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(54) **Title:** ANTIFUNGAL ACTIVITY OF IRON SEQUESTERING POLYMERS

(57) **Abstract:** A polymeric metal sequestrant providing antifungal activity is provided. The polymeric metal sequestrant comprises a polyamine polymer covalently coupled to a chelator, wherein the chelator has a benzene ring with more than one hydroxyl group at any position that is free. The polymeric metal sequestrant is effective in inhibiting and preventing fungal infections and displays synergistic effects in combination with traditional antifungal agents.



ANTIFUNGAL ACTIVITY OF IRON SEQUESTERING POLYMERS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims the benefit of U.S. Provisional Application No. 62/321,275 filed on April 12, 2016, the contents of which are incorporated herein by reference.

BACKGROUND

[0002] Fungal infections are common and the treatment options are limited. For example, *Candida albicans* remains the most commonly isolated species in clinical trials capable of causing infections throughout the body, including mucosal surfaces (oropharyngeal candidiasis (OPC)), within the bloodstream (candidemia), as well as disseminated infections (e.g., brain, liver, kidneys). Although this species is also normally found as a commensal in the oral cavity, gastrointestinal (GI) tract, and vagina of most healthy individuals, in immunocompromised and critically ill patients, *C. albicans* can transition from commensal to opportunistic pathogen via dissemination from the GI tract. Little is known about the mechanisms that *Candida* uses to invade and translocate across the oral and gastric mucosa. Unfortunately, dissemination of *Candida* from the gastrointestinal tract to systemic organs can lead to invasive candidiasis. Not only are the treatment options for fungal infections such as *Candida* limited, these options are often associated with toxicity concerns. Thus, a novel approach to anti-fungal therapy is needed that provides effective treatment without toxicity concerns.

SUMMARY

[0003] The present disclosure is based on the discovery that certain polymeric materials are highly effective against fungal infections and furthermore, provide a synergistic effect when combined with known antifungal agents. A polymeric chelator composition is therefore provided comprising a polymer covalently coupled to one or more chelators, wherein the polymer comprises a polyamine, and wherein the one or more chelators has a benzene ring with more than one hydroxyl group at any position that is free, or a derivative of the chelator, or a salt of the chelator. The composition may further comprise an antifungal agent.

[0004] In certain embodiments, the polymers of the polymeric chelator composition are cross-linked to one another independent of any cross-linking that may occur as a result of metal

binding to the chelator of the polymeric chelator composition. In certain embodiments, the polymer is a polyamine including, but is not limited to polyallylamine (PAA or PAI), polylysine (PLL), polyethylenimine (PEI), or the like.

[0005] In certain embodiments, the chelator is capable of chelating a metal, a heavy metal, and more specifically, one or more of aluminum, arsenic, cadmium, chromium, copper, iron, lead, manganese, and mercury. In one particular embodiment, the chelator is 2,3 dihydroxybenzoic acid (DHBA). In another particular embodiment, the chelator is 2,3 dihydroxybenzaldehyde.

[0006] In certain embodiments, the chelator is covalently coupled to a primary amine of the polyamine through an amide bond. In certain embodiments, the chelator is covalently coupled to a primary amine of the polyamine through an amine bond. The chelator may be present in the composition in an amount to provide a molar ratio of chelator to amine of from about 0.03 to about 0.40, from about 0.03 to about 0.22, from about 0.05 to about 0.20, from about 0.07 to about 0.175, and from about 0.10 to about 0.15.

[0007] In one particular embodiment, the polyamine is polyallylamine and the chelator is 2,3 dihydroxybenzoic acid. In one particular embodiment, the polyamine is polyallylamine and the chelator is 2,3 dihydroxybenzaldehyde. In one particular embodiment, the polyamine is polylysine and the chelator is 2,3 dihydroxybenzoic acid. In one particular embodiment, the polyamine is polylysine and the chelator is 2,3 dihydroxybenzaldehyde. In another particular embodiment, the polyamine is polyethylenimine and the chelator is 2,3 dihydroxybenzoic acid. In yet another particular embodiment, the polyamine is polyethylenimine and the chelator is 2,3 dihydroxybenzaldehyde.

[0008] In certain embodiments, the antifungal agent is selected from the group comprising amphotericin B, abifungin, candicidin, filipin, hamycin, natamycin, nystatin, rimocidin, bifonazole, butoconazole, clotrimazole, econazole, fenticonazole, isoconazole, ketoconazole, luliconazole, miconazole, omoconazole, oxiconazole, sertaconazole, sulconazole, tioconazole, albaconazole, efinaconazole, epoxiconazole, fluconazole, itraconazole, propiconazole, ravuconazole, terconazole, voriconazole, posaconazole, amorolfine, butenafine, naftifine, anidulafungin, caspofungin, micafungin, terbinafine, cyclopirox, flucytosine, griseofulvin, haloprogin, tolinaftate, and undecylenic acid.

[0009] In certain embodiments, the polymeric chelator composition is a hydrogel. In certain embodiments, the polymeric chelator composition is a topical formulation for topical administration.

[00010] The present disclosure also provides a method for treating a subject with a fungal infection. The method comprises administering a polymeric chelator composition to a site on the subject harboring the fungal infection, wherein the composition comprises a polymer covalently coupled to a chelator, wherein the polymer comprises a polyamine, and wherein the chelator has a benzene ring with more than one hydroxyl group at any position that is free. In another embodiment, the polymeric chelator composition used in the method comprises a plurality of cross-linked polymers, wherein the plurality of cross-linked polymers comprise a polyamine, with one or more chelators covalently coupled to one or more primary amines, respectively, of the polyamine through one or more amide bonds or amine bonds, wherein each of the one or more chelators has a benzene ring with more than one hydroxyl group at any position that is free, or a derivative of the chelator, or a salt of the chelator. In particular embodiments, the polyamine includes, but is not limited to polyallylamine (PAA), polylysine (PLL), polyethylenimine (PEI), or the like. In any of the above embodiments of the method, the chelator is capable of chelating a metal, a heavy metal, and more specifically, one or more of aluminum, arsenic, cadmium, chromium, copper, iron, lead, manganese, and mercury. In one particular embodiment, the chelator is 2,3 dihydroxybenzoic acid (DHBA). In another particular embodiment, the chelator is 2,3 dihydroxybenzaldehyde. In any of the above embodiments, the composition used in the method is a hydrogel. In any of the above embodiments, the composition can be a topical formulation for topical administration.

[00011] In certain embodiments, the site is an external wound on the subject. In particular embodiments, the site is a mucosal surface such as bronchial, endometrial, gastric, penile, vaginal, olfactory, intestinal, anal, or oral.

[00012] In certain embodiments, the fungal infection is caused by at least one of the group comprising the following: *Coccidioides* species such as *Coccidioides immitis*; *Candida* species such as *Candida parapsilosis*, *Candida krusei*, *Candida glabrata*, *Candida guilliermondii*, and *Candida albicans*; *Paecilomyces* species such as *Paecilomyces variotii*; *Cryptococcus* species such as *Cryptococcus neoformans*; *Cryptococcus gattii*; *Aspergillus* species such as *Aspergillus fumigatus* and *Aspergillus flavus*; *Fusarium* species such as *Fusarium oxysporum* and *Fusarium solani*;

Rhizopus oryzae; *Scedosporium* species such as *Scedosporium prolificans* and *Scedosporium apiospermum*; *Lomentospora prolificans*; *Blasotmyces dermatitidis*; *Pneumocystis jirovecii*; *Sporothrix schenckii*; *Saksenaea* species such as *Saksenaea vasiformis*; *Histoplasma capsulatum*; and ringworm.

[00013] In certain embodiments, the administering step is performed by applying the composition topically to a site of fungal infection on the skin, nail, or on a mucosal surface.

[00014] In certain embodiments, the method can further comprise the step of administering an antifungal agent to the site. In any of the above embodiments, the composition used in the method may further comprise an antifungal agent. In certain embodiments, the antifungal agent is selected from the group comprising amphotericin B, abifungin, candicidin, filipin, hamycin, natamycin, nystatin, rimocidin, bifonazole, butoconazole, clotrimazole, econazole, fenticonazole, isoconazole, ketoconazole, luliconazole, miconazole, omoconazole, oxiconazole, sertaconazole, sulconazole, tioconazole, albaconazole, efinaconazole, epoxiconazole, fluconazole, itraconazole, propiconazole, ravuconazole, terconazole, voriconazole, posaconazole, amorolfine, butenafine, naftifine, anidulafungin, caspofungin, micafungin, terbinafine, cyclopirox, flucytosine, griseofulvin, haloprogin, tolnaftate, and undecylenic acid.

[00015] The compositions and methods of the present disclosure may also be used to prevent fungal infections in subjects at risk for exposure to a fungal or having a wound that is susceptible to fungal infection.

[00016] In certain embodiments, the subject was previously treated with an antifungal agent prior to administering the composition.

[00017] The present disclosure further provides a process for preparing a polymeric composition. The process comprises the following steps: obtaining a first solution comprising an activated chelator; obtaining a second solution comprising a polymer and a cross-linker, wherein the polymer comprises a polyamine; adding the first solution to the second solution at a desired ratio to form a third solution; mixing the third solution until it is transparent; and incubating the third solution at room temperature. In certain embodiments of the process, the activated chelator comprises 2,3-dihydroxybenzoic acid activated with N-hydroxysuccinimide. In certain embodiments, the polyamine is selected from the group consisting of polyallylamine, polylysine,

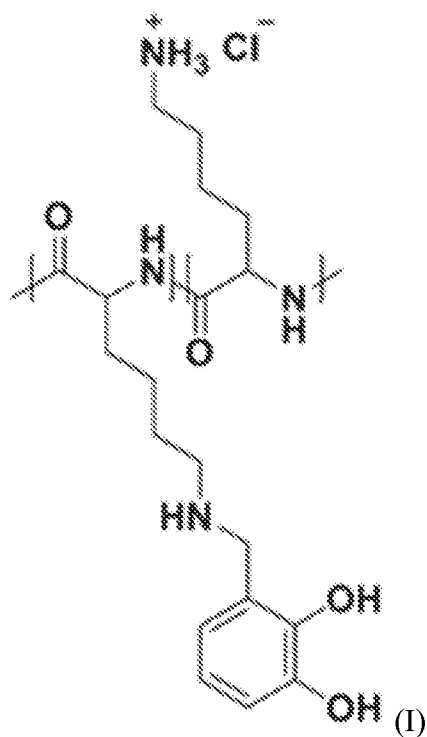
and polyethylenimine. In certain embodiments, the cross-linker is N,N-methylene bisacrylamide.

[00018] In any of the above embodiments of the process, the first solution is added to the second solution in an amount sufficient to provide a composition having a molar ratio of chelator to amine of from about 0.03 to about 0.40, from about 0.03 to about 0.22, from about 0.05 to about 0.20, from about 0.07 to about 0.175, and from about 0.10 to about 0.15. In an embodiment, the first solution is added to the second solution in an amount sufficient to provide a molar ratio of chelator to amine of from about 5% to about 40%.

[00019] In any of the above embodiments of the process, the third solution is incubated at room temperature for at least 48 hours.

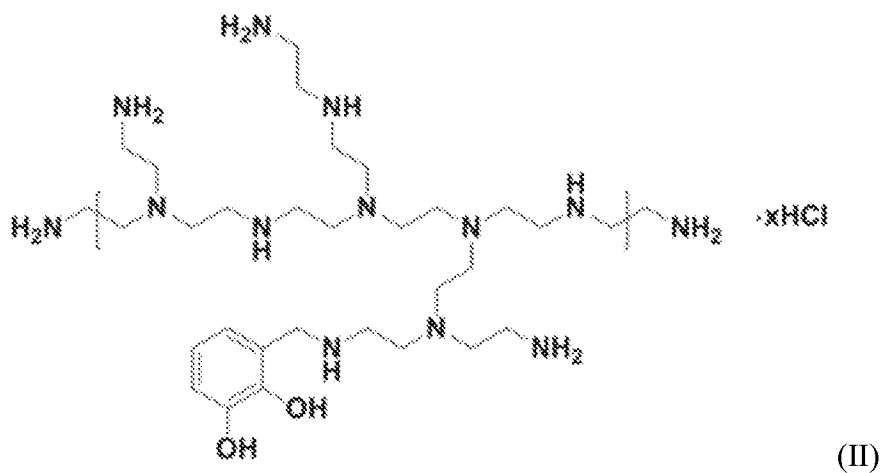
[00020] In some embodiments, a polymeric chelator comprises a plurality of polyamine polymer backbone chains and one or more chealtors, wherein the polamine polymer backbone chains are polyallylamine or polylysine, wherein the one or more chealtors are covalently coupled to the one or more primary amines, respectively, of at least one of the plurality of polyamine polymer backbone chains through one or more amide bonds, respectively, wherein each of the one or more chealtors has a benzene ring with more than one hydroxyl group at any position that is free, and wherein the plurality of polyamine polymer backbone chains are cross-linked to one another independent of any cross-linking that may result from interactions with the one or more chelators.

[00021] In another embodiment, a polymeric metal sequestrant can have a structure (I), wherein the structure (I) is



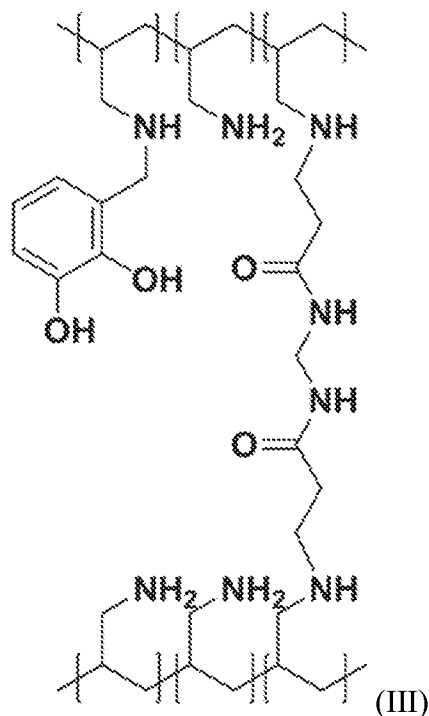
[00022] In certain embodiments, a method for treating a subject with a fungal infection comprises administering a composition to a site on the subject harboring the fungal infect, wherein the composition comprises the polymeric metal sequestrant of structure (I).

[00023] In another embodiment, a polymeric metal sequestrant can have a structure (II), wherein the structure (II) is



[00024] In certain embodiments, a method for treating a subject with a fungal infection comprises administering a composition to a site on the subject harboring the fungal infect, wherein the composition comprises the polymeric metal sequestrant of structure (II).

[00025] In another embodiment, a polymeric metal sequestrant can have a structure (III), wherein the structure (III) is



[00026] In certain embodiments, a method for treating a subject with a fungal infection comprises administering a composition to a site on the subject harboring the fungal infect, wherein the composition comprises the polymeric metal sequestrant of structure (III).

[00027] In some embodiments, the foregoing compositions of polymeric metal sequestrants may further comprise an antifungal agent. In certain embodiments, the antifungal agent is selected from the group comprising amphotericin B, abifungin, candicidin, filipin, hamycin, natamycin, nystatin, rimocidin, bifonazole, butoconazole, clotrimazole, econazole, fenticonazole, isoconazole, ketoconazole, luliconazole, miconazole, omoconazole, oxiconazole, sertaconazole, sulconazole, tioconazole, albaconazole, efinaconazole, epoxiconazole, fluconazole, itraconazole, propiconazole, ravuconazole, terconazole, voriconazole, posaconazole, amorolfi, butenafine, naftifine, anidulafungin, caspofungin, micafungin, terbinafine, cyclopirox, flucytosine, griseofulvin, haloprogin, tolnaftate, and undecylenic acid. Such compositions, further comprising an antifungal agent may be used in methods for treating a subject with a fungal infection comprising administering such composition to a site on the subject harboring the fungal infection.

[00028] Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating specific embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description. For example, features of certain aspects or embodiments for compositions and/or methods may be combined as applicable within the spirit of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[00029] FIGURE 1 provides the reaction scheme for synthesis of cross-linked PAI-DHBA polymer.

[00030] FIGURE 2 provides the iron affinity indexes of PAI-DHBA polymers as measured using a ligand competition assay.

[00031] FIGURE 3 provides iron sequestration capacities of PAI-DHBA polymers, expressed as mg Fe/g PAI-DHBA. (■) Theoretical Fe Sequestration Capacity; (▼) Experimental Fe Sequestration Capacity.

[00032] FIGURE 4 provides the results of metal selective studies for essential metals, expressed as mmol metals/g PAI-DHBA.

[00033] FIGURE 5 provides the results of metal selectivity studies for PAI-DHBA polymers in M63 media.

[00034] FIGURE 6 depicts the structure of various polymeric metal sequestrants of the present disclosure. Structure I is an exemplary polymeric metal sequestrant comprising polyallylamine covalently coupled to 2,3 dihydroxybenzaldehyde. Structure II is an exemplary polymeric metal sequestrant comprising polyethylenimine covalently coupled to 2,3 dihydroxybenzaldehyde. Structure III is an exemplary polymeric metal sequestrant comprising polylysine covalently coupled to 2,3 dihydroxybenzaldehyde.

[00035] FIGURE 7 depicts the structure of various polymeric metal sequestrants of the present disclosure. Structure I is an exemplary polymeric metal sequestrant comprising polyallylamine covalently coupled to 2,3 dihydroxybenzoic acid. Structure II is an exemplary polymeric metal sequestrant comprising polyethylenimine covalently coupled to 2,3

dihydroxybenzoic acid. Structure III is an exemplary polymeric metal sequestrant comprising polylysine covalently coupled to 2,3 dihydroxybenzoic acid.

[00036] FIGURE 8 depicts the results of metal selectivity studies for polyallylamine (PAA)-DHBA8, polyethylenimine (PEI)-DHBA9, and polylysine (PLL)-DHBA10 in simulated intestinal fluid.

DESCRIPTION

[00037] The present disclosure provides a composition that is effective in the treatment of fungal infections. The composition is a polymeric metal sequestrant (i.e. polymers that bind and retain metals such as iron). Polymers, especially cross-linked polymeric materials, cannot be absorbed through skin, thereby limiting concerns of toxicity that have plagued the prior art. Moreover, the combination of polymeric metal sequestrant with traditional antifungals provide a synergistic therapeutic effect and reduce the minimum inhibitory concentrations (MICs) of antifungals.

[00038] The polymeric metal sequestrants described herein are designed with several key features such as high affinity, a large binding capacity, and selectivity for iron. In one embodiment, primary amine groups on polyallylamine (PAI or PAA) were simultaneously cross-linked by, for example, methylenebisacrylamide (MBA) and conjugated with 2,3-dihydroxybenzoic acid (DHBA) molecules or 2,3 dihydroxybenzaldehyde molecules, which serve as iron chelation sites. The resultant iron-sequestering polymer demonstrates strong affinity and high selectivity for iron. Further, the polymeric metal sequestrants are demonstrated herein as an effective antifungal alone and in combination with traditional antifungals such as imidazoles, triazoles, thiazoles, and echinocandins, which are commonly used for treatment of a variety of fungal infections.

[00039] The polymeric metal sequestrants of the present disclosure comprise a polymer covalently coupled to a chelator, wherein the polymer comprises a polyamine. In some embodiments, the chelator coupled to the polymer may include 2,3 dihydroxybenzoic acid (DHBA) and other iron chelators, such as 2,3 dihydroxybenzaldehyde. FIG. 6 depicts various polymers comprising PAA, PLL, or PEI each covalently coupled to 2,3 dihydroxybenzaldehyde providing exemplary polymeric metal sequestrants of the present disclosure. FIG. 7 depicts various polymers comprising PAA, PLL, or PEI each covalently coupled to 2,3

dihydroxybenzoic acid providing additional exemplary polymer metal sequestrants of the present disclosure. DHBA acid is a fraction of the natural iron chelator Enterobactin ($\text{Log } K = 52$) which is a high affinity siderophore that acquires iron for microbial systems. Chelators of other metals, including heavy metals that can be coupled to a polymer may also be included. As used herein, “heavy metals” are chemical elements with a specific gravity that is at least 5 times the specific gravity of water. In certain embodiments, the polymeric metal sequestrants selectively bind iron.

[00040] In some embodiments, the chelator may be coupled to the polymer via a carboxyl group of the chelator. In some embodiments, the chelator may be coupled to the polymer via a peptide bond. In some embodiments, the chelators can include a feature for coupling with the polymer, such as carboxy groups that can be coupled to the amines of the polymer through amide bonds. In other embodiments, the chelator, such as or 2,3 dihydroxybenzaldehyde, can be coupled to the amines of the polymer through an amine bond. Other crosslinking or coupling reagents can be included in the polymer and chelator system in order to prepare a polymeric chelator having the ability to chelate iron.

[00041] In some embodiments, the present disclosure provides a polymeric chelator, polymer or hydrogel, made by reacting 2,3 dihydroxybenzoic acid (DHBA) or 2,3 dihydroxybenzaldehyde to a polymer comprising a polyamine. In some embodiments, the polymeric chelators, in polymer or hydrogel form, can be fabricated as solids or equilibrated in aqueous solution as a solution or suspension. In some examples the polyamine polymer may comprise PVAm and PAAm. PVAm and PAAm are polycation hydrogels consisting of reactive primary amine side groups for the conjugation of DHBA. Cross-linked PVAm hydrogel may be synthesized by hydrolyzing a precursor polymer, PNVE, in a basic medium. Cross-linked PAAm hydrogel may be synthesized by cross-linking the precursor PAAm chains.

[00042] In other embodiments, thioglycolic acids (TGA) in combination with the siderophore moiety dihydroxybenzoic acid (DHBA) may be introduced onto PAAm and PVA to from the polymeric chelator.

[00043] In certain embodiments, the polymeric metal sequestrants of the present disclosure comprise a plurality of cross-linked polyamine-containing polymers covalently coupled to one or more chelators that form hydrogels. The polymeric metal sequestrants, including all embodiments disclosed herein, may comprise a swelling ratio of from about 5 to about 20, or alternatively less than 5 wherein the swelling ratio is determined by $(W_s - W_d)/W_d$

where W_s and W_d represent the weight of polymer after full swelling in PBS, and the weight of dried polymer, respectively.

[00044] The polymeric metal sequestrants, including all embodiments disclosed herein, may further comprise a molar ratio of chelator to amine of from about 0.03 to about 0.40, from about 0.03 to about 0.22, from about 0.05 to about 0.20, from about 0.07 to about 0.175, and from about 0.10 to about 0.15. In other embodiments, the molar ratio of chelator to amine is 0.07, 0.08, 0.09, 0.10, 0.11, 0.12, 0.13, 0.14, 0.15, 0.16, 0.17, 0.18, 0.19, 0.20, 0.21, 0.22, 0.23, 0.24, or 0.25.

[00045] The polymeric metal sequestrants, including all embodiments disclosed herein, possesses an iron affinity index from about 25 to about 35 and more preferably from about 28 to about 32. Determination of the iron affinity index is described in the Examples herein below.

[00046] The polymeric metal sequestrants, including all embodiments disclosed herein, possesses an iron sequestration capacity of from about 5 mg Fe/g polymeric metal sequestant to about 25 mg Fe/g polymeric metal sequestant, and more preferably about 20 mg Fe/g polymeric metal sequestant. Iron sequestration capacity, as used herein, describes the maximum iron adsorption by the polymeric metal sequestrants.

[00047] The polymeric metal sequestrants can be fabricated as solids, gels, pastes, liquids, such as being equilibrated in aqueous solution as a solution or suspension. The polymeric metal sequestrants may further be formulated for topical administration. Compositions for topical administration may include the polymeric metal sequestrants formulated for a medicated application such as an ointment, paste, cream or powder. Ointments include all oleaginous, adsorption, emulsion and water-soluble based compositions for topical application, while creams and lotions are those compositions that include an emulsion base only. Topically administered medications may contain a penetration enhancer to facilitate adsorption of the active ingredients through the skin. Suitable penetration enhancers include glycerin, alcohols, alkyl methyl sulfoxides, pyrrolidones and laurocapram. Possible bases for compositions for topical application include polyethylene glycol, lanolin, cold cream and petrolatum as well as any other suitable absorption, emulsion or water-soluble ointment base. Topical preparations may also include emulsifiers, and gelling agents, as necessary to preserve the composition and provide for a homogenous mixture. Transdermal administration of the present invention may also comprise the use of a "patch". For example, the patch may supply one or more active substances at a

predetermined rate and in a continuous manner over a fixed period of time. Topical preparation, such as, for example, 5% PLL-DHBA10 iron binding polymer or 5% PAA-DHBA8 iron binding polymer formulations, may include excipients for topical formulation which include, by way of example, but not limitation, aloe barbadensis leaf juice, avena sativa (oat) kernel extract, benzyl alcohol, pyrogallol, butylated hydroxytoluene, cetostearyl alcohol, cetyl alcohol, chamomilla recutita (matricaria) flower extract, diazolidinyl urea, dimethicone, distearyldimonium chloride, edetate disodium, glycerin, glyceryl monostearate, hydrolyzed collagen, hydrozed elastin, hydrolyzed jojoba esters, jojoba esters, magneiusm ascorbyl phosphate, menthyl lactate, methyl gluceth-20, methylparaben, petrolatum, polysorbate 60, potassium hydroxide, PPG-12/SMDI copolymer, propylparaben, purified water, retinyl palmitate, stearamidopropyl PG-dimonium chloride phosphate, steareth-2, steareth-21, stearyl alcohol, and tocopheryl acetate.

[00048] In certain embodiments, the polymeric metal sequestrants can be incorporated into textiles, fabrics, absorbent members, gauze, wipes, bandages, or the like.

[00049] The polymeric metal sequestrants can be present in the compositions at a range of from about 1 mg/ml to about 2,000 µg/ml, from about 10 µg/ml to about 1,000 µg/ml, from about 20 µg/ml to about 500 µg/ml, from about 30 µg/ml to about 400 µg/ml, from about 40 µg/ml to about 300 µg/ml, from about 50 µg/ml to about 200 µg/ml, and from about 100 µg/ml to about 150 µg/ml, and any range there between.

[00050] In certain embodiments, the composition of the present disclosure further comprises an antifungal agent in addition to the polymeric metal sequestrant. Suitable antifungal agents of the composition include, but are not limited to amphotericin B, abifungin, candicidin, filipin, hamycin, natamycin, nystatin, rimocidin, bifonazole, butoconazole, clotrimazole, econazole, fenticonazole, isoconazole, ketoconazole, luliconazole, miconazole, omoconazole, oxiconazole, sertaconazole, sulconazole, tioconazole, albaconazole, efinaconazole, epoxiconazole, fluconazole, itraconazole, propiconazole, ravuconazole, terconazole, voriconazole, posaconazole, amorolfine, butenafine, naftifine, anidulafungin, caspofungin, micafungin, terbinafine, cyclopirox, flucytosine, griseofulvin, haloprogin, tolnaftate, and undecylenic acid.

[00051] The antifungal agent may be dispersed in the polymeric metal sequestrant by methods known for administering antifungals in other polymeric hydrogels. Examples for comparison could include topical gels or creams containing clotrimazole, intravaginal gels

containing miconazole, and oral gels containing miconazole. In many of these products, polymers are used to form a viscosified vehicle to facilitate placement or retention of the dose, which could also be achieved by the polymeric metal sequestrant.

[00052] The polymeric metal sequestrants may be formulated as a pharmaceutical composition comprising an effective amount of one or more polymeric metal sequestrants and optionally, one or more antifungals dissolved or dispersed in a pharmaceutically acceptable carrier. The phrases “pharmaceutical or pharmacologically acceptable” refers to molecular entities and compositions that do not produce an adverse, allergic or other untoward reaction when administered to an animal, such as, for example, a human, as appropriate. The preparation of a pharmaceutical composition that contains at least one polymeric metal sequestrants and optionally, one or more antifungals will be known to those of skill in the art in light of the present disclosure.

[00053] As used herein, “pharmaceutically acceptable carrier” includes any and all solvents, dispersion media, coatings, surfactants, antioxidants, preservatives, isotonic agents, absorption delaying agents, salts, preservatives, stabilizers, gels, binders, excipients, disintegration agents, lubricants, dyes, such like materials and combinations thereof, as would be known to one of ordinary skill in the art.

[00054] In accordance with the present invention, the composition is combined with the carrier in any convenient and practical manner, i.e., by solution, suspension, emulsification, admixture, encapsulation, absorption and the like. Such procedures are routine for those skilled in the art.

[00055] The polymeric metal sequestrants of the present disclosure can be used as an antifungal therapy to treat or prevent fungal infections in connection with external wounds and burns and also in the treatment or prevention of fungal infections present on mucosal membranes. In one embodiment, the polymeric metal sequestrants are applied topically to the wound or burn surface or directly on the mucosal surface.

[00056] In certain embodiments, a traditional antifungal can be added to the site of fungal infection simultaneously with the polymeric metal sequestrant or following an initial treatment with the polymeric metal sequestrant. More generally, such an agent would be provided in a combined amount with a polymeric metal sequestrant effective to kill or inhibit proliferation of the fungal infection. This process may involve contacting the cell(s) with an antifungal agent and

the polymeric metal sequestrant at the same time or within a period of time wherein separate administration of the polymeric metal sequestrant and antifungal to a cell, tissue or organism produces a desired therapeutic benefit. This may be achieved by contacting the cell, tissue or organism with a single composition or pharmacological formulation that includes both a polymeric metal sequestrant and one or more antifungals, or by contacting the wound or infection site with two or more distinct compositions or formulations, wherein one composition includes a polymeric metal sequestrant and the other includes one or more antifungals.

[00057] The polymeric metal sequestrants may be applied daily, or one to four times daily, based on the location of the infection, the severity of the infection, or the fungus causing the infection.

[00058] It is contemplated that any embodiment discussed in this specification can be implemented with respect to any method or composition of the invention, and vice versa. Furthermore, compositions of the invention can be used to achieve methods of the invention.

EXAMPLES

EXAMPLE 1: SYNTHESIS AND CHARACTERIZATION OF PAI-DHBA POLYMER

Materials and Methods

[00059] NHS-activated DHBA was synthesized before preparing the polymer. A solution of DHBA (770 mg, 5 mmol) and NHS (690 mg, 6 mmol) in 5 mL of DMF was mixed with a solution of EDC (1200 mg, 6.2 mmol) in 5 mL of DMF. The mixture was stirred at room temperature for 8 h and used for the next step without any purification. The PAI cross-linking and DHBA conjugation were conducted in a single step. Briefly, a 15% w/w PAI hydrochloride (56 kDa) solution containing a predetermined amount of N,N-methylene bisacrylamide (BMA; 5%, molar ratio of cross-linker to total amines) was prepared in H₂O/DMF (50/50 v/v) mixture. Then, the NHS-activated DHBA solution with a desired DHBA/amine molar ratio (5-40%) was added to the solution. After sonication for 2 min to get a transparent solution, TEA was added to the solution and mixed thoroughly, and then the solution was incubated at room temperature for 48 h. The cross-linked polymer gels were washed with 0.1 M sodium hydroxide for several days under the protection of nitrogen, and then lyophilized. The polymer gels were ground to powder

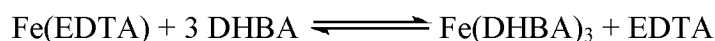
for subsequent studies. The particle size of the ground powder was ~100 µm measured by optical microscopy. As the polymers were cross-linked particles, the DHBA conjugation ratios could not be characterized directly by NMR analysis. Instead, the unconjugated DHBA left in the solution after the reaction was determined by NMR. The real conjugated DHBA ratio was calculated by deducting the unconjugated DHBA ratio from the feed DHBA ratio.

[00060] The swelling behavior of cross-linked polymers was studied in PBS buffer (pH 7.4). Dried polymer samples were placed in PBS buffer at room temperature. The weight of the swollen polymer samples was determined at different time points until there was no weight gain, indicative of complete swelling. The swelling ratio was defined as the fractional increase in the weight of the cross-linked polymer due to water absorption (Park, et al., *Effect of swelling ratio of injectable hydrogel composites on chondrogenic differentiation of encapsulated rabbit marrow mesenchymal stem cells in vitro*, Biomacromolecules, 2009;10(3):541-6). The swelling ratio of the cross-linked polymers was determined by the following equation:

$$\text{Swellingratio} = \frac{W_s - W_d}{W_d}$$

Here, W_s , and W_d represent the weight of polymer after full swelling in PBS, and the weight of dried polymer, respectively.

[00061] The iron affinity index of the polymer was measured using a ligand competition assay. The competitive chelation of iron by the polymers in equilibrium with EDTA (a water-soluble chelator) was used to determine the affinity index. Briefly, 1.5 mL of 10 mM EDTA solution, 2 mL of 5 mM FeCl₃ solution, 21.5 mL PBS and a known mass of polymer were mixed together and rotated at 25 °C for 5 days. Then, the concentration of the soluble iron complex was determined by inductively coupled plasma optical emission spectrometry (ICP-OES; Optima 2000 DV, PerkinElmer, USA). The affinity index of the polymer was determined following the procedure reported in literature (Feng et al., *Iron (III) chelating resins. VI. Stability constants of iron(III)-ligand complexes on insoluble polymeric matrices*, J. Appl. Poly. Sci., 1995;56(10):1231-7). In the equilibrium situation, the system could be represented in the following way (for brevity, all charges have been omitted):



$$Kq = \frac{[\text{Fe}(\text{DHBA})_3][\text{EDTA}]}{[\text{Fe}(\text{EDTA})][\text{DHBA}]^3} = \frac{[\text{Fe}(\text{DHBA})_3]}{[\text{Fe}][\text{DHBA}]^3} \times \frac{[\text{Fe}][\text{EDTA}]}{[\text{Fe}(\text{EDTA})]} \quad (1)$$

The iron stability constant of DHBA (Q) could be defined as follows:

$$Q = \frac{[\text{Fe}(\text{DHBA})_3]}{[\text{Fe}][\text{DHBA}]^3} \quad (2)$$

The iron stability constant of EDTA (K) could be defined as:

$$K = \frac{[\text{Fe}(\text{EDTA})]}{[\text{Fe}][\text{EDTA}]} \quad (3)$$

Herein, Iron affinity index was defined as LogQ. Based on equation (1), (2) and (3):

$$\text{Iron affinity index} = \text{LogQ} = \text{Log}(Kq \times K) = \text{LogKq} + \text{LogK}$$

The value of K was known, and Kq could be easily calculated based on the iron concentration, EDTA concentration, and the concentration of DHBA groups in polymers as described in literature (Feng, et al.). The iron affinity index showed how strong the polymers chelated with iron versus EDTA.

[00062] A known mass of polymer was incubated in a 5 mM FeCl₃ solution in the presence of 5 mM EDTA as a stabilizer at 25 °C for a week. The remaining iron concentration was determined by ICP-OES.

Results

[00063] Previous preparations of PAI-DHBA polymer used a two-step synthesis strategy. PAI hydrochloride was first cross-linked with N,N-methylene bisacrylamide (BMA) by a Michael-type addition reaction and then the formed PAI hydrogel was further conjugated to DHBA via EDC/NHS conjugation chemistry. This two-step strategy was time consuming, and in the second step, DHBA conjugation may be favored near the particle surface. In this report, the polymer cross-linking and DHBA conjugation were conducted in a single step. DHBA conjugation was controlled by adjusting the DHBA/polymer feed ratios. Several PAI-DHBA polymers with various DHBA content (5-40% of total amines) but the same cross-linking density (5%) were prepared via this one step strategy as shown in Figure 1. DHBA conjugation ratios which are shown in Table 1 were determined by NMR analysis. As the DHBA content increased from 5% to 30%, the swelling ratios decreased from 11.8 to 5.3, indicating that the gel became more hydrophobic as DHBA conjugation increased. When incubated with Fe³⁺ solution, all the

PAI-DHBA samples exhibited dark color indicating chelation with Fe^{3+} , while the PAI gel did not show a color change.

Table 1. Synthesis and characterization of PAI-DHBA polymers.

Sample	Cross-linking Density ^a	Feed DHBA/amine ^b	Found DHBA/amine ^c	Swelling ratio pH 7.4
G0	0.05	0	0	17.2±2.1
G5	0.05	0.05	0.0311	11.8±1.9
G10	0.05	0.10	0.0700	8.2±1.1
G15	0.05	0.15	0.1117	6.9±0.8
G20	0.05	0.20	0.1488	5.6±1.2
G25	0.05	0.25	0.1743	5.4±0.6
G30	0.05	0.30	0.2216	5.3±0.4
G35	0.05	0.35	0.2689	-
G40	0.05	0.40	0.3216	-

a. Feed molar ratio of cross-linker to total amines

b. Feed molar ratio of DHBA to total amines

c. Found molar ratio of DHBA to total amines by a modified NMR analysis

[00064] The strength of iron chelation is an important parameter for iron chelating materials; however, affinity cannot be calculated for materials in the conventional sense. Since the polymers are cross-linked particles, the chelation between the polymer materials and iron ions presents a heterogeneous system and direct equilibrium constants are not obtainable. Thus, the term iron affinity index was used to assess how strong the polymers bind and trap iron relative to a reference iron chelator with a documented stability constant. The iron affinity index was determined by a ligand competition method in equilibrium with EDTA (iron stability constant 10^{25}). The iron affinity index was calculated based on the equation for the calculation of stability constant as described in the preceding materials and methods part entitled “Determination of the Iron Affinity Index”. All the polymers with various DHBA contents showed higher iron affinity indices than EDTA (Fig. 2). The G10 sample had the highest iron affinity index (32.2), which indicated that the iron affinity of G10 polymer is 10^7 times stronger than EDTA (Log stability constant is 25.1, also shown in Fig. 2 for comparison). For all the other samples tested, they all showed at least 10^3 times stronger of iron affinity than EDTA. As the DHBA content increased from 5 to 30%, the affinity indexes of polymers decreased from

32.2 to 28.1. It should be noted that, theoretically, all the samples should have almost the same affinity indices, since the intrinsic affinity indexes of the DHBA groups in different samples were the same. As the DHBA content increased; however, the increased hydrophobicity of the polymers may have hindered Fe^{3+} access or coordination, hence reducing the apparent iron affinity indexes based on the calculation.

[00065] The iron sequestration capacity, which describes the maximum iron adsorption by the polymers, was also investigated. In order to reach the maximum iron sequestration, all the samples were incubated in a Fe^{3+} solution for one week. The theoretical and experimental iron sequestration capacities of the polymers with various DHBA contents were determined (Fig. 3). As the DHBA content increased, the experimental iron sequestration capacities also went up for low DHBA conjugation (5-20%), and reached a plateau (20-30%) around 20 mg Fe/g polymer. For all the samples tested, only the samples with low DHBA content achieved the theoretical iron sequestration capacities. The increased hydrophobicity of the polymers at higher DHBA conjugation percentages probably limited Fe^{3+} access to the gel particle interior. It is noteworthy that after the polymers containing chelated iron were incubated with fresh PBS containing 2 mM EDTA for 1 week, iron was not detectable in the medium by ICP-OES (data not shown), indicating that iron sequestration by the polymers is not reversible.

EXAMPLE 2: PAI-DHBA EXHIBITED HIGH SELECTIVITY FOR IRON

Materials and Methods

[00066] The iron selectivity of PAI-DHBA polymer was determined in the presence of copper, zinc, manganese, calcium, nickel and potassium. A solution containing all these metal ions, each at a concentration of 0.4 mM, was prepared in a phosphate buffer at pH 7.2 containing 2 mM EDTA. A predetermined amount of polymer was added into the solution and incubated at 25 °C for 5 days. The concentration of each metal ion remaining in solution was determined by ICP-OES. For the selectivity in M63 media, a predetermined amount of polymer was added into the M63 solution and incubated at 25 °C for 3 days. The concentrations of the metals (Fe^{3+} and Mg^{2+}) remaining in the solution were determined by ICP-OES.

Results

[00067] Selectivity to iron is especially important for the application of iron sequestering polymers in the biological field. Poor selectivity may affect the bioavailability or the balance of essential metal ions such as Cu^{2+} , Zn^{2+} , Ca^{2+} , Mn^{2+} , Ni^{2+} , or K^{+} . The influence of other metals on the sequestration of Fe^{3+} by the polymers was investigated using a multi-metal system. The concentration of each metal was fixed at 0.4 mM, and the metal/polymer ratio was fixed at 0.2 mmol per gram of polymer. All the samples absorbed almost 100% of the iron present in the media while typically the absorption for other essential metals was considerably lower, demonstrating high selectivity for iron (Fig. 4).

[00068] The selectivity of the polymer (G25) was also tested in the M63 media used in the *P.aeruginosa* studies. The M63 media only contained Fe^{3+} and Mg^{2+} metals. All the Fe^{3+} and only about 12% of the Mg^{2+} in the solution were sequestered by the polymer (Fig. 5). When considering swelling of the polymer, Mg^{2+} is likely primarily physically absorbed with imbibed water, rather than specifically chelated.

EXAMPLE 3: SYNTHESIS AND CHARACTERIZATION OF PAA-DHBA, PEI-DHBA, AND PLL-DHBA

Materials and Methods

Preparation of water soluble PAA-DHBA8

[00069] NHS-activated DHBA was synthesized before preparing the polymer. A solution of DHBA (308 mg, 2 mmol) and NHS (345 mg, 3 mmol) in 5 mL of DMF was mixed with a solution of EDC (402 mg, 2.1 mmol) in 3 mL of DMF. The mixture was stirred at room temperature for 8 h and used for the next step without any purification. Polyallylamine (1140 mg, 20 mmol amine groups) was dissolved in 200 mL $\text{H}_2\text{O}/\text{DMF}$ (50/50 v/v) solution by stirring. Under the protection of nitrogen, the as-prepared NHS-activated DHBA solution was added dropwise into the polyallylamine solution with vigorous stirring. After purged with nitrogen for 1 h, the solution was sealed and stirred at room temperature under the protection of light for 24 h. Then the solution was acidified by adding 15 mL of 2M HCl and transferred into a dialysis bag (MWCO 3400) to remove unreacted agents under the protection of nitrogen. After 3 days of

dialysis, the purified polymer was obtained by lyophilization. The DHBA conjugated ratio was 8% as analyzed by NMR.

Preparation of water soluble PLL-DHBA10

[00070] NHS-activated DHBA was synthesized before preparing the polymer. A solution of DHBA (308 mg, 2 mmol) and NHS (345 mg, 3 mmol) in 5 mL of DMF was mixed with a solution of EDC (402 mg, 2.1 mmol) in 3 mL of DMF. The mixture was stirred at room temperature for 8 h and used for the next step without any purification. Poly-L-Lysine (1460 mg, 10 mmol amine groups) was dissolved in 300 mL H₂O/DMF (50/50 v/v) solution by stirring. Under the protection of nitrogen, the as-prepared NHS-activated DHBA solution was added dropwise into the Poly-L-Lysine (PLL) solution with vigorous stirring. After purged with nitrogen for 1 h, the solution was sealed and stirred at room temperature under the protection of light for 24 h. Then the solution was acidified by adding 10 mL of 2M HCl and transferred into a dialysis bag (MWCO 3400) to remove unreacted agents under the protection of nitrogen. After 3 days of dialysis, the purified polymer was obtained by lyophilization. The DHBA conjugated ratio was 10% as analyzed by NMR.

Preparation of water soluble PEI-DHBA13

[00071] NHS-activated DHBA was synthesized before preparing the polymer. A solution of DHBA (308 mg, 2 mmol) and NHS (345 mg, 3 mmol) in 5 mL of DMF was mixed with a solution of EDC (402 mg, 2.1 mmol) in 3 mL of DMF. The mixture was stirred at room temperature for 8 h and used for the next step without any purification. Polyethyleneimine (1460 mg, 10 mmol amine groups) was dissolved in 300 mL H₂O/DMF (50/50 v/v) solution by stirring. Under the protection of nitrogen, the as-prepared NHS-activated DHBA solution was added dropwise into the polyethylenimine solution with vigorous stirring. After purged with nitrogen for 1 h, the solution was sealed and stirred at room temperature under the protection of light for 24 h. Then the solution was acidified by adding 20 mL of 2M HCl and transferred into a dialysis bag (MWCO 3400) to remove unreacted agents under the protection of nitrogen. After 3 days of dialysis, the purified polymer was obtained by lyophilization. The DHBA conjugated ratio was 13% as analyzed by NMR. The preceding methods are one example of synthesizing the

polymeric chelators of the present invention, and one of skill in the art would appreciate that other methods could be used.

Iron Stability Constant Determination

[00072] The polymers, as prepared above, were tested to determine the iron stability constant for each polymer. The competitive chelation of iron by the polymers in equilibrium with EDTA was used to determine the stability constant. Briefly, competition experiments were performed with 10 mL samples that contained ferric ion (0.1 mM), iron binding polymer (0.3 mM) DHBA groups on the polymer), and EDTA (0.5 mM). The pH of the solution was adjusted to 5.0 and the solution was incubated at 25°C under the protection of light for 5 h. The concentration of Ferric-DHBA complex ($\text{Fe}(\text{DHBA})_3$) was determined using UV-vis spectroscopy, and the concentration of ferric-EDTA complex ($\text{Fe}(\text{EDTA})$), DHBA, and EDTA could be calculated accordingly based on equations (1), (2) and (3) above.

Selectivity Studies

[00073] PAA-DHBA8, PEI-DHBA9 and PLL-DHBA10 were subjected to iron selectivity testing. The selectivity of the polymers was determined in the presence of copper, zinc, manganese, nickel, calcium and magnesium. A solution (20 mL) containing all of these metal ions (1 mM), iron binding polymer (total DHBA concentration 3 mM) and EDTA (6 mM) was prepared in a simulated intestinal fluid (pH 6.5, without lecithin and sodium taurocholate). The solution was incubated at 37°C under the protection of light for 8 h. Then the solution was transferred to a dialysis bag (MWCO 3400) and dialysed for 48 h. The metal concentration in the solution was determined by ICP-OES.

Results

[00074] Iron binding capacities and iron stability constants for the polymers are shown in Table 2 for different conjugation ratios and different polymers.

Table 2: Iron Binding Capacities and Iron Stability Constants for Various Polymers

Polymer	DHBA Conjugation Ratio	Molecular Weight (kDa)	Iron Binding Capacity (mg/g)	Iron Stability Constant
PAA-DHBA8	8%	17.5	14.5	49
PAA-DHBA-10	10%	17.5	17.3	49
PEI-DHBA9	9%	25	18.1	46
PEI-DHBA13	13%	25	25.1	46
PLL-DHBA10	10%	19.5	10	47
PLL-DHBA12	12%	19.5	11.8	47

[00075] The results of the iron selectivity testing are shown in Fig. 8.

EXAMPLE 3: IRON DEPLETION USING PAI-DHBA or PLL-DHBA INHIBITS FUNGAL GROWTH

Materials and Methods

[00076] Susceptibility testing was performed according to the CLSI M-27 A3 broth microdilution reference method for yeasts and M38-A2 method for molds. For *Blastomyces*, *Histoplasma*, and *Cryptococcus* isolates testing was performed by macro dilution but still using the M38-A2 method. Results of investigational agents reported in mg/ml. Those of comparators fluconazole (FLU), posaconazole (POS), and voriconazole (VOR) are reported in mcg/ml. Numbers after the identified test article indicate the time point measured (e.g. 24 hours, 48 hours, etc.).

Table 3: Effect of PLL on Fungal Growth

ISOLATES		PLL 24	PLL 24	PLL 48	PLL 48	PLL 72	PLL 72	PLL 96	PLL 96
		50%	100 %	50%	100 %	50%	100 %	50%	100 %
CANDIDA PARAPSILOSIS	QC	≤0.0 1	≤0.0 1	≤0.0 1	≤0.0 1	---	---	---	---
CANDIDA KRUSEI	QC	≤0.0 1	≤0.0 1	≤0.0 1	≤0.0 1	---	---	---	---
PAEC	QC	≤0.0 1	≤0.0 1	≤0.0 1	≤0.0 1	---	---	---	---
CANDIDA ALBICANS	CA 1	>5.0	>5.0	>5.0	>5.0	---	---	---	---
CANDIDA ALBICANS	CA 2	>5.0	>5.0	>5.0	>5.0	---	---	---	---
CANDIDA ALBICANS	CA 3	>5.0	>5.0	>5.0	>5.0	---	---	---	---
CRYPTOCOCCUS NEOFORMANS	CN 1	---	---	≤0.0 1	≤0.0 1	≤0.0 1	≤0.0 1	---	---
CRYPTOCOCCUS NEOFORMANS	CN 2	---	---	≤0.0 1	≤0.0 1	≤0.0 1	≤0.0 1	---	---
CRYPTOCOCCUS NEOFORMANS	CN 3	---	---	≤0.0 1	≤0.0 1	≤0.0 1	≤0.0 1	---	---
A. FUMIGATUS	AF 1	>5.0	>5.0	>5.0	>5.0	---	---	---	---
A. FUMIGATUS	AF 2	>5.0	>5.0	>5.0	>5.0	---	---	---	---
A. FUMIGATUS	AF 3	>5.0	>5.0	>5.0	>5.0	---	---	---	---
FUSARIUM OXYSPORUM	FO 1	≤0.0 1	≤0.0 1	≤0.0 1	≤0.0 1	---	---	---	---
FUSARIUM OXYSPORUM	FO 2	≤0.0 1	≤0.0 1	≤0.0 1	≤0.0 1	---	---	---	---
FUSARIUM SOLANI	FS1	≤0.0 1	≤0.0 1	≤0.0 1	≤0.0 1	---	---	---	---
RHIZOPU ORYZAE	RO 1	≤0.0 1	≤0.0 1	≤0.0 1	≤0.0 1	---	---	---	---
RHIZOPU ORYZAE	RO 2	≤0.0 1	≤0.0 1	≤0.0 1	≤0.0 1	---	---	---	---
RHIZOPU ORYZAE	RO 3	≤0.0 1	≤0.0 1	>5	>5	---	---	---	---
SCEDOSPORIUM SP.	S1	---	---	---	---	≤0.0 1	≤0.0 1	≤0.0 1	≤0.0 1
SCEDOSPORIUM SP.	S2	---	---	---	---	≤0.0	≤0.0	≤0.0	≤0.0

						1	1	1	1
LOMENTOSPORA PROLIFICANS	LP1	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	---	---	---	---
BLASTOMYCES DERMATITIDIS	BD 1	---	---	---	---	---	---	≤ 0.0 1	≤ 0.0 1
BLASTOMYCES DERMATITIDIS	BD 2	---	---	---	---	---	---	≤ 0.0 1	≤ 0.0 1
BLASTOMYCES DERMATITIDIS	BD 3	---	---	---	---	---	---	≤ 0.0 1	≤ 0.0 1

Table 4: Effect of PLL-DHBA on Fungal Growth

ISOLATES		PLL- DHB A 24	PLL- DHB A 24	PLL- DHB A 48	PLL- DHB A 48	PLL- DHB A 72	PLL- DHB A 72	PLL- DHB A 96	PLL- DHB A 96
		50%	100 %	50%	100 %	50%	100 %	50%	100 %
CANDIDA PARAPSILOSIS	QC	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	---	---	---	---
CANDIDA KRUSEI	QC	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	---	---	---	---
PAEC	QC	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	---	---	---	---
CANDIDA ALBICANS	CA 1	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	---	---	---	---
CANDIDA ALBICANS	CA 2	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	---	---	---	---
CANDIDA ALBICANS	CA 3	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	---	---	---	---
CRYPTOCOCCUS NEOFORMANS	CN 1	---	---	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	---	---
CRYPTOCOCCUS NEOFORMANS	CN 2	---	---	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	---	---
CRYPTOCOCCUS NEOFORMANS	CN 3	---	---	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	---	---
A. FUMIGATUS	AF 1	---	---	>5	>5	---	---	---	---
A. FUMIGATUS	AF 2	---	---	>5	>5	---	---	---	---
A. FUMIGATUS	AF 3	---	---	>5	>5	---	---	---	---
FUSARIUM OXYSPOURUM	FO 1	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	---	---	---	---

FUSARIUM OXYSPOURUM	FO 2	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	---	---	---	---
FUSARIUM SOLANI	FS1	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	---	---	---	---
RHIZOPU ORYZAE	RO 1	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	---	---	---	---
RHIZOPU ORYZAE	RO 2	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	---	---	---	---
RHIZOPU ORYZAE	RO 3	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	---	---	---	---
SCEDOSPORIUM SP.	S1	---	---	---	---	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1
SCEDOSPORIUM SP.	S2	---	---	---	---	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1
LOMENTOSPORA PROLIFICANS	LP1	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	---	---	---	---
BLASTOMYCES DERMATITIDIS	BD 1	---	---	---	---	---	---	≤ 0.0 1	≤ 0.0 1
BLASTOMYCES DERMATITIDIS	BD 2	---	---	---	---	---	---	≤ 0.0 1	≤ 0.0 1
BLASTOMYCES DERMATITIDIS	BD 3	---	---	---	---	---	---	≤ 0.0 1	≤ 0.0 1

Table 5: Effect of PAAm-DHBA on Fungal Growth

ISOLATES		PAA m- DHB A 24	PAA m- DHB A 24	PAA m- DHB A 48	PAA m- DHB A 48	PAA m- DHB A 72	PAA m- DHB A 72	PAA m- DHB A 96	PAA m- DHB A 96
		50%	100 %	50%	100 %	50%	100 %	50%	100 %
CANDIDA PARAPSILOSIS	QC	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	---	---	---	---
CANDIDA KRUSEI	QC	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	---	---	---	---
PAEC	QC	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	---	---	---	---
CANDIDA ALBICANS	CA 1	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	---	---	---	---
CANDIDA ALBICANS	CA 2	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	---	---	---	---
CANDIDA ALBICANS	CA 3	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	---	---	---	---
CRYPTOCOCCUS NEOFORMANS	CN 1	---	---	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	---	---

CRYPTOCOCCUS NEOFORMANS	CN 2	---	---	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	---	---
CRYPTOCOCCUS NEOFORMANS	CN 3	---	---	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	---	---
A. FUMIGATUS	AF 1	---	---	≤ 0.0 1	≤ 0.0 1	---	---	---	---
A. FUMIGATUS	AF 2	---	---	≤ 0.0 1	≤ 0.0 1	---	---	---	---
A. FUMIGATUS	AF 3	---	---	≤ 0.0 1	≤ 0.0 1	---	---	---	---
FUSARIUM OXYSPOURUM	FO 1	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	---	---	---	---
FUSARIUM OXYSPOURUM	FO 2	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	---	---	---	---
FUSARIUM SOLANI	FS1	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	---	---	---	---
RHIZOPU ORYZAE	RO 1	0.15 6	5	0.31 2	>5	---	---	---	---
RHIZOPU ORYZAE	RO 2	≤ 0.0 1	2.5	≤ 0.0 1	>5	---	---	---	---
RHIZOPU ORYZAE	RO 3	1.25	5	0.62 4	>5	---	---	---	---
SCEDOSPORIUM SP.	S1	---	---	---	---	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1
SCEDOSPORIUM SP.	S2	---	---	---	---	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1
LOMENTOSPORA PROLIFICANS	LP1	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	≤ 0.0 1	---	---	---	---
BLASTOMYCES DERMATITIDIS	BD 1	---	---	---	---	---	---	≤ 0.0 1	≤ 0.0 1
BLASTOMYCES DERMATITIDIS	BD 2	---	---	---	---	---	---	≤ 0.0 1	≤ 0.0 1
BLASTOMYCES DERMATITIDIS	BD 3	---	---	---	---	---	---	≤ 0.0 1	≤ 0.0 1

Table 6: Effect of Fluconazole, Voriconazole, and Posaconazole on Fungal Growth

ISOLATES		FLU	POS	VOR I
CANDIDA PARAPSILOSIS	QC	0.5	0.06	≤ 0.0 3
CANDIDA KRUSEI	QC	16	0.25	0.12 5
PAEC	QC	ND	---	0.12 5

CANDIDA ALBICANS	CA 1	>64	---	---
CANDIDA ALBICANS	CA 2	0.25	---	---
CANDIDA ALBICANS	CA 3	≤ 0.1 25	---	---
CRYPTOCOCCUS NEOFORMANS	CN 1	2	---	---
CRYPTOCOCCUS NEOFORMANS	CN 2	2	---	---
CRYPTOCOCCUS NEOFORMANS	CN 3	4	---	---
A. FUMIGATUS	AF 1	---	---	0.25
A. FUMIGATUS	AF 2	---	---	0.25
A. FUMIGATUS	AF 3	---	---	0.5
FUSARIUM OXYSPOURUM	FO 1	---	---	2
FUSARIUM OXYSPOURUM	FO 2	---	---	8
FUSARIUM SOLANI	FS1	---	---	8
RHIZOPU ORYZAE	RO 1	---	0.5	---
RHIZOPU ORYZAE	RO 2	---	0.25	---
RHIZOPU ORYZAE	RO 3	---	0.5	---
SCEDOSPORIUM SP.	S1	---	---	0.12 5
SCEDOSPORIUM SP.	S2	---	---	0.5
LOMENTOSPORA PROLIFICANS	LP1	---	---	>16
BLASTOMYCES DERMATITIDIS	BD 1	---	---	0.25
BLASTOMYCES DERMATITIDIS	BD 2	---	---	0.25
BLASTOMYCES DERMATITIDIS	BD 3	---	---	0.25

Table 7: Effect on Fungal Growth: PLL-DHBA vs. Voriconazole and Posaconazole

Species	No.	50%	100%	VOR 100%	POS 100%
<i>P. variotii</i>	PAEC	≤0.01	≤0.01	0.125	ND
<i>Fusarium sp.</i>	FS-1	≤0.01	≤0.01	16	ND
<i>Fusarium sp.</i>	FS-2	≤0.01	≤0.01	>16	ND
<i>Fusarium sp.</i>	FS-3	≤0.01	≤0.01	8	ND
<i>Fusarium sp.</i>	FS-4	≤0.01	≤0.01	8	ND
<i>Fusarium sp.</i>	FS-5	≤0.01	≤0.01	8	ND
<i>Fusarium sp.</i>	FS-6	≤0.01	≤0.01	>16	ND
<i>Fusarium sp.</i>	FS-7	≤0.01	≤0.01	>16	ND
<i>Fusarium sp.</i>	FS-8	≤0.01	≤0.01	>16	ND
<i>Fusarium sp.</i>	FS-9	≤0.01	≤0.01	>16	ND
<i>Fusarium sp.</i>	FS-10	≤0.01	≤0.01	16	ND
<i>Rhizopus oryzae</i>	RO-1	≤0.01	≤0.01	ND	0.5
<i>Rhizopus oryzae</i>	RO-2	≤0.01	0.02	ND	0.25
<i>Rhizopus oryzae</i>	RO-3	≤0.01	0.02	ND	2
<i>Rhizopus oryzae</i>	RO-4	≤0.01	0.02	ND	4
<i>Rhizopus oryzae</i>	RO-5	≤0.01	0.02	ND	1
<i>Rhizopus oryzae</i>	RO-6	≤0.01	≤0.01	ND	0.25
<i>Rhizopus oryzae</i>	RO-7	≤0.01	≤0.01	ND	0.5
<i>Rhizopus oryzae</i>	RO-8	≤0.01	≤0.01	ND	0.25
<i>Rhizopus oryzae</i>	RO-9	≤0.01	≤0.01	ND	0.5
<i>Rhizopus oryzae</i>	RO-10	≤0.01	0.02	ND	1
<i>Saksenaea sp.</i>	SV-1	0.04	0.15	ND	0.5
<i>Saksenaea sp.</i>	SV-2	0.02	0.04	ND	1
<i>Scedosporium sp.</i>	Ss-1	≤0.01	≤0.01	0.25	ND
<i>Scedosporium sp.</i>	Ss-2	≤0.01	≤0.01	1	ND
<i>Scedosporium sp.</i>	Ss-3	≤0.01	≤0.01	1	ND
<i>Scedosporium sp.</i>	Ss-4	≤0.01	≤0.01	0.5	ND
<i>Scedosporium sp.</i>	Ss-5	≤0.01	≤0.01	>16	ND
<i>Scedosporium sp.</i>	Ss-6	≤0.01	≤0.01	1	ND
<i>Scedosporium sp.</i>	Ss-7	≤0.01	≤0.01	>16	ND
<i>Scedosporium sp.</i>	Ss-8	≤0.01	≤0.01	0.25	ND
<i>Scedosporium sp.</i>	Ss-9	≤0.01	≤0.01	0.125	ND
<i>Scedosporium sp.</i>	Ss-10	≤0.01	≤0.01	0.5	ND
<i>Blastomyces dermatitidis</i>	BD-1	≤0.01	≤0.01	0.125	ND
<i>Blastomyces dermatitidis</i>	BD-2	≤0.01	≤0.01	0.25	ND
<i>Blastomyces dermatitidis</i>	BD-3	≤0.01	≤0.01	0.125	ND
<i>Blastomyces dermatitidis</i>	BD-4	≤0.01	≤0.01	ND	ND
<i>Blastomyces</i>	BD-5	≤0.01	≤0.01	ND	ND

<i>dermatitidis</i>					
<i>Blastomyces dermatitidis</i>	BD-6	≤ 0.01	≤ 0.01	ND	ND
<i>Blastomyces dermatitidis</i>	BD-7	≤ 0.01	≤ 0.01	ND	ND
<i>Blastomyces dermatitidis</i>	BD-8	≤ 0.01	≤ 0.01	ND	ND
<i>Blastomyces dermatitidis</i>	BD-9	≤ 0.01	≤ 0.01	ND	ND
<i>Blastomyces dermatitidis</i>	BD-10	≤ 0.01	≤ 0.01	0.125	ND
<i>Coccidioides sp.</i>	Cs-1	≤ 0.01	≤ 0.01	0.125	ND
<i>Coccidioides sp.</i>	Cs-2	≤ 0.01	≤ 0.01	0.25	ND
<i>Coccidioides sp.</i>	Cs-3	≤ 0.01	≤ 0.01	0.125	ND
<i>Histoplasma capsulatum</i>	HC-1	≤ 0.01	≤ 0.01	0.125	ND
<i>Histoplasma capsulatum</i>	HC-2	≤ 0.01	≤ 0.01	<0.03	ND
<i>Histoplasma capsulatum</i>	HC-3	≤ 0.01	≤ 0.01	0.125	ND

Table 8: Effect on Fungal Growth: PLL-DHBA vs. Fluconazole

Species	No.	50%	100%	FLU 50%
<i>Candida parapsilosis</i>	QC	≤ 0.01	≤ 0.01	1
<i>Candida krusei</i>	QC	≤ 0.01	≤ 0.01	16
<i>Candida albicans</i>	CA-1	≤ 0.01	0.3	>64
<i>Candida albicans</i>	CA-2	≤ 0.01	0.02	>64
<i>Candida albicans</i>	CA-3	≤ 0.01	0.04	64
<i>Candida albicans</i>	CA-4	≤ 0.01	1.25	0.25
<i>Candida albicans</i>	CA-5	≤ 0.01	>5	0.25
<i>Candida albicans</i>	CA-6	≤ 0.01	5	≤ 0.125
<i>Candida albicans</i>	CA-7	≤ 0.01	1.25	≤ 0.125
<i>Candida albicans</i>	CA-8	≤ 0.01	2.5	≤ 0.125
<i>Candida albicans</i>	CA-9	≤ 0.01	2.5	>64
<i>Candida albicans</i>	CA-10	≤ 0.01	1.25	>64
<i>Candida glabrata</i>	CG-1	≤ 0.01	2.5	16
<i>Candida glabrata</i>	CG-2	≤ 0.01	0.02	32
<i>Candida glabrata</i>	CG-3	≤ 0.01	0.04	>64
<i>Candida parapsilosis</i>	CP-1	≤ 0.01	0.08	0.25

<i>Candida parapsilosis</i>	CP-2	≤ 0.01	0.04	4
<i>Candida parapsilosis</i>	CP-3	≤ 0.01	0.08	0.25
<i>Candida guilliermondii</i>	Cgu-1	≤ 0.01	0.15	2
<i>Candida guilliermondii</i>	Cgu-2	≤ 0.01	≤ 0.01	1
<i>Candida guilliermondii</i>	Cgu-3	≤ 0.01	≤ 0.01	1
<i>Cryptococcus neoformans</i>	CN-1	≤ 0.01	≤ 0.01	2
<i>Cryptococcus neoformans</i>	CN-2	≤ 0.01	≤ 0.01	1
<i>Cryptococcus neoformans</i>	CN-3	≤ 0.01	≤ 0.01	2
<i>Cryptococcus neoformans</i>	CN-4	≤ 0.01	≤ 0.01	0.5
<i>Cryptococcus neoformans</i>	CN-5	≤ 0.01	≤ 0.01	4
<i>Cryptococcus neoformans</i>	CN-6	≤ 0.01	≤ 0.01	0.5
<i>Cryptococcus neoformans</i>	CN-7	≤ 0.01	≤ 0.01	1
<i>Cryptococcus neoformans</i>	CN-8	≤ 0.01	≤ 0.01	16
<i>Cryptococcus neoformans</i>	CN-9	≤ 0.01	≤ 0.01	4
<i>Cryptococcus neoformans</i>	CN-10	≤ 0.01	≤ 0.01	2

Table 9: Effect on Fungal Growth: PAAm-DHBA vs. Voriconazole

Species	No.	50%	100%	VOR 100%
<i>P. variotii</i>	QC	≤ 0.01	≤ 0.01	0.125
<i>Aspergillus fumigatus</i>	AF-1	0.02	0.02	0.5
<i>Aspergillus fumigatus</i>	AF-2	0.02	0.02	0.25
<i>Aspergillus fumigatus</i>	AF-3	0.02	0.04	0.25
<i>Aspergillus</i>	AF-4	0.02	0.02	0.5

<i>fumigatus</i>				
<i>Aspergillus fumigatus</i>	AF-5	0.02	0.04	1
<i>Aspergillus fumigatus</i>	AF-6	0.02	0.02	0.5
<i>Aspergillus fumigatus</i>	AF-7	0.02	0.02	0.5
<i>Aspergillus fumigatus</i>	AF-8	0.02	0.02	0.25
<i>Aspergillus fumigatus</i>	AF-9	0.02	0.02	0.5
<i>Aspergillus fumigatus</i>	AF-10	0.02	0.02	0.5
<i>Aspergillus flavus</i>	Afl-1	0.02	0.08	1
<i>Aspergillus flavus</i>	Afl-2	0.02	0.04	0.5
<i>Aspergillus flavus</i>	Afl-3	0.02	0.04	0.5

Table 10: Effect on Fungal Growth: PLL vs. Fluconazole

Species	No.	50%	100%	FLU 50%
<i>Candida parapsilosis</i>	CP	≤0.01	≤0.01	1
<i>Candida krusei</i>	CK	≤0.01	≤0.01	16
<i>Candida albicans</i>	CA-1	5	>5	>64
<i>Candida albicans</i>	CA-2	0.08	1	>64
<i>Candida albicans</i>	CA-3	>5	>5	64
<i>Candida albicans</i>	CA-4	>5	>5	0.25
<i>Candida albicans</i>	CA-5	>5	>5	0.25
<i>Candida albicans</i>	CA-6	>5	>5	≤0.125
<i>Candida albicans</i>	CA-7	>5	>5	≤0.125
<i>Candida albicans</i>	CA-8	>5	>5	≤0.125
<i>Candida albicans</i>	CA-9	>5	>5	>64
<i>Candida albicans</i>	CA-10	2.5	>5	>64
<i>Candida glabrata</i>	CG-1	5	>5	16
<i>Candida glabrata</i>	CG-2	0.3	2.5	32
<i>Candida glabrata</i>	CG-3	2.5	>5	>64
<i>Candida parapsilosis</i>	CP-1	≤0.01	>5	0.25
<i>Candida parapsilosis</i>	CP-2	≤0.01	2.5	4
<i>Candida parapsilosis</i>	CP-3	≤0.01	5	0.25

<i>Candida guilliermondii</i>	Cgu-1	≤ 0.01	2.5	2
<i>Candida guilliermondii</i>	Cgu-2	≤ 0.01	≤ 0.01	1
<i>Candida guilliermondii</i>	Cgu-3	≤ 0.01	≤ 0.01	1

Results

[00077] Polymers that had the conjugated iron chelator DHBA showed a significant increase in antifungal activity when compared to polymers without iron chelator. For example, the concentrations required to eradicate or slow the growth of fungi was lower for PLL-DHBA than for PLL. In addition, polymers with conjugated DHBA showed broadspectrum activity across most all of these tested fungi, even highly drug resistant strains.

EXAMPLE 4: FORMULATIONS FOR TOPICAL USE

[00078] Antioxidants will be titrated into a topical formulation and to reduce the oxidation of DHBA. In particular, antioxidants that improve DHBA stability and do not interfere with iron binding will be identified. In addition, antioxidants that did not improve the solubility of iron will be identified.

EXAMPLE 5: EVALUATION OF AN IRON SEQUESTERANT POLYMER FOR THE PREVENTION OF CANDIDA DISSEMINATION FROM THE GASTROINTESTINAL TRACT

[00079] The objective of this study is to assess the ability of a polymeric chelator of the present disclosure in preventing dissemination of *C. albicans* from the GI tract in the setting of immunosuppression. To accomplish this, an established murine model of diet-associated GI colonization and dissemination will be utilized as described below.

[00080] Male BALB/c mice weighing ~25 grams will be used. Mice will be housed 5 animals per cage and will have access to food and water *ad libitum* throughout the course of the evaluations.

[00081] For immunosuppression, cyclophosphamide (150 mg/kg intraperitoneally) and prednisolone sodium succinate (50 mg/kg, subcutaneously) will be administered on days 14 and 17 post-infection relative to inoculation of *Candida* cells into the gastrointestinal tract. Starting on day 14 when immunosuppression begins, mice will receive drinking water with enrofloxacin

(50 ppM) as antibacterial prophylaxis. Mice will be placed on one of three purified rodent diets based on the commercially available AIN-93G diet (Dyets, Inc., Table 11) beginning 14 days prior to inoculation (day 0). One diet group will be the standard AIG-93G diet (3-8 ppm iron citrate base + 35 ppm iron citrate added), another the AIN-93G diet with a reduced iron citrate content (3-8 ppm), the third diet will consist of the AIG-93G diet with reduced iron citrate content plus the polymeric chelator of the present disclosure at concentrations of 0.5%, 1, %, 2.5%, or 5%, the fourth diet will consist of the AIG-93G diet with reduced iron citrate content plus an anti-fungal agent such as amphotericin B, abifungin, candicidin, filipin, hamycin, natamycin, nystatin, rimocidin, bifonazole, butoconazole, clotrimazole, econazole, fenticonazole, isoconazole, ketoconazole, luliconazole, miconazole, omoconazole, oxiconazole, sertaconazole, sulconazole, tioconazole, albaconazole, efinaconazole, epoxiconazole, fluconazole, itraconazole, propiconazole, ravuconazole, terconazole, voriconazole, posaconazole, amorolfen, butenafine, naftifine, anidulafungin, caspofungin, micafungin, terbinafine, cyclopirox, flucytosine, griseofulvin, haloprogin, tolinaftate, and undecylenic acid or similar antifungal agent, the fifth diet will consist of the AIG-93G diet with reduced iron citrate content plus a combination treatment comprising the anti-fungal agent and the polymeric chelator of the present disclosure at concentrations of 0.5%, 1, %, 2.5%, or 5%, and the sixth diet will consist of the AIG-93G diet with reduced iron citrate content plus an iron chelating compound such as deferoxamine or ethylenediamine tetraacetic acid (EDTA) (referred to herein as an "iron-chelating agent"). The various groups of infected and uninfected groups of mice are shown in Table 12.

[00082] Mice that receive the Group 1 and 2 diets will receive the standard diet beginning two weeks prior to inoculation. These diets will continue through the end of the study (day 21). Mice that receive the Groups 3-6 diet will be administered the Group 2 diet (AIN-93G with 3-5 ppm iron citrate) for 11 days prior to inoculation. Three days prior to inoculation, these mice will be switched to one of the Groups 3-6 diet (addition of the polymeric chelator, anti-fungal agent or combination thereof, and iron-chelating agent, respectively). They will then be maintained on this diet until day 21 post-inoculation.

Food and water will be removed from the cages at approximately 16 and 4 hours, respectively, prior to infection. *C. albicans* SC5314 will be the strain, as this is the most commonly used wild-type *C. albicans* strain and reference strain from which most other *C. albicans* strains are derived. In addition, this strain was used to establish this diet associated model of GI candidiasis.

The isolate will be sub-cultured at 37°C for 48 hours on Sabouraud dextrose agar twice. Prior to inoculation, the isolate taken from the second subculture will be placed into brain heart infusion broth and grown overnight at 37°C with shaking at 200 rpm; cells will then be collected by centrifugation and washed three times in sterile saline. Cells will then be serially diluted and number of cells/mL will be determined using a hemocytometer. The inoculum will be adjusted to 1.0×10^8 cells/mL in sterile PBS and 0.5 mL of a target inoculum size of 5×10^7 *C. albicans* cells will be used to inoculate each mouse by oral gavage. Inoculum viability will be confirmed by carrying out serial dilutions of the inoculum in sterile PBS, plating 100 µL in duplicate onto Sabouraud dextrose agar, and counting the number of colonies following growth at 37°C overnight.

[00083] Throughout the course of the experiment, mice will be monitored at least twice daily to prevent and minimize unnecessary pain or distress post-inoculation. Moribund animals will be identified by the following criteria: (1) ruffled/matted fur; (2) hypothermia; (3) weight loss (e.g., $\geq 20\%$); (4) inability to eat or drink; and (5) hunched posture. Any animal demonstrating ≥ 1 of these criteria will be humanely euthanized by isoflurane anesthesia followed by exsanguination via cardiac puncture and cervical dislocation.

Table 11. Composition of the purified diet

Ingredient	g/kg
Cornstarch	529.5
Casein	200.0
Sucrose	100.0
Soybean oil	70.0
Cellulose	50.0
Mineral mix	35.0
Vitamin mix	10.0
L-Cystine	3.0
Choline bitartrate	2.5

Table 12. Diet groups, time points, and number of mice per group

Diet Group	Time Points & Mice Per Group		
	Day 0 (day of inoculation)	Day 14	Day 21
<i>Infected Mice</i>			
AIN-93G Standard Diet (Group 1)	5	5	10
AIN-93G reduced iron citrate (Group 2)	5	5	10
AIN93G reduced iron citrate + 0.5%, 1, %, 2.5%, or 5% polymeric chelator (Group 3)	5	5	10
AIN93G reduced iron citrate + anti-fungal agent (Group 4)	5	5	10
AIN93G reduced iron citrate + anti-fungal agent + 0.5%, 1, %, 2.5%, or 5% polymeric chelator (Group 5)	5	5	10
AIN93G reduced iron citrate + iron-chelating agent (Group 6)	5	5	10
<i>Uninfected Control Mice</i>			
AIN-93G Standard Diet (Group 1)	---	---	5
AIN-93G reduced iron citrate (Group 2)	---	---	5
AIN93G reduced iron citrate + 0.5%, 1, %, 2.5%, or 5% polymeric chelator (Group 3)	---	---	5
AIN93G reduced iron citrate + anti-fungal agent (Group 4)			5
AIN93G reduced iron citrate + anti-fungal agent + 0.5%, 1, %, 2.5%, or 5% polymeric chelator (Group 5)			5
AIN93G reduced iron citrate + iron-chelating agent (Group 6)			5

[00084] Mice will be placed in wired bottom cages with paper liners for 24 hours so that feces could be collected immediately prior to inoculation (Day 0) and on days 14 and 21 to determine the level of colonization by measuring colony-forming units*. On days 0 (prior to inoculation), and 14 (start of immune suppression) 5 mice in each of the infected groups will be humanely euthanized as described above. On day 21, 10 mice in each of the infected groups and 5 in each of the uninfected groups will be humanely euthanized. On each of the days of

euthanasia, the liver and kidneys will be aseptically removed to measure fungal burden and assess for dissemination of *C. albicans* from the GI tract. The organs will be weighed, and will then be homogenized and plated onto Sabouraud dextrose agar. The plates will be incubated at 37°C, and the number of CFU/g will be calculated.

[00085] Differences in fungal burden (CFU/g) between two groups of mice will be compared using a t-test or the Wilcoxon matched-pairs test. For comparisons involving more than two groups, ANOVA with Tukey's post-test for multiple comparisons for normally distributed data or the Kruskal-Wallis Test with Dunn's post-test for multiple comparisons for non-normally distributed data will be used. A p-value < 0.05 will be considered statistical significance for all comparisons.

[00086] Therefore, the present invention is well adapted to attain the ends and advantages mentioned as well as those that are inherent therein. While numerous changes may be made by those skilled in the art, such changes are encompassed within the spirit of this invention as illustrated, in part, by the appended claims.

What is claimed is:

1. A composition comprising:
a polymer covalently coupled to a chelator, wherein the polymer comprises a polyamine, wherein the chelator has a benzene ring with more than one hydroxyl group at any position that is free; and
an antifungal agent.
2. The composition of claim 1 wherein the chelator is covalently coupled to a primary amine of the polyamine through an amide bond.
3. The composition of claim 1 wherein the polyamine is selected from the group consisting of polyallylamine (PAA), polylysine (PLL), and polyethylenimine (PEI).
4. The composition of claim 1 wherein the polyamine is polyallylamine.
5. The composition of claim 1 wherein the chelator is covalently coupled to a primary amine of the polyamine through an amine bond.
6. The composition of claim 1 wherein the chelator is 2,3 dihydroxybenzaldehyde or 2,3 dihydroxybenzoic acid.
7. The composition of claim 1 wherein the chelator is capable of chelating iron.
8. The composition of claim 1 wherein the antifungal agent is selected from the group consisting of amphotericin B, abifungin, candicidin, filipin, hamycin, natamycin, nystatin, rimocidin, bifonazole, butoconazole, clotrimazole, econazole, fenticonazole, isoconazole, ketoconazole, luliconazole, miconazole, omoconazole, oxiconazole, sertaconazole, sulconazole, tioconazole, albaconazole, efinaconazole, epoxiconazole, fluconazole, itraconazole, propiconazole, ravuconazole, terconazole, voriconazole, posaconazole, amorolfine, butenafine, naftifine, anidulafungin, caspofungin, micafungin, terbinafine, cyclopirox, flucytosine, griseofulvin, haloprogin, tolnaftate, and undecylenic acid.
9. The composition of any of claims 1-8 wherein the composition is formulated for topical administration.

10. The composition of any of claims 1-8 is a hydrogel.
11. A composition comprising:
 - a plurality of cross-linked polymers, wherein the plurality of cross-linked polymers comprise a polyamine;
 - one or more chelators, wherein the one or more chelators are covalently coupled to one or more primary amines, respectively, of the polyamine through one or more amide or amine bonds, respectively, wherein each of the one or more chelators has a benzene ring with more than one hydroxyl group at any position that is free, wherein the plurality of cross-linked polymers are cross-linked independent from any cross-linking due to the chelator binding a metal ion; and
 - an antifungal agent.
12. The composition of claim 11, wherein the polyamine is selected from the group consisting of polyallylamine (PAA), polylysine (PLL), and polyethylenimine (PEI).
13. The composition of claim 11 wherein the one or more chelators is 2,3 dihydroxybenzaldehyde or 2,3 dihydroxybenzoic acid.
14. The composition of claim 11 wherein the antifungal agent is selected from the group consisting of amphotericin B, abifungin, candicidin, filipin, hamycin, natamycin, nystatin, rimocidin, bifonazole, butoconazole, clotrimazole, econazole, fenticonazole, isoconazole, ketoconazole, luliconazole, miconazole, omoconazole, oxiconazole, sertaconazole, sulconazole, tioconazole, albaconazole, efinaconazole, epoxiconazole, fluconazole, itraconazole, propiconazole, ravuconazole, terconazole, voriconazole, posaconazole, amorolfine, butenafine, naftifine, anidulafungin, caspofungin, micafungin, terbinafine, cyclopirox, flucytosine, griseofulvin, haloprogin, tolnaftate, and undecylenic acid.
15. The composition of claim 11 wherein the polyamine is polyallylamine and the one or more chelators is 2,3 dihydroxybenzaldehyde.
16. The composition of any of claims 11-15 wherein the composition is formulated for topical administration.

17. The composition of any of claims 11-15 is a hydrogel.
18. A method for treating a subject with a fungal infection comprising:

administering a composition to a site on the subject harboring the fungal infection, wherein the composition comprises a polymer covalently coupled to a chelator, wherein the polymer comprises a polyamine, and wherein the chelator has a benzene ring with more than one hydroxyl group at any position that is free.
19. The method of claim 18 wherein the site is an external wound on the subject.
20. The method of claim 18 wherein the site is a mucosal surface.
21. The method of claim 20 wherein the mucosal surface is bronchial, endometrial, gastric, penile, vaginal, olfactory, intestinal, anal, or oral.
22. The method of claim 18 wherein the fungal infection is caused by at least one of the group consisting of: *Coccidioides* species such as *Coccidioides immitis*; *Candida* species such as *Candida parapsilosis*, *Candida krusei*, *Candida glabrata*, *Candida guilliermondii*, and *Candida albicans*; *Paecilomyces* species such as *Paecilomyces variotii*; *Cryptococcus neoformans*; *Cryptococcus gattii*; *Aspergillus* species such as *Aspergillus fumigatus* and *Aspergillus flavus*; *Fusarium* species such as *Fusarium oxysporum* and *Fusarium solani*; *Rhizopus oryzae*; *Scedosporium* species such as *Scedosporium prolificans* and *Scedosporium apiospermum*; *Lomentospora prolificans*; *Blasotmyces dermatitidis*; *Pneumocystis jirovecii*; *Sporothrix schenckii*; *Saksenaea* species such as *Saksenaea vasiformis*; *Histoplasma capsulatum*; and ringworm.
23. The method of claim 18 wherein the subject was previously treated with an antifungal agent prior to administering the composition.
24. The method of claim 23 wherein the antifungal agent is selected from the group consisting of amphotericin B, abifungin, candicidin, filipin, hamycin, natamycin, nystatin, rimocidin, bifonazole, butoconazole, clotrimazole, econazole, fenticonazole, isoconazole, ketoconazole, luliconazole, miconazole, omoconazole, oxiconazole, sertaconazole, sulconazole,

tioconazole, albaconazole, efinaconazole, epoxiconazole, fluconazole, itraconazole, propiconazole, ravuconazole, terconazole, voriconazole, posaconazole, amorolfine, butenafine, naftifine, anidulafungin, caspofungin, micafungin, terbinafine, cyclopirox, flucytosine, griseofulvin, haloprogin, tolnaftate, and undecylenic acid.

25. The method of claim 18 wherein the administering step is by topical application to the site.

26. The method of any of claims 18-25 wherein the chelator is covalently coupled to a primary amine of the polyamine polymer through an amine bond.

27. The method of any of claims 18-25 wherein the polyamine is selected from the group consisting of polyallylamine (PAA), polylysine (PLL), and polyethylenimine (PEI).

28. The method of any of claims 18-25 wherein the polyamine is polyallylamine.

29. The method of any of claims 18-25 wherein the chelator is 2,3 dihydroxybenzoic acid.

30. The method of any of claims 18-25 wherein the chelator is 2,3 dihydroxybenzaldehyde.

31. The method of any of claims 18-25 wherein the chelator is capable of chelating iron.

32. The method of any of claims 18-25 wherein the chelator is 2,3 dihydroxybenzaldehyde and the polyamine is polyallylamine.

33. The method of any of claims 18-25 further comprising the step of administering an antifungal to the site.

34. The method of any of claims 18-25 wherein the composition further comprises an antifungal.

35. The method of any of claims 18-25 further comprising the step of administering an antifungal to the site following administration of the composition.

36. The method of any of claims 18-25 wherein the composition is a hydrogel.

37. A method for preventing a fungal infection in a subject comprising:

administering a composition to a site on the subject that is susceptible to the fungal infection, wherein the composition comprises a polymer covalently coupled to a chelator, wherein the polymer is a polyamine, and wherein the chelator has a benzene ring with more than one hydroxyl group at any position that is free.

38. The method of claim 37 wherein the site is a wound on the skin of the subject.

39. The method of claim 37 wherein the site is a mucosal surface.

40. The method of claim 39 wherein the mucosal surface is bronchial, endometrial, gastric, penile, vaginal, olfactory, intestinal, anal, or oral.

41. The method of claim 37 wherein the administering step is by topical application to the site.

42. The method of any of claims 37-41 wherein the chelator is covalently coupled to a primary amine of the polyamine polymer through an amine bond.

43. The method of any of claims 37-41 wherein the polyamine is selected from the group consisting of polyallylamine (PAA), polylysine (PLL), and polyethylenimine (PEI).

44. The method of any of claims 37-41 wherein the polyamine is polyallylamine.

45. The method of any of claims 37-41 wherein the chelator is 2,3 dihydroxybenzaldehyde.

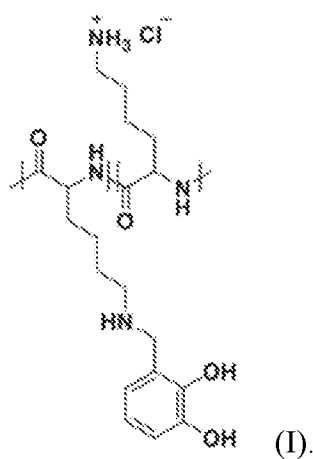
46. The method of any of claims 37-41 wherein the chelator is 2,3 dihydroxybenzoic acid.

47. The method of any of claims 37-41 wherein the chelator is capable of selectively chelating iron.

48. The method of any of claims 37-41 wherein the chelator comprises 2,3 dihydroxybenzaldehyde and the polyamine is polyallylamine.

49. The method of any of claims 37-41 wherein the composition is a hydrogel.

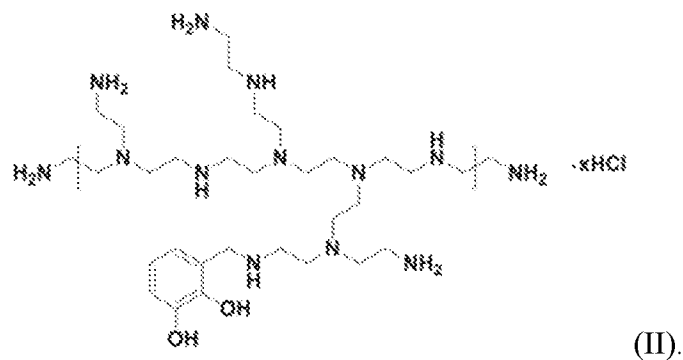
50. A polymeric metal sequestrant having a structure (I), wherein the structure (I) is



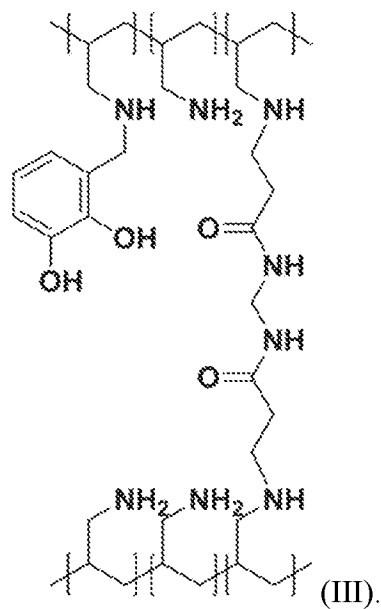
51. A method for treating a subject with a fungal infection comprising:
administering a composition to a site on the subject harboring the fungal infection, wherein the composition comprises the polymeric metal sequestrant of claim 50.

52. A composition comprising the polymeric metal sequestrant of claim 50 and an antifungal agent.

53. A polymeric metal sequestrant having a structure (II), wherein the structure (II) is



54. A method for treating a subject with a fungal infection comprising:
 administering a composition to a site on the subject harboring the fungal infection, wherein the composition comprises the polymeric metal sequestrant of claim 53.
55. A composition comprising the polymeric metal sequestrant of claim 53 and an antifungal agent.
56. A polymeric metal sequestrant having a structure (III), wherein the structure (III) is



57. A method for treating a subject with a fungal infection comprising:

administering a composition to a site on the subject harboring the fungal infection, wherein the composition comprises the polymeric metal sequestrant of claim 56.

58. A composition comprising the polymeric metal sequestrant of claim 56 and an antifungal agent.

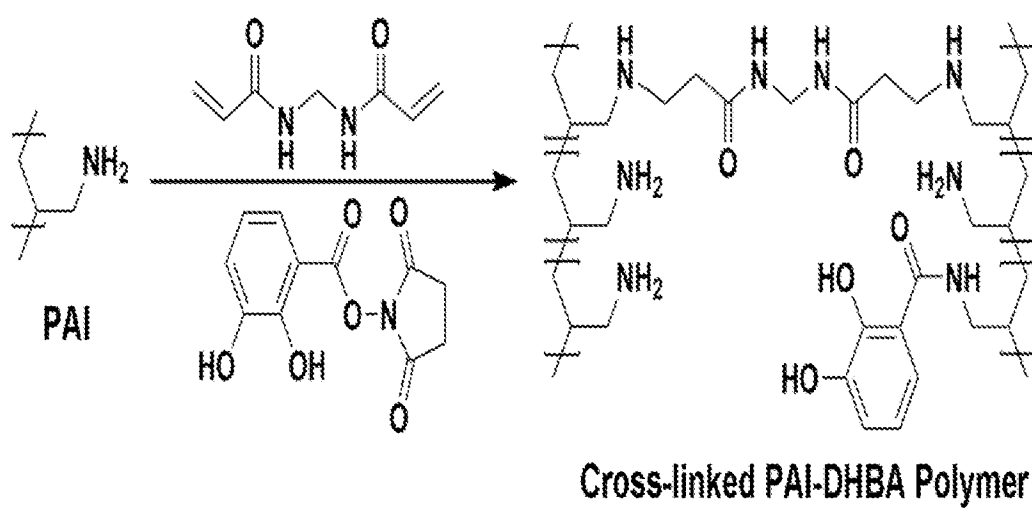
59. The composition of any of claims 52, 55, or 58 wherein the antifungal agent is selected from the group consisting of amphotericin B, abifungin, candicidin, filipin, hamycin, natamycin, nystatin, rimocidin, bifonazole, butoconazole, clotrimazole, econazole, fenticonazole, isoconazole, ketoconazole, luliconazole, miconazole, omoconazole, oxiconazole, sertaconazole, sulconazole, tioconazole, albaconazole, efinaconazole, epoxiconazole, fluconazole, itraconazole, propiconazole, ravuconazole, terconazole, voriconazole, posaconazole, amorolfine, butenafine, naftifine, anidulafungin, caspofungin, micafungin, terbinafine, cyclopirox, flucytosine, griseofulvin, haloprogin, tolnaftate, and undecylenic acid.

60. A method for treating a subject with a fungal infection comprising:

administering a composition to a site on the subject harboring the fungal infection, wherein the composition is that of claims 52, 55, or 58.

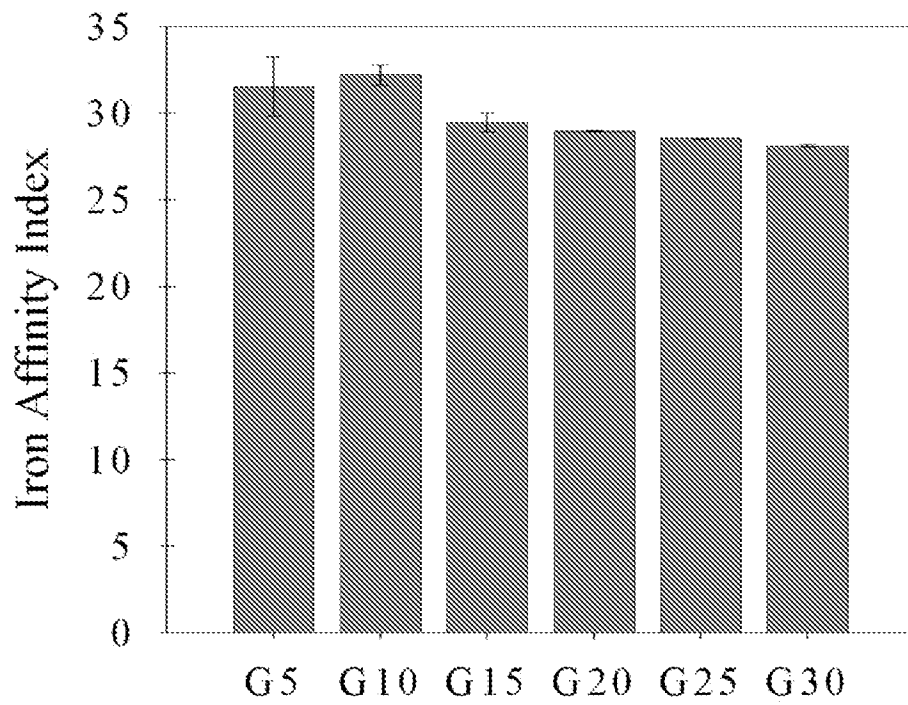
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Figure 1



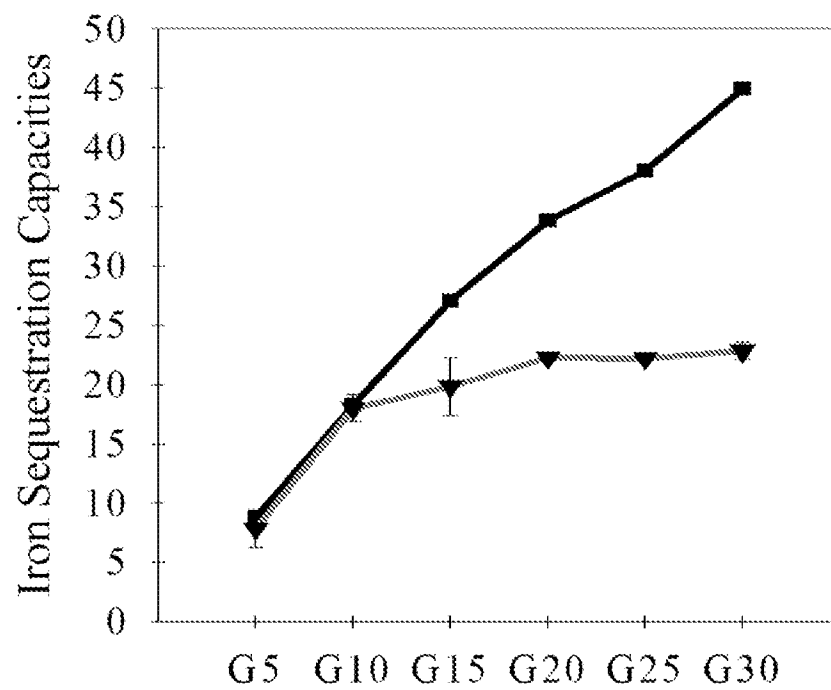
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Figure 2



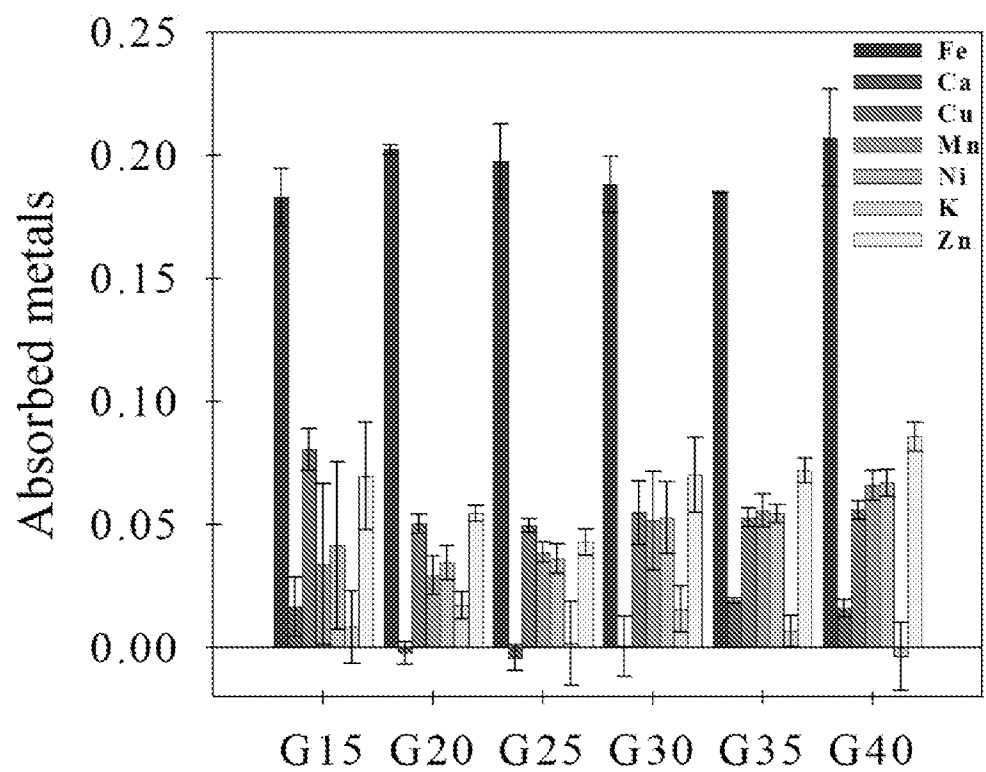
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Figure 3



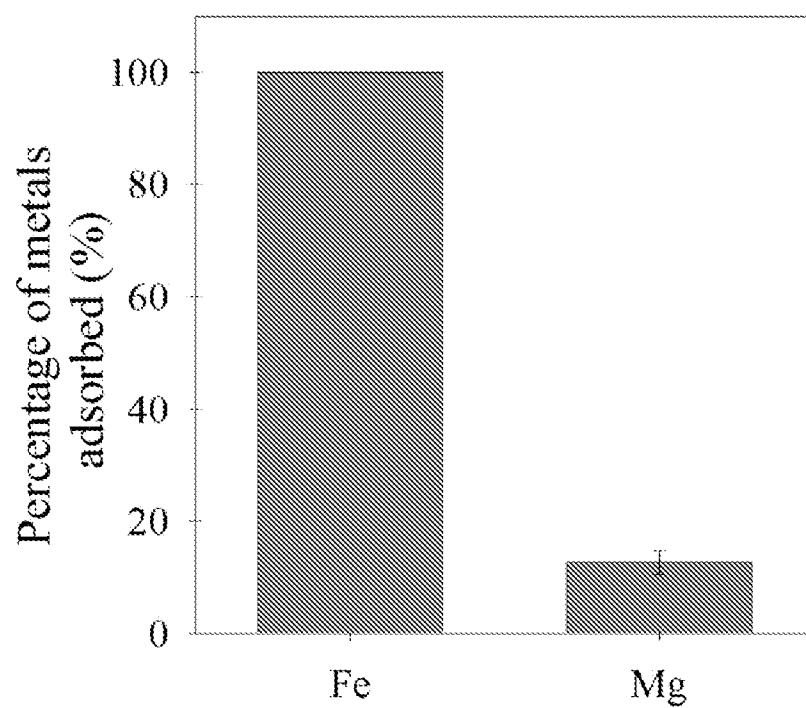
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Figure 4



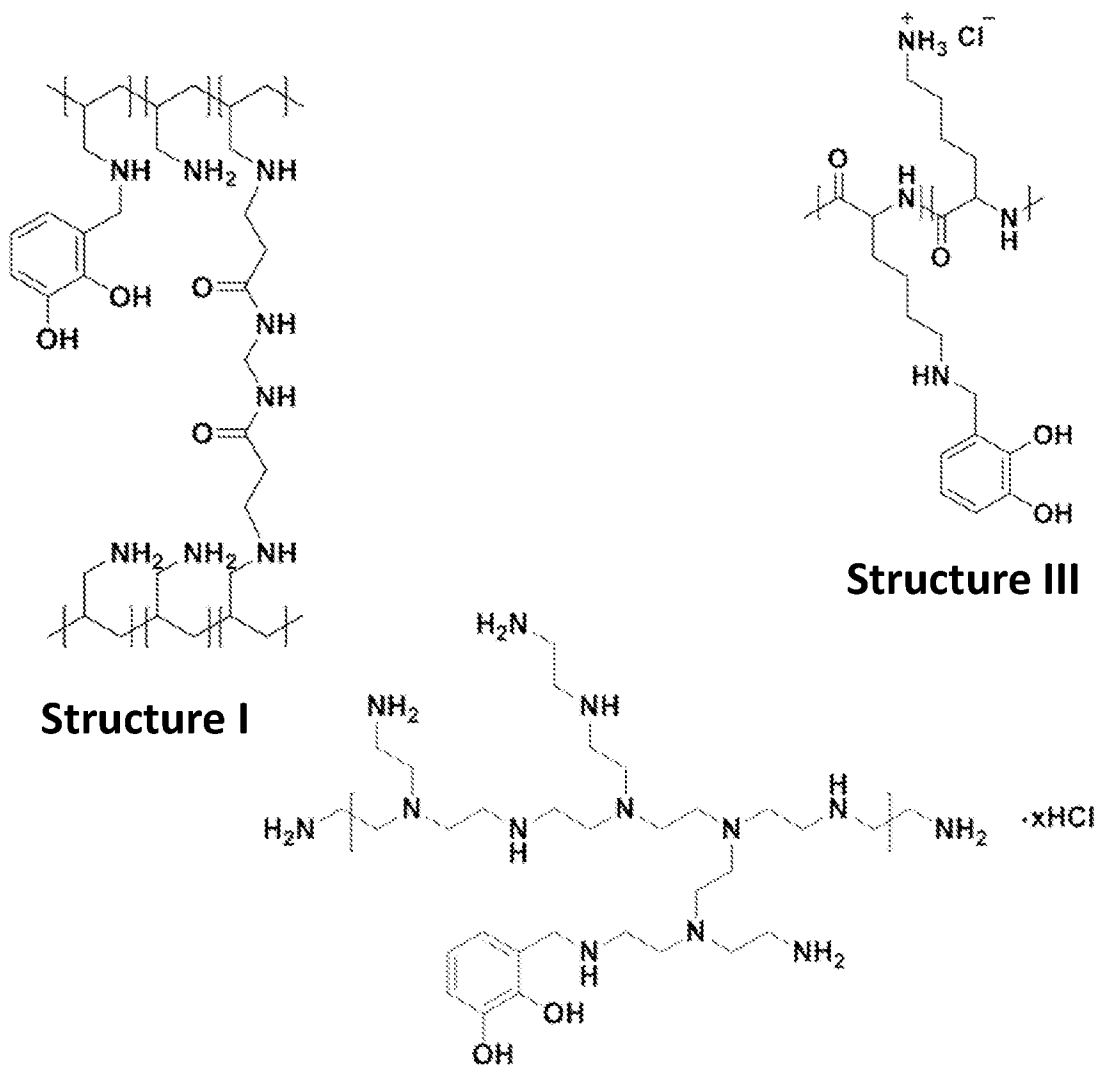
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Figure 5



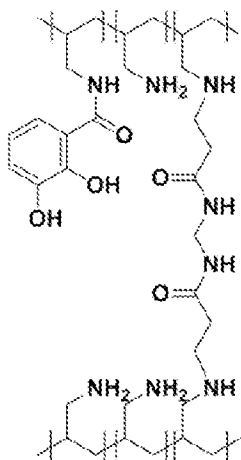
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Figure 6

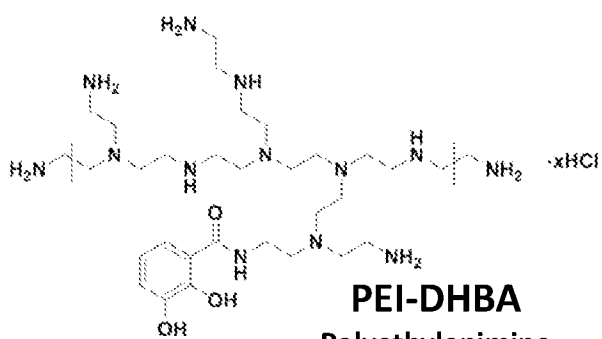
**Structure II**

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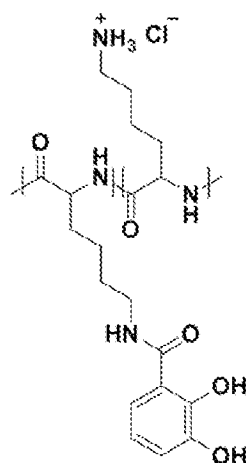
Figure 7



PAAm-DHBA
Polyallylamine
Hydrogel material



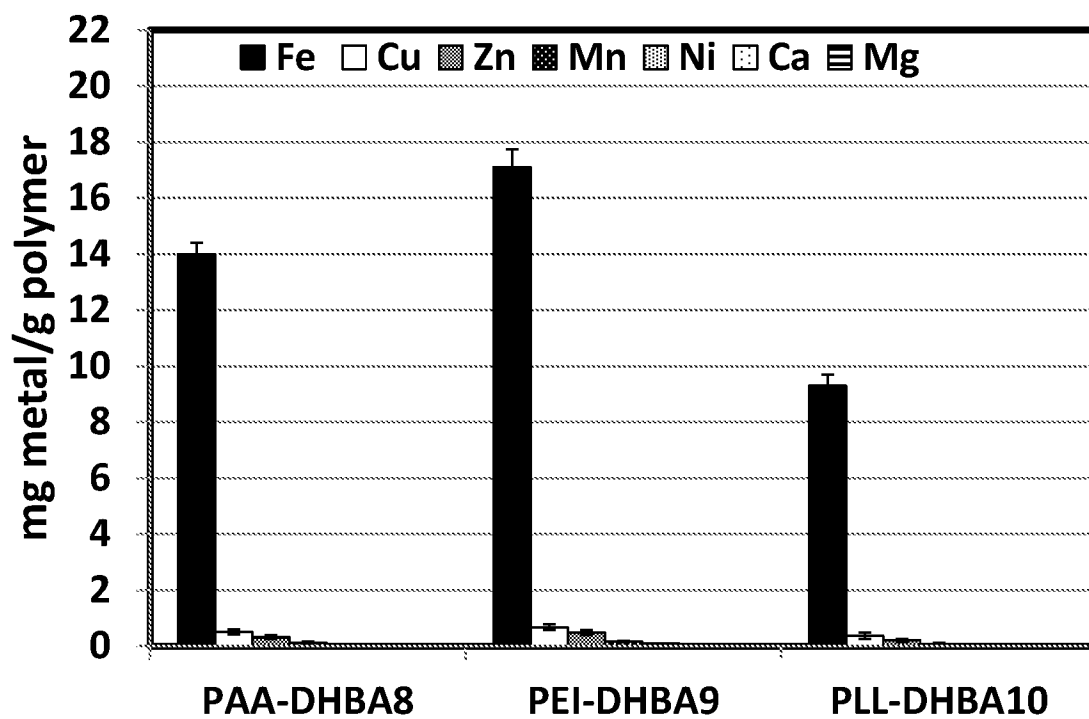
PEI-DHBA
Polyethylenimine
Soluble polymer, non-degradable



PLL-DHBA
Poly-L-Lysine
Soluble polymer, biodegradable

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Figure 8



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/27158

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 31/785; A61K 47/48 (2017.01)

CPC - A61K31/785; A61K47/48076; A61K47/48207; C08F126/02; C08G69/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2013/0056211 A1 (BERKLAND et al.) 07 March 2013 (07.03.2013) para [0075]-[0078];[0081]-[0087];[0112]-[0115]; [0134]-[0135]; [0160];[0174]; abstract; Claim 3	1-60
Y	WO 2014/059417 A1 (ARCH CHEMICAL, INC.) 17 April 2014 (17.04.2014) para [0013];[0014];[0021];[0028];[0029];[0032]-[0034];[0037]	1-49; 51; 52; 54; 55; 57-60
Y	US 2003/0125528 A1 (HAY et al.) 03 July 2003 (03.07.2003) para [0018]; [0028];[0079]	5; 9; 10; 15-17; 26; 30; 32; 42; 45; 48; 50-60

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

07 June 2017

Date of mailing of the international search report

14 JUL 2017

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