microbes of interest were completely eliminated (see Figure 4) after exposure to the ZIF-coated zinc (Zn/ZIF) particles after an 18-hour incubation period (see Table 2(b)). A 5-log reduction of microbe population was observed for *E. coli, S. aureus* and *C. albicans,* respectively (Table 2(a)).

Table 2(a): Antibacterial and antifungal properties of glass slides coated with ZIF-coated zinc (Zn/ZIF) composite.

Sample	**Log_{10} reduction**		
	E. coli	*S. aureus*	*C. albicans*
Glass control	0	0	0
Zn/ZIF (HIm)	>5	>5	>5
Zn/ZIF (MeIm)	>5	>5	>5
Zn/ZIF (BnIm)	>5	>5	>5

Table 2(b)

	t = 0	t =18h			
	Starting titer	**Glass blank**	**H-Im**	**Me-Im**	**Bn-Im**
S.aureus	6.28	8.39	0*	0*	0*
E.coli	5.56	8.25	0*	0*	0*
C.albicans	4.71	7.65	0*	0*	0*

Log_{10} $CFUmL^{-1}$, 10% media in H_2O

* No colonies observed at the highest dilution factor, >5 log reduction in CFU

Example 4 - Evaluation of Antiviral Efficacy

The antiviral efficacy of the ZIF-coated zinc (Zn/ZIF) particles were assessed against Influenza A virus, based on a modified ISO 21702 protocol.

Similar to the antibacterial efficacy tests, 20 mg of ZIF-coated zinc particles (500) were dispersed homogenously in ethanol and deposited on a glass slide (510) measuring 2.5 cm by 2.5 cm. Ethanol was allowed to evaporate completely, and blank glass slides were included as a control.

A 100 uL aliquot of Influenza A (10^5 PFU mL^{-1}) (520) was introduced onto the Zn/ZIF (MeIm) coated glass slides and blank glass slides. Subsequently a thin parafilm (530) was used to cover onto each slide to ensure virus contact with the coated slide. Supernatants were harvested for plaque assay at 6, 12 and 24 h post-incubation.

MDCK cells were inoculated on 12-well plates separately. Influenza-treated supernatants were serially diluted to 10^{-4} and 250 µL of the diluted supernatant were added to MDCK cells. Plates were incubated for 1 h with 15 mins rocking intervals. Plates were then washed with 1x PBS twice and 0.3% agarose overlay media were added to each well. Plates were incubated for 2 days. Lastly, overlay media was removed, and crystal violet was added to stain for countable plaques. Plaques were then calculated using plaque forming units per mL. As shown in Figure 4, full elimination of influenza A viral titer was observed upon exposure to the Zn/ZIF(MeIm) material, for the 3 timepoints evaluated. The (*) in Figure 4 denotes full elimination of viral titer.

Example 5 - Skin Sensitization Tests OECD 406

Zn/ZIF(MeIm) was tested to determine the materials' skin sensitization potential, according to the test protocol described in OECD Guideline for Testing of Chemicals 406: Skin Sensitization. The materials did not produce skin sensitization in guinea pigs when Buehler Test was conducted.

The method of the present invention involves using imidazole, which is a base, to react with zinc powder. Imidazole reacts with zinc/zinc oxide to form ZIF-coated zinc (Zn/ZIF) composite. It was found that Zn/ZIF composite materials exhibit excellent antibacterial, antifungal, and antiviral properties. The antimicrobial property is due to the release of reactive oxygen species (ROS). These new materials could have broad range of applications in healthcare, consumer care and cosmetic industries.

Although embodiments of the invention have been shown and described, the invention is not limited to the described embodiments. Instead, it would be appreciated by those skilled in the art that various modifications and variations can be made to the embodiments of the invention without departing from the scope of the invention, the scoop of which is set forth in the following claims.

CLAIMS

1. An antimicrobial composite comprising a zinc core with a zeolitic imidazolate framework (ZIF) shell.

2. The antimicrobial composite according to claim 1, wherein the zeolitic imidazolate framework shell comprises an imidazole or an imidazole derivative linking moiety.

3. The antimicrobial composite according to claim 2, wherein the imidazole or imidazole derivative linking moiety is from one imidazole or imidazole derivative selected from the group consisting of 2H-imidazole (HIm), 2-methylimidazole (MeIm) and benzimidazole (BnIm).

4. The antimicrobial composite according to claim 3, wherein the composite is a Zn/ZIF (HIm) core/shell composite, a Zn/ZIF (MeIm) core/shell composite, or a Zn/ZIF (BnIm) core/shell composite.

5. The antimicrobial composite according to claim 4, wherein the antimicrobial composite has an antimicrobial mechanism involving spontaneous reactive oxygen species generation based on electron donation from the zinc core to the ZIF shell through self-corrosion of the zinc core.

6. The antimicrobial composite according to any one of claims 1 to 5, wherein the antimicrobial composite is in powder form.

7. A ZIF-based antimicrobial material comprising a substrate and an antimicrobial composite as claimed in any one of the preceding claims 1 to 6.

8. A method of preparing an antimicrobial composite comprising a zinc core with a zeolitic imidazolate framework shell, the method comprising:

mixing zinc powder with an imidazole selected from the group consisting of 2H-imidazole (HIm), 2-methylimidazole (MeIm) and benzimidazole (BnIm) at a

temperature ranging from 80°C to 160°C for a time duration in presence of a solvent to obtain an antimicrobial zeolitic imidazolate framework (ZIF) coated zinc composite.

9. The method according to claim 8, further comprising drying the zeolitic imidazolate framework (ZIF) coated zinc composite at a temperature ranging from 55°C to 65°C.

10. The method according to claim 8, wherein the time duration is 1 to 20 hours.

11. The method according to claim 8, wherein the imidazole is in an amount of less than 5 wt% based on the total weight of the antimicrobial composite.