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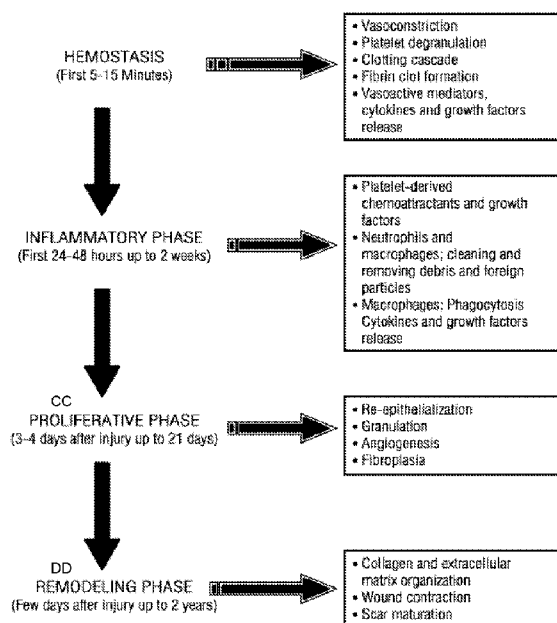


FIG. 1

(57) Abstract: The present invention relates to antimicrobial, disinfecting, and wound healing compositions and methods for producing and using the same. The compositions may comprise one or more of a peracid, a hydroperoxide, a bis(hydroperoxide), or an epoxide.

**ANTIMICROBIAL, DISINFECTING AND WOUND HEALING COMPOSITIONS
AND METHODS FOR PRODUCING AND USING SAME**

CROSS-REFERENCE TO RELATED APPLICATION

[0001] The present application claims priority under 35 U.S.C. §119(e) to U.S. Provisional Application Serial No. 62/530,045 filed July 7, 2017, the disclosure of which is incorporated by reference herein in its entirety.

FIELD OF THE INVENTION

[0002] The present invention relates to antimicrobial, disinfecting, and wound healing compositions and methods for producing and using the same. The compositions may comprise one or more of a peracid, a hydroperoxide, a bis(hydroperoxide), or an epoxide.

BACKGROUND OF THE INVENTION

[0003] The skin is the body's largest organ and serves as the primary protective barrier to the outside world. Any physical disruption (i.e., wound) to this organ must therefore be quickly and efficiently repaired in order to restore tissue integrity and function. Quite often proper wound healing is impaired with devastating consequences such as severe morbidity, amputations, or death. In humans and animals, protection from mechanical injury, chemical hazards, and bacterial invasion is provided by the skin because the epidermis is relatively thick and covered with keratin. Secretions from sebaceous glands and sweat glands also benefit this protective barrier. In the event of an injury that damages the skin's protective barrier, the body triggers a wound healing cascade of events.

[0004] The classical model of wound healing is divided into three or four sequential, yet overlapping, phases: (1) hemostasis, (2) inflammatory, (3) proliferative and (4) remodeling. The hemostasis phase involves platelets (thrombocytes) to form a fibrin clot to control active bleeding. The inflammatory phase involves migration of phagocytes to the wound to kill microorganisms and release of subsequent signaling factors to involve the migration and division of cells involved in the proliferative phase. The proliferative phase involves vascular cell production for angiogenesis, fibroblast cells to excrete collagen and fibronectin to form an extracellular matrix, and epithelial cells to reform the external epidermis. In addition, the wound is made smaller by myofibroblasts. Finally, collagen is remodeled and cells that are no longer needed are removed by programmed cell death (i.e., apoptosis).

[0005] The process of wound healing can be divided into two major phases: early phase and cellular phase. See FIG. 1. The early phase involves hemostasis which involves vasoconstriction, temporary blockage of a break by a platelet plug, and blood coagulation, or formation of a clot that seals the hole until tissues are repaired. The early phase also involves the generation of stimuli to attract the cellular responses needed to instigate inflammation. In the inflammation phase (see FIG. 2), white blood cells, or leukocytes, are attracted to the wound site by platelet-derived growth factor (PDGF), and these cells of the immune system are involved in defending the body against both infectious disease and foreign materials.

[0006] There are 18 other known proteins involved in the inflammatory phase which interact to regulate this response. For example, IL-4, IL-10, and IL-13 are potent activators of B lymphocytes. However, IL-4, IL-10, and IL-13 are also potent anti-inflammatory agents. The phagocytic cells engulf and then digest cellular debris and pathogens and stimulate lymphocytes and other immune cells to respond to the wound area. Once the invading microorganisms have been brought under control, the skin proceeds through the proliferative and remodeling stage by a complex cascade of biochemical events orchestrated to repair the damage. This involves the formation of a scab within several hours. The scab temporarily restores the integrity of the epidermis and restricts the entry of microorganisms.

[0007] After the scab is formed, cells of the stratum basale begin to divide by mitosis and migrate to the edges of the scab. A week after the injury, the edges of the wound are pulled together by contraction. Contraction is an important part of the healing process when damage has been extensive, and involves shrinking in size of underlying contractile connective tissue, which brings the wound margins toward one another. In a major injury, if epithelial cell migration and tissue contraction cannot cover the wound, suturing the edges of the injured skin together, or even replacement of lost skin with skin grafts, may be required to restore the skin. Interruption of this healing process by a breakdown in any of these wound healing processes will lead to a chronic wound.

[0008] Other skin wounds involve burns. Major burns are relatively common injuries that require multidisciplinary treatment for patient survival and recovery. More than 30,000 people die each year worldwide because of fire-related burn injuries. Many more are seriously injured, disabled, or disfigured because of all types of burns. There have been significant advances in medical care for burns over the last 15 years due to fluid resuscitation, wound cleaning, skin replacement, infection control, and nutritional support. These changes have

primarily resulted from the use of early burn wound excision, early adequate nutrition, and the use of surgical techniques that minimize blood and heat loss. Since modern treatment of burns has greatly advanced, sepsis has become the leading cause of death after a burn injury. Multiple antibiotic resistant bacteria and fungus now account for the bulk of deaths due to sepsis in burns, the etiology of which is due to antibiotic resistant bacteria and biofilm formation in the wound and extraneous nosocomial infections.

[0009] Impediments to wound healing include hypoxia, infection, presence of debris and necrotic tissue, use of inflammatory medications, a diet deficient in vitamins or minerals or general nutrition, tumors, environmental factors, and metabolic disorders such as diabetes mellitus. The primary impediments to acute wound healing are hypoxia, infection, wound debris, and anti-inflammatory medications. The molecular events in the wound healing process of acute, chronic and burn wounds continues to be studied and exhibits an extremely complex array of biochemical events imposing a regulated cascade of inter and intra cellular events.

[0010] A rapidly growing field of wound healing research is centered around cellular growth factors and the use of these factors for the treatment of wounds. The biochemical response at the cellular level is a process involving intricate interactions among different cell functions which include energy production, structural proteins, growth factors, and proteinases. The treatment of wounds with known cellular growth factors has the potential ability to help heal wounds by stimulating the cellular processes involved in angiogenesis, cellular proliferation, regulating the production and degradation of the extracellular matrix, and being the signal for attracting the inflammatory cells and fibroblasts. Obviously, this complexity requires a plethora of biochemical reactions to provide the functions necessary to accomplish healing of the wound and is not completely understood at this point.

[0011] One emerging area of research is the metabolic effect of the alpha keto acids on wound healing. U.S. Patent No. 6,329,343 discloses the use of a composition of salts of pyruvic acid and/or salts of pyruvic acid and alpha keto glutaric acid, a mixture of fatty acids, and an effective amount of an antibacterial agent as a bioadhesive antibacterial wound healing composition.

[0012] Several strategies have been employed to combat the significant infectious complication rates associated with wounds. However, to-date, these strategies have been mainly limited to improved surgical asepsis, surgical technique, and administrative regimens of peri-operative systemic antibiotics and local antibiotic irrigation procedures which have not

been well defined. New approaches are emerging in the clinic, including vacuum-sealed dressings, transparent film dressings, irrigation with antimicrobial agents, use of the port and cap, use of new agents such as deuteroporphyrin, gamma interferon (IFN- γ), silver sulfadiazone water soluble gel, geomagnetic therapy, and natural remedies such as milliacynic oil and lysozyme.

[0013] Unfortunately, few of these innovations have made a major impact on infection and fatality rates and have been shown to have cellular toxicity issues. Most new approaches involve delivery of antimicrobial compounds, to which many wound pathogens are resistant, in some form of salve or in dressings. These treatments lend themselves to continued production of antibiotic resistant bacteria which will negatively affect future therapies against resistive bacteria such as Methicillin-Resistant *Staphylococcus aureus* (MRSA), Vancomycin-resistant enterococci (VRE) and *Acinetobacter baumannii*. *A. baumannii* accounts for 6% of Gram-negative infections in intensive care facilities in the USA, with mortality rates as high as 54% having been reported. Isolation of MDR *Acinetobacter* soared from 6.7% in 1993 to 29.9% by 2004, emphasizing the need for newer and better drugs. Out of 1,040 antibiotics tested only 20 (1.92%) exhibited significant antimicrobial activity and only five compounds exhibited activity against the more resistant BAA-1605 *A. baumannii*.

[0014] Today, MRSA and *C. difficile* are the leading causes of nosocomial infection in most parts of the world. In 2003, *S. aureus* was the leading pathogen associated with skin and soft tissue infections. In the last 20 years, MRSA has moved from an exclusively hospital-acquired pathogen (HA-MRSA) to another type known as a community-acquired pathogen, CA-MRSA. In fact, it has been stated that topical application of antibiotic solutions for lower-limb open fracture wounds offers no advantage over the use of a nonsterile soap and may increase the risk of wound-healing problems.

[0015] Wound healing and "good" care of wounds has been synonymous with topical prevention and management of microbial contamination. Today's primary therapy involves the use of either topical application of antiseptics or systemic and topical use of antibiotics. The general perspective is that topical application of antibiotics to wounds has no advantages over the use of other antiseptic methods and may increase the risk of wound-healing by producing a sovereign bacteria that is resistant within the wound. The use of silver-based dressings for therapy against infections are widely used in chronic wound and burn therapy. There are several of these commercially available such as ActicoattTM, Aquacels Age,

Contreet® Foam, PolyMem® Silver, Urgotul® SSD). These silver containing dressings do not kill spores or biofilms and require long exposure times that may become cytotoxic over time. The major cause of sepsis in burn wounds, *Aspergillus niger* has a 70% fatality and is not susceptible to silver compounds.

[0016] The cytotoxic effect would explain, in part, the clinical observation of delayed wound healing or inhibition of wound epithelialization after the use of certain topical silver dressings.

[0017] There are a myriad of solutions available that claim to kill 99.9% of MRSA and other vegetative bacteria and some spores on surfaces and skin (e.g., hand sanitizers). Therefore, these solutions leave one viable bacterium, or spore, in a thousand or a thousand viable bacteria, or spores, in a million after treatment. However, contaminated surfaces can contain millions of bacteria, some of which can be contained within complex matrices such as blood drops, thus making them difficult to kill. Other types of bacteria, such as *Bacillus subtilis*, form biofilms on surfaces of endoscopes and other medical devices for insertion into the body, which affects the kill efficacy of most disinfectants. These low level disinfectants, often called sanitizers, that claim to kill 99.9% of the bacteria present will not completely kill all bacteria which are present in higher populations (colonized), contained within a complex matrix, or existing as a biofilm.

[0018] There are currently several topical antiseptics on the market that are used to diminish the growth of bacterial infections in wounds. Most antiseptics are not suitable for open wounds because they may impede wound healing by direct cytotoxic effects to keratinocytes and fibroblasts. In general, current topical antiseptics have limited bactericidal effect (e.g., 3 log reduction in 30 minute exposure) and nearly all have some cytotoxicity effect which varies with concentration and application time.

[0019] There are primarily five high level disinfectants/sterilants in use today. These include glutaraldehyde, orthophthalaldehyde, hypochlorite, hydrogen peroxide, and peracetic acid. The aldehydes are highly toxic and take a very long time to affect a >99.9999% (or 6 log kill). The most successful high level disinfectants used today are oxidizers such as hypochlorites, hydrogen peroxide and peracetic acid. The reactive advantage for disinfection by oxidation is the nonspecific free radical damage to all components of the microbe, including proteins, lipids, and DNA. Therefore, microbial resistance to oxidation at high enough solution concentration is virtually non-existent.

[0020] Safe and non-toxic concentrations of hydrogen peroxide are not capable of killing spores or high populations of microbes. Hypochlorous acid, which is formed by PMN by myeloperoxidase-mediated peroxidation of chloride ions, is easily neutralized at physiological pH by nitrite, a major end-product of cellular nitric oxide (NO) metabolism, and its bactericidal effects subsequently diminished and it is not as effective as silver sulfadiazine, a common topical wound sanitizer.

[0021] However, it appears that hypochlorous acid does not inhibit wound healing at the concentrations for the effective biocidal levels used. That may be because it is a natural compound found in the inflammatory phase of wound healing. Peracetic acid is used mainly in the food industry, where it is applied as a cleanser and as a disinfectant. Since the early 1950's, acetic acid was applied for bacteria and fungi removal from fruits and vegetables. It was also used for the disinfection of recycled rinsing water for foodstuffs. Nowadays peracetic acid is applied for the disinfection of medical supplies and to prevent biofilm formation in pulp industries. It can be applied during water purification as a disinfectant and for plumbing disinfection. Peracetic acid is produced by a reaction between hydrogen peroxide and acetic acid or it can also be produced by oxidation of acetaldehyde. Peracetic acid is a very powerful oxidant; the oxidation potential outranges that of chlorine and chlorine dioxide. Peracetic acid has not been tested in wound healing. However, it is not known to be involved in any significant cellular metabolism and is typically produced with toxic sulfuric acid catalyst. Thus, many conventional topical wound sanitizers have various limitations.

[0022] As stated above, a drawback of the peroxyacid-based chemical disinfectants is their inherent lack of stability, which poses a challenge for shelf-life when used for long term applications. Thus, a need exists for a peracid-based disinfectant, which is an effective broad spectrum antimicrobial, is in an easily removable homogenous antimicrobial coating composition providing both short-term and extended long-term antimicrobial efficacy after application to a surface or a wound.

[0023] In addition, there is a continuing need for new topical wound sanitizers, healers or both, and in particular there is a need to develop peroxyacids that are effective sporocides, bactericides and virucides for wounds which are easy to handle and store. Moreover, there is a need for peroxyacids that are easy to handle and store and that have a low corrosive nature. It is therefore desirable to develop a sanitizer that does not decompose rapidly and violently and that can be used as a topical wound sanitizer or as an antimicrobial coating.

[0024] The present invention is directed toward overcoming one or more of the problems discussed above.

SUMMARY OF THE INVENTION

[0025] In some aspects, the present invention relates to novel antimicrobial, disinfecting, and/or wound healing compositions and methods for producing and using the same. The compositions may comprise one or more of a keto acid, a peroxyacid, a hydroperoxide, a bis(hydroperoxide), or an epoxide.

[0026] In one aspect, the present invention provides a wound healing composition made by a method comprising contacting a keto acid or a salt or anhydride thereof with an oxidizing agent while stirring and under conditions sufficient to produce one or more of a peroxyacid, a hydroperoxide, a bis(hydroperoxide), or an epoxide. In some embodiments, the conditions are sufficient to produce a peroxyacid and a bis(hydroperoxide). In some embodiments, the conditions are sufficient to produce a peroxyacid, a hydroperoxide, a bis(hydroperoxide), and an epoxide.

[0027] In some embodiments, the keto acid may be an alpha-, beta-, or gamma-keto acid. In other embodiments, the keto acid is an alpha-keto acid. In some embodiments, the keto acid is pyruvic acid or a salt or anhydride thereof. In other embodiments, the keto acid is parapyruvuc acid or a salt or anhydride thereof. In other embodiments, the keto acid is acetoacetic acid or a salt or anhydride thereof. In some embodiments, the keto acid salt may be a lithium, sodium, potassium, rubidium, cesium, zinc, magnesium, or calcium salt, or a mixture thereof. In other embodiments, the process further comprises contacting the keto acid or salt thereof and the oxidizing agent with maleic acid or anhydride, citraconic acid or anhydride, or a mixture thereof.

[0028] In some embodiments, the reaction temperature is about 10° C. or less. In other embodiments, the reaction temperature ranges from about -10° C. to 10° C. In some embodiments, the molar ratio of oxidizing agent to keto acid typically ranges from 1:1 to about 4:1.

[0029] In one embodiment, the method comprises stirring the oxidizing agent at a shear rate between 150 s⁻¹ and 850 s⁻¹, cooling the oxidizing agent to between -10° C. to 0° C, and adding the keto acid at a rate sufficient to maintain the temperature between -10° C. to 0° C during addition of the keto acid to form a reaction solution. A shear rate between about 150 and about 850 sec⁻¹ equates to stirring at a rate between about 90 and about 500 RPM. In some

embodiments, the method further comprises continually stirring the reaction solution for 10 to 12 hours at a temperature -10° C. to 0° C.. In other embodiments, the method further comprises warming the reaction solution to between 14° C and 27° C. In some embodiments, the method further comprises cooling the reaction solution to maintain this temperature for 30 days. In some embodiments, the oxidizing agent is hydrogen peroxide and the keto acid is pyruvic acid.

[0030] In another aspect, the present invention provides a wound healing composition made by a method comprising contacting citramalic acid or a salt thereof with an oxidizing agent while stirring under conditions sufficient to produce one or more of a peroxyacid, a hydroperoxide, a bis(hydroperoxide), or an epoxide. In some embodiments, the conditions are sufficient to produce a peroxyacid, a hydroperoxide, a bis(hydroperoxide), and an epoxide. In some embodiments, the citramalic acid salt may be a lithium, sodium, potassium, rubidium, cesium, zinc, magnesium, or calcium salt, or a mixture thereof. In other embodiments, the process further comprises contacting citramalic acid or salt thereof and the oxidizing agent with acetic acid or anhydride thereof, maleic acid or anhydride thereof, citraconic acid or anhydride thereof, or a mixture thereof.

[0031] In another aspect, the present invention provides a wound healing composition made by a method comprising contacting an acetoacetate ester or a salt thereof with an oxidizing agent while stirring under conditions sufficient to produce one or more of a peroxyacid, a hydroperoxide, a bis(hydroperoxide), or an epoxide. In some embodiments, the conditions are sufficient to produce a peroxyacid, a hydroperoxide, a bis(hydroperoxide), and an epoxide. In some embodiments, the acetoacetate ester may be methyl acetoacetate or ethylacetoacetate, or a mixture thereof. In other embodiments, the acetoacetate ester salt may be a lithium, sodium, potassium, rubidium, cesium, zinc, magnesium, or calcium salt, or a mixture thereof. In some embodiments, the process further comprises adding citramalic acid.

[0032] While a variety of oxidizing agents may be used in such methods, typical oxidizing agents may comprise hydrogen peroxide, barium peroxide, sodium carbonate peroxide, potassium superoxide, or a mixture thereof. In some embodiments, the oxidizing agent is hydrogen peroxide.

[0033] In another aspect, the present invention provides a wound healing composition comprising a peroxyacid and a bis(hydroperoxide). In some embodiments, the composition further comprises a hydroperoxide. In other embodiments, the composition further comprises an epoxide. In some embodiments, the composition comprises peracetic acid and 3,3-

bis(hydroperoxy)butanoic acid. In other embodiments, the composition comprises peracetic acid and 3,3-bis(hydroperoxy)butaneperoxoic acid. In other embodiments, the composition further comprises at least one of methylhydroperoxide and hydroxymethyl hydroperoxide. In some embodiments, the composition further comprises 5-hydroperoxy-5-methyl-1,2-dioxolan-3-one. In some embodiments, the composition further comprises hydrogen peroxide.

[0034] In some embodiments, the wound healing composition further comprises peroxyitraconic acid. The peroxyitraconic acid may be either (2Z)-4-hydroperoxy-3-methyl-4-oxobut-2-enoic acid, (2Z)-4-hydroperoxy-2-methyl-4-oxobut-2-enoic acid, or a mixture thereof. In other embodiments, the compositions may comprise diperoxyitraconic acid, i.e., (2Z)-2-methylbut-2-enediperoxoic acid. In other embodiments, the composition further comprises peroxyitramalic acid. The peroxyitramalic acid may be either 4-hydroperoxy-2-hydroxy-2-methyl-4-oxobutanoic acid, 4-hydroperoxy-3-hydroxy-3-methyl-4-oxo-butanoic acid, or a mixture thereof.

[0035] In some embodiments, the present invention provides a wound healing composition comprising 3,3-bis(hydroperoxy)butanoic acid, 3,3-bis(hydroperoxy)butaneperoxoic acid, or 3-oxobutaneperoxoic acid, or a mixture thereof. In other embodiments, the compositions further comprise 5-hydroperoxy-5-methyl-1,2-dioxolan-3-one. In some embodiments, the composition further comprises one or more of hydrogen peroxide, an organic hydroperoxide, an organic peroxide, an organic peracid, an inorganic peracid, an organic acid, or an inorganic acid. In some embodiments, the composition further comprises hydrogen peroxide.

[0036] In some embodiments, the present invention provides a wound healing composition comprising acetoacetic acid, or a salt of acetoacetic acid. The salt of acetoacetic acid may be a lithium, sodium, potassium, rubidium, cesium, zinc, magnesium, or calcium salt. The composition may further comprise a hydroperoxide, including hydrogen peroxide and/or an organic hydroperoxide. In other embodiments, the composition may further comprise a keto acid. The keto acid may be an alpha-, beta- or gamma-keto acid. In some embodiments, the composition may further comprise pyruvic acid, parapyruvic acid, or citramalic acid, any of their salts, or mixtures thereof. In other embodiments, the composition may further comprise an acetoacetate ester such as methyl acetoacetate, ethyl acetoacetate, or acetoacetic anhydride. In some embodiments, the composition further comprises hydrogen peroxide.

[0037] In some embodiments, the present invention provides a wound healing composition comprising hydroperoxyacetic acid. In other embodiments, the composition further comprises hydrogen peroxide.

[0038] In another aspect, the present invention provides an antimicrobial, chemical oxidizer, or disinfecting products comprising one or more of the above-described compositions. In some embodiments, the antimicrobial product is a household care product. Within such embodiments, in some cases the household care product is selected from the group consisting of hard surface cleaners, deodorizers, fabric care compositions, fabric cleaning compositions, manual dish detergents, automatic dish detergents, floor waxes, kitchen cleaners, bathroom cleaners, and combinations thereof. In other embodiments, the anti-microbial product is selected from the group consisting of hard surface cleaners, deodorizers, fabric care compositions, fabric cleaning compositions, manual dish detergents, automatic dish detergents, floor waxes, kitchen cleaners, bathroom cleaners, and combinations thereof. Antimicrobial products of the invention can be used in a wide variety of settings including, but not limited to, in health care facilities such as hospitals, rehabilitation, assisted living facilities, etc.

[0039] In other embodiments, the antimicrobial product is a medical device disinfectant. Still in other embodiments, the antimicrobial product is used as a disinfectant for aseptic filling equipment. Yet in other embodiments, the antimicrobial product is used in an aseptic food processing system. In other embodiments, the antimicrobial product is used as a disinfectant for biofilms in water systems. Still in other embodiments, the antimicrobial product is used as a disinfectant for waste water treatment.

[0040] In yet another aspect the present invention provides a method of making a wound healing composition comprising contacting a keto acid or a salt or anhydride thereof with an oxidizing agent while stirring and under conditions sufficient to produce one or more of a peroxyacid, a hydroperoxide, a bis(hydroperoxide), or an epoxide. In some embodiments the conditions are sufficient to produce a peroxyacid, a hydroperoxide, a bis(hydroperoxide), and an epoxide.

[0041] In some embodiments, the keto acid may be an alpha-, beta-, or gamma-keto acid. In some embodiments, the keto acid is pyruvic acid or a salt or anhydride thereof. In other embodiments, the keto acid is parapyruvic acid or a salt or anhydride thereof. In other embodiments, the keto acid is acetoacetic acid or a salt or anhydride thereof. In some embodiments, the keto acid salt may be a lithium, sodium, potassium, rubidium, cesium, zinc,

magnesium, or calcium salt, or a mixture thereof. In other embodiments, the process further comprises contacting the keto acid or salt thereof and the oxidizing agent with maleic acid or anhydride, citraconic acid or anhydride, or a mixture thereof.

[0042] In some embodiments, the reaction temperature is about 10° C. or less. In other embodiments, the reaction temperature ranges from about -10° C. to 10° C. In some embodiments, the molar ratio of oxidizing agent to keto acid typically ranges from 1:1 to about 4:1. In some embodiments, the stirring is at a shear rate between 150 s⁻¹ and 850 s⁻¹.

[0043] In one embodiment, the method comprises stirring the oxidizing agent at a shear rate between 150 s⁻¹ and 850 s⁻¹, cooling the oxidizing agent to between -10° C. to 0° C, and adding the keto acid at a rate sufficient to maintain the temperature between -10° C. to 0° C during addition of the keto acid to form a reaction solution. A shear rate between about 150 and about 850 sec⁻¹ equates to stirring at a rate between about 90 and about 500 RPM. In some embodiments, the method further comprises continually stirring the reaction solution for 10 to 12 hours. In other embodiments, the method further comprises warming the reaction solution to between 14° C and 27° C. In some embodiments, the method further comprises cooling the reaction solution to maintain this temperature for 30 days. In some embodiments the temperature is room temperature (between 20° C and 22° C.) In some embodiments, the oxidizing agent is hydrogen peroxide and the keto acid is pyruvic acid.

[0044] In another aspect, the present invention provides a method of making a wound healing composition comprising contacting citramalic acid or a salt thereof with an oxidizing agent while stirring under conditions sufficient to produce one or more of a peroxyacid, a hydroperoxide, a bis(hydroperoxide), or an epoxide. In some embodiments, the conditions are sufficient to produce a peroxyacid, a hydroperoxide, a bis(hydroperoxide) and an epoxide. In some embodiments the citramalic acid salt may be a lithium, sodium, potassium, rubidium, cesium, zinc, magnesium, or calcium salt, or a mixture thereof. In other embodiments, the process further comprises contacting citramalic acid or salt thereof and the oxidizing agent with acetic acid, maleic acid or anhydride, citraconic acid or anhydride, or a mixture thereof.

[0045] In another aspect, the present invention provides a method of making a wound healing composition comprising contacting an acetoacetate ester or a salt thereof with an oxidizing agent while stirring under conditions sufficient to produce one or more of a peroxyacid, a hydroperoxide, a bis(hydroperoxide), or an epoxide. In some embodiments, the conditions are sufficient to produce a peroxyacid, a hydroperoxide, a bis(hydroperoxide), and

an epoxide. In some embodiments, the acetoacetate ester may be methyl acetoacetate or ethylacetoacetate, or a mixture thereof. In other embodiments, the acetoacetate ester salt may be a lithium, sodium, potassium, rubidium, cesium, zinc, magnesium, or calcium salt, or a mixture thereof. In some embodiments, the process further comprises adding citramalic acid.

[0046] While a variety of oxidizing agents may be used in such methods, typical oxidizing agents may comprise hydrogen peroxide, barium peroxide, sodium carbonate peroxide, potassium superoxide, or a mixture thereof. In some embodiments, the oxidizing agent is hydrogen peroxide.

[0047] In another aspect, the present invention provides a method of making a wound healing composition comprising combining one or more of a peroxyacid, a hydroperoxide, a bis(hydroperoxide), or an epoxide in an aqueous solution. In some embodiments, the method comprises combining a peroxyacid and bis(hydroperoxide) in an aqueous solution. In some embodiments, the peroxyacid is peracetic acid. In other embodiments, the bis(hydro-peroxide) is 3,3-bis(hydroperoxy)butanoic acid or 3-bis(hydroperoxy)butaneperoxoic acid.

[0048] In some embodiments, the method further comprises adding a hydroperoxide to the aqueous solution. In some embodiments, the hydroperoxide is one of methylhydroperoxide and hydroxymethyl hydroperoxide. In other embodiments, the method further comprises adding an epoxide to the aqueous solution. In some embodiments, the epoxide is 5-hydroperoxy-5-methyl-1,2-dioxolan-3-one. In some embodiments, the method further comprises adding hydrogen peroxide to the aqueous solution.

[0049] In other embodiments, the peroxyacid is peroxyitraconic acid. The peroxyitraconic acid may be either (2Z)-4-hydroperoxy-3-methyl-4-oxobut-2-enoic acid, (2Z)-4-hydroperoxy-2-methyl-4-oxobut-2-enoic acid, or a mixture thereof. In other embodiments, the peroxyacid is diperoxyitraconic acid, i.e., (2Z)-2-methylbut-2-enediperoxoic acid. In other embodiments, the peroxyacid is peroxycitramalic acid. The peroxycitramalic acid may be either 4-hydroperoxy-2-hydroxy-2-methyl-4-oxobutanoic acid, 4-hydroperoxy-3-hydroxy-3-methyl-4-oxobutanoic acid, or a mixture thereof.

[0050] In another aspect, the present invention provides methods of making antimicrobial, chemical oxidizer, and disinfecting solutions comprising any of the above-described methods.

[0051] In another aspect, present invention provides methods for treating a wound infection in a subject comprising contacting the infected wound in the subject with a therapeutically effective amount of an above-described composition. Methods of the invention can be used to treat surgical wound, battle wound, accidental wound, thermal burn wound, chemical burn wound, chronic wound, decubitus ulcer, foot ulcer, venous ulcer, laser treatment wound, sunburn, and/or an abrasion.

[0052] Generally, the composition is applied to the infected wound at least once, often at least twice a day initially.

[0053] In other embodiments, the composition is formulated as a gel, a liquid, lotion, skin patch, irrigation gel, a liquid, lotion, skin patch, a spray, application granules, or a combination thereof.

[0054] In yet another aspect, the present invention provides methods for reducing the number of microbes on a surface. Such methods typically include contacting the surface with an antimicrobial product comprising an above-described composition. Yet other aspects of the invention provide a method for reducing the number of infectious vegetative bacteria on a substrate comprising contacting the substrate with an antimicrobial solution comprising an effective amount of an above-described composition. Other aspects of the invention provide a method for reducing the number of bacterial spores on a substrate comprising contacting the substrate with an antimicrobial solution comprising an effective amount of an above-described composition.

[0055] In some embodiments, the microbe comprises vegetative bacteria. Within these embodiments, in some instances the microbe comprises bacterial spores, mycobacteria, gram-negative bacteria, vegetative gram-positive bacteria, or a combination thereof.

[0056] Yet other aspects of the invention provide methods for preventing and/or reducing bacteria-related diseases in a mammal that result from the mammal's contact with a bacteria-infected substrate. Such methods can include contacting the substrate with an above-described composition.

[0057] The details of one or more embodiments of the invention are set forth in the description below. Other features, objectives, and advantages of the invention will be apparent from the description and from the claims.

BRIEF DESCRIPTION OF THE FIGURES

[0058] The accompanying drawings, which are incorporated herein and constitute part of this specification, illustrate the presently preferred embodiments of the invention, and, together with the general description above and the detailed description given below, serve to explain the features of the invention. In the drawings:

[0059] FIG. 1 is a graphic illustration of phases of wound healing.

[0060] FIG. 2 is a schematic illustration of inflammatory phases of wound healing.

[0061] FIG. 3 is a reaction scheme for a reaction comprising pyruvic acid and hydrogen peroxide according to an embodiment of the present invention.

[0062] FIG. 4 is a reaction scheme for a reaction comprising acetoacetic acid and hydrogen peroxide according to an embodiment of the present invention.

[0063] FIG. 5 is a reaction scheme for a reaction comprising maleic acid and hydrogen peroxide according to an embodiment of the present invention.

[0064] FIG. 6 is a reaction scheme for a reaction comprising citraconic acid and hydrogen peroxide according to an embodiment of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

[0065] While various aspects and features of certain embodiments have been summarized above, the following detailed description illustrates a few embodiments in further detail to enable one of skill in the art to practice such embodiments. The described examples are provided for illustrative purposes and are not intended to limit the scope of the invention.

[0066] In the following description, for the purposes of explanation, numerous specific details are set forth in order to provide a thorough understanding of the described embodiments. It will be apparent to one skilled in the art, however, that other embodiments of the present invention may be practiced without some of these specific details. In other instances, certain structures and devices are shown in block diagram form. Several embodiments are described herein, and while various features are ascribed to different embodiments, it should be appreciated that the features described with respect to one embodiment may be incorporated with other embodiments as well. By the same token, however, no single feature or features of any described embodiment should be considered essential to every embodiment of the invention, as other embodiments of the invention may omit such features.

[0067] Unless otherwise indicated, all numbers expressing quantities of ingredients, dimensions, reaction conditions, and so forth, used in the specification and claims, are to be understood as being modified in all instances by the term “about”.

[0068] In this application and the claims, the use of the singular includes the plural unless specifically stated otherwise. In addition, use of “or” means “and/or” unless stated otherwise. Moreover, the use of the term “including”, as well as other forms, such as “includes” and “included”, is not limiting. Also, terms such as “element” or “component” encompass both elements and components comprising one unit and elements and components that comprise more than one unit unless specifically stated otherwise.

[0069] In some aspects, the present invention relates to antimicrobial, disinfecting, and/or wound healing compositions and methods for producing and using the same. The compositions may comprise one or more of a keto acid, a peracid, a hydroperoxide, a bis(hydroperoxide), or an epoxide.

[0070] Some aspects of the present invention provide methods for treating a wound on a subject comprising contacting the wound with a therapeutically effective amount of a composition comprising a peracid and a bis(hydroperoxide). In some aspects, the present invention also relates to compositions comprising a peracid and a bis(hydroperoxide), as well as methods for making and using such compositions and mixtures thereof. In some embodiments, the composition further comprises a hydroperoxide, an epoxide, or both.

[0071] In general, peracids are compounds of oxidized form of a base organic acid (generally a carboxylic acid) that exist in equilibrium with an oxidizer (generally hydrogen peroxide) and water. One species of peracid with superior regeneration properties are peroxy alpha-keto acid (PKCA) compounds (*see* U.S. Patent Application Publication No. 2010/0261792). PKCA compounds would generally be composed of an α -keto carboxylic acid, the anion of that α -keto acid, a buffer, and hydrogen peroxide, and the oxidized form of the carboxylic acid. Peroxy pyruvate acid (PPA), for example, may be in equilibrium with pyruvic acid, acetic acid and peracetic acid and other peracids. Peracids may be oxidized from other carboxylic acids, e.g. citric acid, succinic acid, short chain fatty acids, etc.

[0072] As used herein, “peracid,” “peroxyacid,” “percarboxylic,” and “peroxy-carboxylic acid,” and are used interchangeably herein and refer to a compounds generally have the formula $R(\text{CO}_3\text{H})_n$, where, for example, R is an alkyl, arylalkyl, cycloalkyl, aromatic, or

heterocyclic group, and n is one, two, or three, and named by prefixing the parent acid with "peroxy-." The R group can be saturated or unsaturated as well as substituted or unsubstituted. Peroxycarboxylic acids can be made by the direct action of an oxidizing agent on a carboxylic acid, by autoxidation of aldehydes, or from acid chlorides, and hydrides, or carboxylic anhydrides with hydrogen or sodium peroxide.

[0073] Peroxycarboxylic acids useful in the compositions and methods of the present invention include peroxyformic, peroxyacetic, peroxypropionic, peroxybutanoic, peroxy-pentanoic, peroxyhexanoic, peroxyheptanoic, peroxyoctanoic, peroxy-nonanoic, peroxy-decanoic, peroxyundecanoic, peroxydodecanoic, or the peroxyacids of their branched chain isomers, peroxylactic, peroxymaleic, peroxyascorbic, peroxyhydroxyacetic, peroxyoxalic, peroxymalonic, peroxysuccinic, peroxyglutaric, peroxyadipic, peroxypimelic and peroxy-suberic acid and mixtures thereof. In some embodiments, the compositions of the invention utilize a combination of several different peroxycarboxylic acids. For example, in some embodiments, the composition includes one or more C1 to C4 peroxycarboxylic acids and one or more C5 to C11 peroxycarboxylic acids. Especially preferred is an embodiment in which the peroxycarboxylic acid is peracetic acid (C2), peroxy propionic acid (C3), peroxybutanoic acid (C4), peroxysuccinic and peroxymalonic acid. It should be noted that both the peroxy-succinic and peroxymalonic acid may come from the alpha-keto dicarboxylic acids. Furthermore, because these acids exist in the Krebs cycle they are metabolically active.

[0074] In some embodiments, the compositions and methods of the present invention include peroxyacetic acid. Peroxyacetic (or peracetic) acid is a peroxycarboxylic acid having the formula: CH_3COOH . Generally, peroxyacetic acid is a liquid having an acrid odor at higher concentrations and is freely soluble in water, alcohol, ether, and sulfuric acid.

[0075] For clarity, terms used herein are to be understood as described herein or as such term would be understood by one of ordinary skill in the art of the invention. Additional explanation of certain terms used herein, are provided below:

[0076] "wt %" refers to the weight percent relative to the total weight of the solution or dispersion.

[0077] "Microorganism" is meant to include any organism comprised of the phylogenetic domains of bacteria and archaea, as well as unicellular (e.g., yeasts) and

filamentous (e.g., molds) fungi, unicellular and filamentous algae, unicellular and multicellular parasites, viruses, viroids, and viroids.

[0078] "Film-forming agent" or "water soluble or water dispersible coating agent," which may be used interchangeably herein, refer to agents that form a film and are employed to provide protective coating to the surface of interest. These agents are either water soluble or water dispersible. These agents are described in further detail below.

[0079] "Antimicrobial agent" as used herein refers to a compound or substance having antimicrobial properties

[0080] "Biocide," as used herein, refers to a chemical agent, typically broad spectrum, which inactivates or destroys microorganisms. A chemical agent that exhibits the ability to inactivate or destroy microorganisms is described as having "biocidal" activity.

[0081] "Biofilm" refers to a structured community of microorganisms encapsulated within a self-developed polymeric matrix and adherent to a living or inert surface. "Drying" refers to a process by which the inert solvent or any other liquid present in the formulation is removed by evaporation.

[0082] "Disinfectant" as used herein is a chemical that kills 99.9% of the specific test microorganisms in 10 minutes under the conditions of the test. (Germicidal and Detergent Sanitizing Action of Disinfectants, Official Methods of Analysis of the Association of Official Analytical Chemists, paragraph 960.09 and applicable sections, 15th Edition, 1990 (EPA Guideline 91-2)).

[0083] "Sterilization" or "sterilant" as used herein refers to the inactivation of all bio-contamination.

[0084] "Locus" as used herein, comprises part or all of a target surface suitable to be coated.

[0085] Some methods of the invention include contacting a keto acid and oxidizing agent while stirring and under conditions sufficient to produce one or more of a peroxyacid, a hydroperoxide, a bis(hydroperoxide), or an epoxide. In some embodiments, the conditions are sufficient to produce a peroxyacid, a hydroperoxide, a bis(hydroperoxide), and an epoxide. As used herein, unless the context requires otherwise, the term "stir" or "stirring" refers to agitating or act of causing a mixing of the reagents by using an external force such as by using a

mechanical stirrer, a magnetic stirrer, a shaker, or any other mechanical, electrical, magnetic, or manual force including simply mixing the reagents manually.

[0086] It should be noted that the yield of the reaction is affected by a variety of reaction conditions and reagents used. One of the factors influencing the yield is the reaction temperature. Generally, the rate of reaction increases as the temperature increases, however, a higher reaction temperature can also increase the yield of side-product(s) and/or decomposition to the non alpha-keto peroxyacid. Therefore, the reaction temperature is typically kept at about 0° C. or below, often at about 10° C. or below, and more often at about -20° C. or below. In some embodiments, the reaction temperature is between -10° C. to 10° C.

[0087] The concentration of the reagents can also affect the rate and the yield of the reaction. The initial concentration of the oxidizing agent is generally about 12 M or less, typically about 7 M or less, and often about 1 M or less.

[0088] The reaction time can also affect the yield. Typically the reaction time ranges from about 4 hours to about 12 hours, often from about 6 hours to about 8 hours, and more often from about 10 hours to about 12 hours.

[0089] Methods of the invention are applicable to a wide variety of keto acids, and in particular alpha-keto carboxylic acids. In fact, generally any alpha-keto carboxylic acid can be used as long as any reactive functional group within the alpha-keto carboxylic acid is properly protected. Suitable protection groups for various chemical reactions are well known to one skilled in the art. See, for example, *Protective Groups in Organic Synthesis*, 3rd ed., T. W. Greene and P. G. M. Wuts, John Wiley & Sons, New York, 1999; *Smith and March, Advanced Organic Chemistry*, 5th ed., John Wiley & Sons, New York, N.Y., 2001; and *Harrison and Harrison et al., Compendium of Synthetic Organic Methods*, Vols. 1-8 (John Wiley and Sons, 1971-1996), which are incorporated herein by reference in their entirety. Exemplary alpha-keto carboxylic acids include, but are not limited to, pyruvic acid, alpha-keto butyric acid, alpha-keto valeric acid, alpha-keto glutaric acid, 2-oxo cyclopentyl acetic acid, etc.

[0090] Exemplary oxidizing agents that are useful in methods of the invention include, but are not limited to, hydrogen peroxide, barium peroxide, sodium carbonate peroxide, calcium peroxide, sodium perborate, lithium peroxide, magnesium peroxide strontium peroxide, zinc peroxide, potassium superoxide, and the like.

[0091] In some embodiments, the methods may comprise additional reagents such as acetic acid or anhydride, maleic acid or anhydride, citraconic acid or anhydride, or a mixture thereof.

[0092] In an exemplary embodiment, the method comprises contacting a mixture of pyruvic acid, maleic acid, and citraconic acid with hydrogen peroxide while stirring at a reaction conditions sufficient to produce the reaction products shown in the reaction schemes of FIGS. 3-6.

[0093] When describing a chemical reaction, the terms "treating," "contacting," and "reacting" are used interchangeably herein, and refer to adding two or more reagents under appropriate conditions to produce the indicated and/or the desired product. It should be appreciated that the reaction which produces the indicated and/or the desired product may not necessarily result directly from the combination of reagents which were initially added, i.e., there may be one or more intermediates which are produced in the mixture which ultimately leads to the formation of the indicated and/or the desired product.

[0094] The reaction is generally conducted in an aqueous solution. Other solvents, such as an organic solvent can also be used in addition to or in place of the aqueous solution. Because it is inexpensive and commercially available in an aqueous solution, typically hydrogen peroxide is used as an oxidizing agent.

[0095] The molar ratio of oxidizing agent to keto acid typically ranges from about 0.5:1 to about 2:1, often about 2:1 to about 6:1. A molar ratio above 1:1 is preferred.

[0096] In another embodiment, instead of reacting a keto acid or a salt or anhydride thereof with an oxidizing agent under the conditions described herein to produce the disclosed mixture of compounds, the present invention also provides compositions that are admixtures of the key composition components. Compositions according to this embodiment contain hydrogen peroxide, a peracid, such as peracetic acid, and one or more optional compounds selected from tartaric acid, formic acid, *cis*-epoxysuccinic acid, methyltartaric acid, acetic acid, *cis*-epoxymethylsuccinic acid, maleic acid, citramalic acid and citraconic acid. Compositions according to this embodiment of the present invention may also optionally include oxidized acetoacetate compounds.

[0097] While various reaction parameters are disclosed herein, it should be appreciated that the scope of the invention is not limited to these particular reaction parameters.

[0098] Various embodiments of the disclosure could also include permutations of the various elements recited in the claims as if each dependent claim was a multiple dependent claim incorporating the limitations of each of the preceding dependent claims as well as the independent claims. Such permutations are expressly within the scope of this disclosure.

[0099] Some aspects of the invention disclose a process for forming a stable aqueous composition containing one or more of a peroxyacid, a hydroperoxide, a bis(hydroperoxide), or an epoxide. In some embodiments, the invention discloses a process for forming a stable aqueous composition comprising a peroxyacid and bis(hydroperoxide). In some embodiments the invention discloses a process for forming a stable aqueous composition comprising a peroxyacid, a hydroperoxide, a bis(hydroperoxide), and an epoxide.

[0100] Environmental concerns about the effects of certain chemicals on the upper atmosphere have led to some unease about the widespread use of certain disinfectants. Hydrogen peroxide, peracetic acid, persulfates and peroxyhydrates, such as sodium perborate are well known as disinfectant compounds but are highly corrosive and sometimes hard to handle and/or store.

[0101] It is therefore particularly desirable that an antimicrobial containing one or more of a peroxyacid, a hydroperoxide, a bis(hydroperoxide), or an epoxide be available for use as a single, stable gel or a viscous solution (sol), although a solid would be satisfactory if it were biodegradable, easily soluble in water, and did not contain significant inorganic dissolved solids such as are provided by sodium persulfate or sodium perborate. It is also desirable for the antimicrobial to have less odor, be non-corrosive and promote wound healing.

[0102] The embodiments disclosed herein overcome the problems of the prior art by providing an aqueous composition comprising stable sols, gels and solids one or more of a peroxyacid, a hydroperoxide, a bis(hydroperoxide), or an epoxide. In some embodiments, the aqueous composition comprises stable sols, gels and solids comprising a peroxy acid and a bis(hydroperoxide). In some embodiments, the peroxyacid is a C2 to C6 peroxycarboxylic acids. In other embodiments, the compositions of the invention provide a combination of several different peroxycarboxylic acids. For example, in some embodiments, the composition includes one or more C1 to C4 peroxycarboxylic acids and one or more C5 to C11 peroxycarboxylic acids. Especially preferred is an embodiment in which the peroxycarboxylic acid is peracetic acid (C2) peroxy propionic acid (C3) peroxybutanoic acid (C4), peroxysuccinic and peroxymalonic acid. Such compositions form carriers for delivering

peroxycarboxylic acids for applications related to high level disinfectants/sterilants of vegetative bacteria, spores and biofilms.

[0103] The compositions are particularly useful for killing vegetative bacteria and spores at the level acceptable to be called disinfectants. Unlike most peroxy carboxylic compounds, it was discovered that the non alpha-keto peroxyacid compounds in combination with keto peroxyacids do not require an acid catalyst for efficient synthesis and are effective against biofilms. Without the need for a toxic catalyst for synthesis, the mixture of the embodiments disclosed herein typically contains water, hydrogen peroxide, a peroxyacid, a hydroperoxide, a bis(hydroperoxide), and an epoxide, all of which work synergistically and are beneficial to healing of a wound.

[0104] Many of the parent compounds (i.e., the corresponding carboxylic acids) of the embodiments disclosed herein are present within nearly all living cells and play significant roles in essential cellular metabolism. For example, the parent carboxylic acid compounds of peroxypyruvic acid, peroxy oxaloacetate, peroxy alpha-keto glutarate, are key compounds within the TCA cycle, the predominant energy producer for cellular metabolism. The parent compound of peroxy alpha keto butyric acid, i.e. alpha keto butyric acid, is involved in the metabolic production of succinyl-CoA which is also used in the TCA cycle and thus contributes to cellular energy production. Alpha keto valeric acid, the parent compound of peroxy alpha keto valeric acid, is an intermediate in protein synthesis and the biosynthesis of the amino acids such as leucine and valine. Alpha keto valeric acid is involved in gluco-neogenesis in cells. Pyruvate is involved in producing energy for hypoxic cells during wound healing through glycolysis. The potential harmful effects of the ROS can be mediated by alpha-keto acids. In addition, pyruvate is involved in protecting DNA during hypoxia and is an indirect metabolic contributor to collagen deposition and angiogenesis in wound healing. Moreover, pyruvic acid accelerates the debridement of dead skin in both wounds and burns.

[0105] In some embodiments, the compositions comprise acetoacetic acid. Acetoacetic acid is one of the ketone bodies (along with 3-hydroxybutyric acid and acetone, although acetone is just a byproduct), which are major energy sources for the body, particularly during starvation. Ketone bodies are involved in pathways related to the Krebs' cycle, lipogenesis, sterol biosynthesis, glucose metabolism, β -oxidation of fatty acids, mitochondrial electron transport chain, intracellular signal transduction pathways, hormonal signaling, and the microbiome (Cotter, D. G., *et al.*, *Am. J. Physiol, Heart Circ. Physiol.*, **2013**, 304, H1060-

H1076). It has been tied to skin formation/biosynthesis in rats (Edmond, J., *J. Biol. Chem.*, **1974**, 249, 72-80). Additionally, it was just recently found to upregulate osteoblasts and increase bone formation (Saito, A., *et al. Biochem. Biophys. Res. Comm.*, **2016**, 473, 537-544).

[0106] Because acetoacetic acid can be converted into acetyl-CoA *in vivo*, its ability to affect biological processes is extremely high. However, its presence in the solution is unexpected because acetoacetic acid is an unstable compound that reacts intramolecularly and irreversibly, producing acetone and carbon dioxide. Thus, it is expected to be unstable in all solvents and even as a solid compound.

[0107] However, acetoacetic acid represents a rather unique case where a compound is stabilized by the addition of hydrogen peroxide, whereas normally the addition of a per-oxide leads to chemical oxidation/degradation. This stabilization is caused by the formation of a range of possible peroxide “adducts” with its ketone functionality and possibly its carboxylic acid. Because both moieties are required for intramolecular “self-destruction”, the formation of these other forms slows down the decomposition of the compound. Peroxide adducts may include 3,3-bis(hydroperoxy)butanoic acid, 3,3-bis(hydroperoxy)butaneperoxoic acid, 3-oxobutaneperoxoic acid, and 5-hydroperoxy-5-methyl-1,2-dioxolan-3-one. This stabilization is shown in the reaction scheme of FIG. 4. The compositions may be further stabilized by citramalic acid or an acetoacetate ester, such as methyl or ethyl acetoacetate.

[0108] In some embodiments, the compositions may comprise peroxycitraconic acid. The peroxycitraconic acid may be either (2Z)-4-hydroperoxy-3-methyl-4-oxobut-2-enoic acid, (2Z)-4-hydroperoxy-2-methyl-4-oxobut-2-enoic acid, or a mixture thereof. In other embodiments, the compositions may comprise diperoxycitraconic acid, i.e., (2Z)-2-methyl-but-2-enediperoxoic acid. In other embodiments, the antimicrobial composition further comprises peroxycitramalic acid. The peroxycitramalic acid may be either 4-hydroperoxy-2-hydroxy-2-methyl-4-oxobutanoic acid, 4-hydroperoxy-3-hydroxy-3-methyl-4-oxobutanoic acid, or a mixture thereof.

[0109] Additionally, it was particularly unexpected that stable compositions of peracids and bis(hydroperoxides) could be prepared, since peracids are very strong oxidizing agents even at a pH of 2 to 8 because the water soluble peracids are decomposing to form free radicals.

[0110] For the purpose of this invention a “stable” composition is one which maintains sufficient physical properties and active oxygen content long enough to be useful, about twelve

months. One important factor that is that “stable” does not imply static. That is, compositions of the present invention may be constantly undergoing a series of internal reactions. This is true of all liquid solutions to a degree, particularly for aqueous ones. However, this is especially true for compositions of the present invention, which have a large number of reversible and effectively irreversible reactions occurring at all times.

[0111] Studies show that many widely used wound antiseptics have undesired cytotoxicity, and while some do kill bacteria at a sufficient level, they often do not promote a relatively fast wound healing. In many cases, irrigation of open fracture wounds with an antibiotic solution offers no significant advantages over the use of a nonsterile soap solution and may in fact increase wound-healing problems.

[0112] To be useful, topical antiseptics should be toxic to bacteria but should have no significant toxicity to underlying tissues, and ideally, they should also preserve or enhance host defense against infection. The present invention provides a method for treating wounds including, but not limited to, surgical, traumatic, chronic and burn wounds. Methods of the invention promote wound healing and typically rapidly kill high levels of viruses, vegetative bacteria, fungi, mycobacteria and spores. Unlike many conventional antiseptics available today, compositions and methods of the invention eliminate bacteria, enhance body's defense system, and enhance the healing process. Without being bound by any theory, it is believed that these benefits are achieved at least in part by the synergistic effect of the parent alpha-keto acids working together with resultant alpha-keto peracid and a non-alpha keto peroxyacid. It is believed that the synergetic effect results in energy generation and serves as intermediates in the generation of other biomolecules that are useful in wound healing.

[0113] In addition, the combination of the peracids and bis(hydroperoxides) disclosed in the present embodiments can kill high levels of bacteria and spores in biofilms and in high protein environments without being corrosive and having virtually no cellular toxicity issues.

[0114] It should be appreciated that because the stability of peracids and bis(hydroperoxides) are often limited, in many instances compositions of the invention can include the presence of the parent carboxylic acid. As used herein, the term "parent carboxylic acid" refers to the corresponding carboxylic acid in which the peracid is derived from or is degraded into under a typical storage or production conditions. In some embodiments, the parent carboxylic acid is present in the composition of the invention in an amount of about 120.4 mM or less, typically, about 12.4 mM or less, more typically, about 6.2 mM or less, often about 2.5 mM or less, more

often, about 1.2 mM or less, still more often about 0.62 mM or less, yet more often about 0.31 mM or less, and most often about 0.062 mM or less.

[0115] Still in other embodiments, compositions of the invention can include hydrogen peroxide. Typically, the amount of hydrogen peroxide present in the wound healing compositions of the invention is about 715 mM or less, typically about 71.5 mM or less, more typically about 35.8 mM or less, often about 14.3 mM or less, more often about 7.2 mM or less, still more often about 3.6 mM or less, yet more often about 1.8 mM or less, and most often about 0.35 mM or less.

[0116] Furthermore, coatings of composition according to the present invention have a tendency to lose their antimicrobial activity over time, which is believed to be the result of evaporation of the neat peracid. One aspect of the present invention adds a magnesium salt to the composition to form a salt of the peracid, which testing has shown to retain antimicrobial activity over a lengthy accelerated aging test. Accordingly, compositions according to the present invention optionally further include a magnesium salt. The magnesium salt can be a salt of the keto acid, or a magnesium salt such as magnesium, hydroxide, magnesium carbonate, magnesium acetate tetrahydrate, and the like.

[0117] Additional objects, advantages, and novel features of this invention will become apparent to those skilled in the art upon examination of the following examples thereof, which are not intended to be limiting. In the Examples, procedures that are constructively reduced to practice are described in the present tense, and procedures that have been carried out in the laboratory are set forth in the past tense.

[0118] The foregoing discussion of the invention has been presented for purposes of illustration and description. The foregoing is not intended to limit the invention to the form or forms disclosed herein. Although the description of the invention has included description of one or more embodiments and certain variations and modifications, other variations and modifications are within the scope of the invention, e.g., as may be within the skill and knowledge of those in the art, after understanding the present disclosure. It is intended to obtain rights which include alternative embodiments to the extent permitted, including alternate, interchangeable and/or equivalent structures, functions, ranges or steps to those claimed, whether

or not such alternate, interchangeable and/or equivalent structures, functions, ranges or steps are disclosed herein, and without intending to publicly dedicate any patentable subject matter.

[0119] While the invention has been particularly shown and described with reference to a number of embodiments, it would be understood by those skilled in the art that changes in the form and details may be made to the various embodiments disclosed herein without departing from the spirit and scope of the invention and that the various embodiments disclosed herein are not intended to act as limitations on the scope of the claims. All references cited herein are incorporated in their entirety by reference.

EXAMPLES

[0120] The present invention is described more fully by way of the following non-limiting examples. Modifications of the examples will be apparent to those skilled in the art.

Example 1

[0121] Five milliliters of 30% hydrogen peroxide at 0-1 °C was stirred using a magnetic stir bar. 84-μL aliquots of pyruvic acid were added approximately every 90 s for 19 aliquots (totaling 27 min). During addition of the pyruvic acid and in the time spent stirring afterwards, temperature of the ice bath was measured in the 0-6 °C range. After the final pyruvic acid addition, the product sat in the bath slowly warming to room temperature. 98 min after the last pyruvic acid addition, stirring was terminated.

[0122] The reaction products were measured by HPLC analysis several times during the first 40 days after the reaction. The first measurement was performed just 2.4 hr after the final pyruvic acid addition.

[0123] Another sample was collected when the reaction was only 5 days old. After 5 days, the majority of the two dimer species have reacted. That citramalic acid is their primary product is evident by the large increase in its peak. Peracetic acid is also a major component at this point. Significant amounts of the oxidized acetoacetic acid species and the main product of their hydrolysis, 2,2-bis(hydroperoxy)propane, are also present.

[0124] Another sample was collected after 40-days. The presence/absence of each individual component directly detectable by HPLC is shown in **Table 1**:

Table 1

Compound	This Work (at 40 Days)
Hydrogen Peroxide (H ₂ O ₂)	Yes
Nitrate	
Hydroxymethyl Hydroperoxide (HMHP)	Yes
Tartaric Acid	Yes
Formic Acid	Yes
Performic Acid	Yes
<i>cis</i> -Epoxysuccinic Acid	
Hydroperoxyacetic acid (HPAA)	Yes
Methyl Hydroperoxide (MHP)	Yes
Methyltartaric Acid	Yes
Peracetic Acid	Yes
Acetic Acid	Yes
Permaleic Acid	Yes
<i>cis</i> -Epoxymethylsuccinic Acid	Yes
Oxidized Acetoacetic Acid-1 (O-AAA-1)	Yes
2,2-Bis(hydroperoxy)propane (2,2-HBPP)	Yes
Unknown 1 (~7.65 min)	Yes
Maleic Acid	Yes
Citramalic Acid	Yes
Oxidized Acetoacetic Acid-2 (O-AAA-2)	Yes
Fumaric Acid	
Oxidized Acetoacetic Acid-3 (O-AAA-3)	Yes
Citraconic Acid	Yes
Unknown 2 (~16.3 min)	Yes
Mesaconic Acid	Yes

Example 2

[0125] Compositions capable of forming shelf-stable coatings containing the magnesium salt of peroxyacetic acid were prepared by drying solutions containing a magnesium salt, acetic acid, hydrogen peroxide, peracetic acid, and poly(ethylene glycol) (PEG). The starting magnesium salt was magnesium hydroxide, magnesium carbonate, or magnesium acetate

tetrahydrate (an anhydrous magnesium acetate salt would also be effective since it is being dissolved in a water-containing mixture). The acetic acid/hydrogen peroxide/peracid source was an aqueous solution (called "PAA Source" in this document) usually containing 8-12 wt% peracid (peracetic acid), 15-22 wt% hydrogen peroxide, and 14-20 wt% acetic acid. Coatings were also be made in the presence of silica particles (up to 2.8%). Finally, the remainder of the solution typically consisted of water, but the short-chain alcohols methanol, ethanol, and isopropanol were also successfully used, with the shortest chains being the most successful.

[0126] A typical coating-solution mixture consisted of the following, which was used immediately after mixing:

- 4.1 wt% magnesium acetate tetrahydrate
- 20 wt% PAA Source
- 20 wt% PEG 3350
- 55.9 wt% water

[0127] Magnesium acetate tetrahydrate concentrations in the 1.8-6.5 wt% range were used successfully, with the best peracid recoveries occurring at higher concentrations. PAA Source concentrations of 8-72 wt% were found to yield stable peracid salts. PEG concentrations of 0-30 wt% were tested successfully. PEG 3350 and PEG 8000 both yielded coatings containing stable peracid salts.

[0128] The composition of the present invention have utility in numerous household products. The present invention thus also provides an antimicrobial product containing the compositions of the present invention. In some embodiments, the product is a household care product. Within such embodiments, in some cases the house hold care product is selected from hard surface cleaners, deodorizers, fabric care compositions, fabric cleaning compositions, manual dish detergents, automatic dish detergents, floor waxes, kitchen cleaners, bathroom cleaners, and combinations thereof. In other embodiments, the antimicrobial product is selected from hard surface cleaners, deodorizers, fabric care compositions, fabric cleaning compositions, manual dish detergents, automatic dish detergents, floor waxes, kitchen cleaners, bathroom cleaners, and combinations thereof. Antimicrobial products of the invention can be used in a wide variety of settings including, but not limited to, in health care facilities such as hospitals, rehabilitation, assisted living facilities, etc.

[0129] In other embodiments, the antimicrobial product is a medical device disinfectant. Still in other embodiments, the antimicrobial product is used as a disinfectant for aseptic filling equipment. Yet in other embodiments, the antimicrobial product is used in an aseptic food processing system. In other embodiments, the antimicrobial product is used as a disinfectant for biofilms in water systems. Still in other embodiments, the antimicrobial product is used as a disinfectant for waste water treatment.

[0130] The foregoing discussion of the invention has been presented for purposes of illustration and description. The foregoing is not intended to limit the invention to the form or forms disclosed herein. Although the description of the invention has included description of one or more embodiments and certain variations and modifications, other variations and modifications are within the scope of the invention, e.g., as may be within the skill and knowledge of those in the art, after understanding the present disclosure. It is intended to obtain rights that include alternative embodiments to the extent permitted, including alternate, interchangeable and/or equivalent structures, functions, ranges or steps to those claimed, whether or not such alternate, interchangeable and/or equivalent structures, functions, ranges or steps are disclosed herein, and without intending to publicly dedicate any patentable subject matter.

CLAIMS

WHAT IS CLAIMED IS:

1. A wound healing composition made by a method comprising:
Contacting a keto acid or a salt or anhydride thereof with an oxidizing agent while string and under conditions sufficient to produce one or more of a peroxyacid, a hydroperoxide, a bis(hydroperoxide), or an epoxide.
2. The composition of claim 1, wherein the conditions are sufficient to produce a peroxyacid and a bis(hydroperoxide)
3. The composition of claim 1, wherein the conditions are sufficient to produce a peroxyacid, a hydroperoxide, a bis(hydroperoxide), and an epoxide.
4. The composition of claim 1, wherein the keto acid is an alpha-, beta-, or gamma-keto acid.
5. The composition of claim 4, wherein the keto acid is an alpha-keto acid.
6. The composition of claim 5, wherein the keto acid is pyruvic acid or a salt or anhydride thereof.
7. The composition of claim 5, wherein the keto acid is parapyruvic acid or a salt or anhydride thereof.
8. The composition of claim 1, wherein the keto acid is a salt.
9. The composition of claim 8, wherein the keto acid salt is lithium, sodium, potassium, rubidium, cesium, zinc, magnesium, or calcium.
10. The composition of claim 1, wherein the oxidizing agent is hydrogen peroxide, barium peroxide, sodium carbonate peroxide, potassium superoxide, or a mixture thereof.
11. The composition of claim 10, wherein the oxidizing agent is hydrogen peroxide.
12. The composition of claim 1, wherein the conditions comprise a reaction temperature of about 10° C. or less.

13. The composition of claim 1, wherein the conditions comprise a reaction temperature between about -10°C . to 10°C
14. The composition of claim 1, wherein the molar ratio of the keto acid to oxidizing agent is between 1:1 to 4:1.
15. The composition of claim 1, wherein the stirring is at shear rate between 150 s^{-1} and 850 s^{-1} .
16. The composition of claim 1, further comprising a magnesium salt.
17. The composition of claim 16, wherein the magnesium salt is magnesium acetate tetrahydrate.
18. A wound healing composition comprising a peroxyacid and a bis(hydroperoxide).
19. The composition of claim 18, wherein the composition further comprises a hydroperoxide.
20. The composition of claim 18, wherein the composition further comprises an epoxide.
21. The composition of claim 18, wherein the composition further comprises a hydroperoxide and an epoxide.
22. The composition of claim 18, wherein the peroxyacid is peracetic acid
23. The composition of claim 18, wherein the bis(hydroperoxide) is 3,3-bis(hydroperoxy)butanoic acid.
24. The composition of claim 18, wherein the the peroxy acid is peracetic acid and the bis(hydroperoxide) is 3,3-bis(hydroperoxy)butanoic acid.
25. The composition of claim 24, wherein the composition further comprises methylhydroperoxide or hydroxymethyl hydroperoxide.
26. The composition of claim 25, further comprising 5-hydroperoxy-5-methyl-1,2-dioxolan-3-one.
27. The composition of claim 18, further comprising a magnesium salt.

28. The composition of claim 27, wherein the magnesium salt is magnesium acetate tetrahydrate
29. A method for treating a wound infection in a subject comprising contacting the infected wound in the subject with a therapeutically effective amount of a composition of any of claims 1 to 26.
30. The method of claim 29, wherein the infected wound is a surgical wound, battle wound, accidental wound, thermal burn wound, chemical burn wound, chronic wound, decubitus ulcer, foot ulcer, venous ulcer, laser treatment wound, sunburn, or an abrasion.
31. The method of claim 29, wherein the infected wound is contacted with the composition once a day.
32. The method of claim 29, wherein the composition is formulated as a gel, a liquid, lotion, skin patch, irrigation gel, a liquid, lotion, skin patch, a spray, application granules, or a combination thereof.

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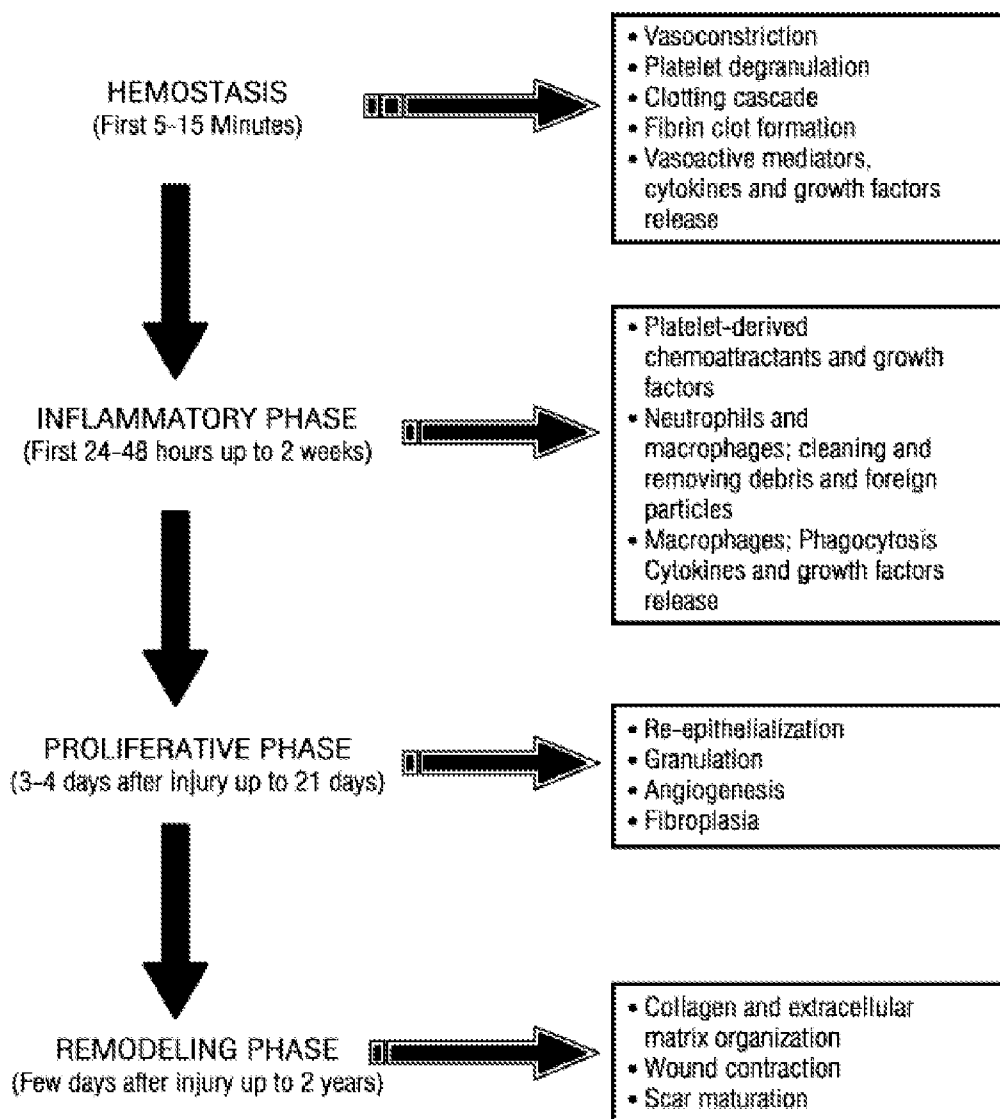
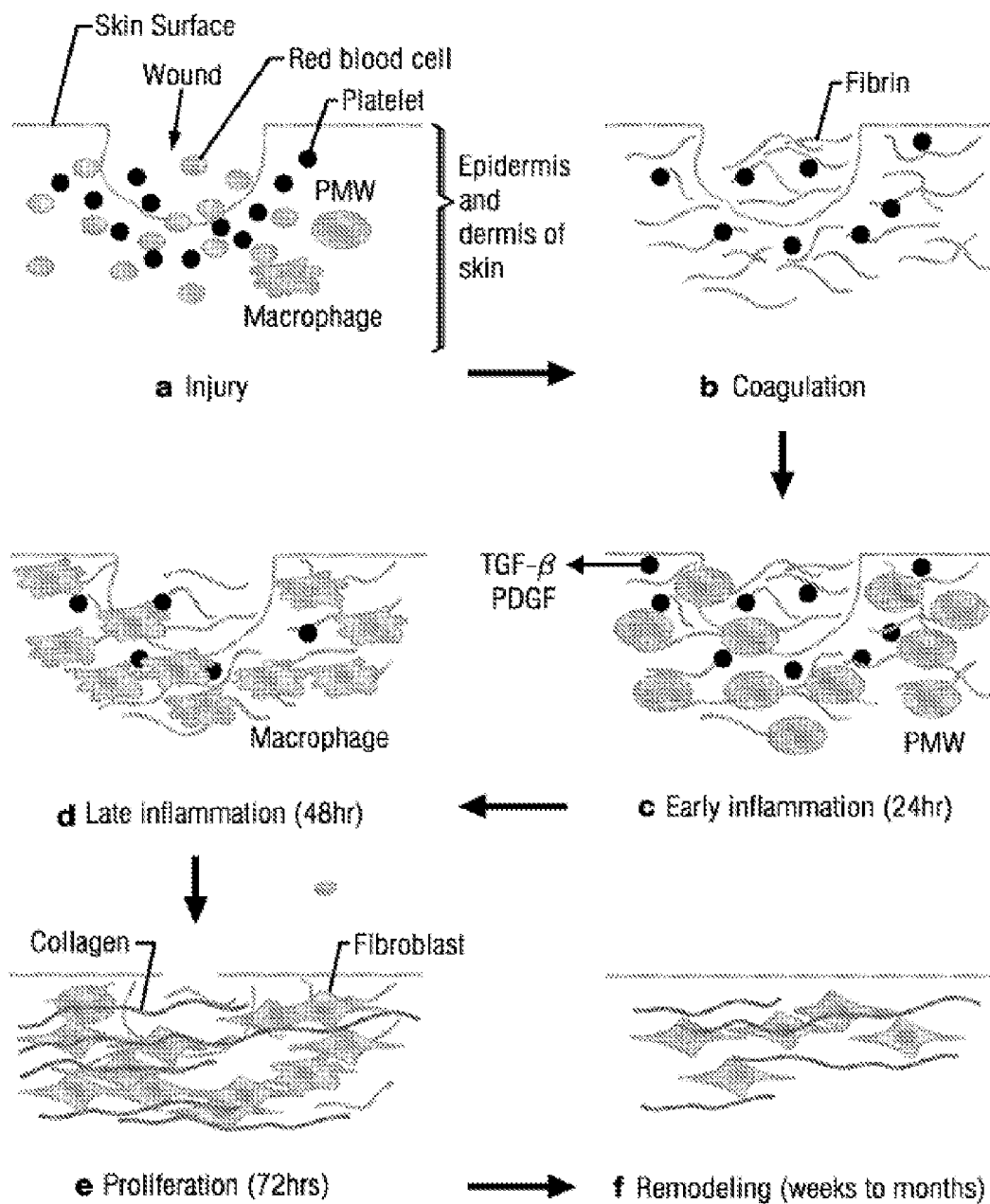


FIG. 1

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The phases of cutaneous wound healing
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FIG. 2

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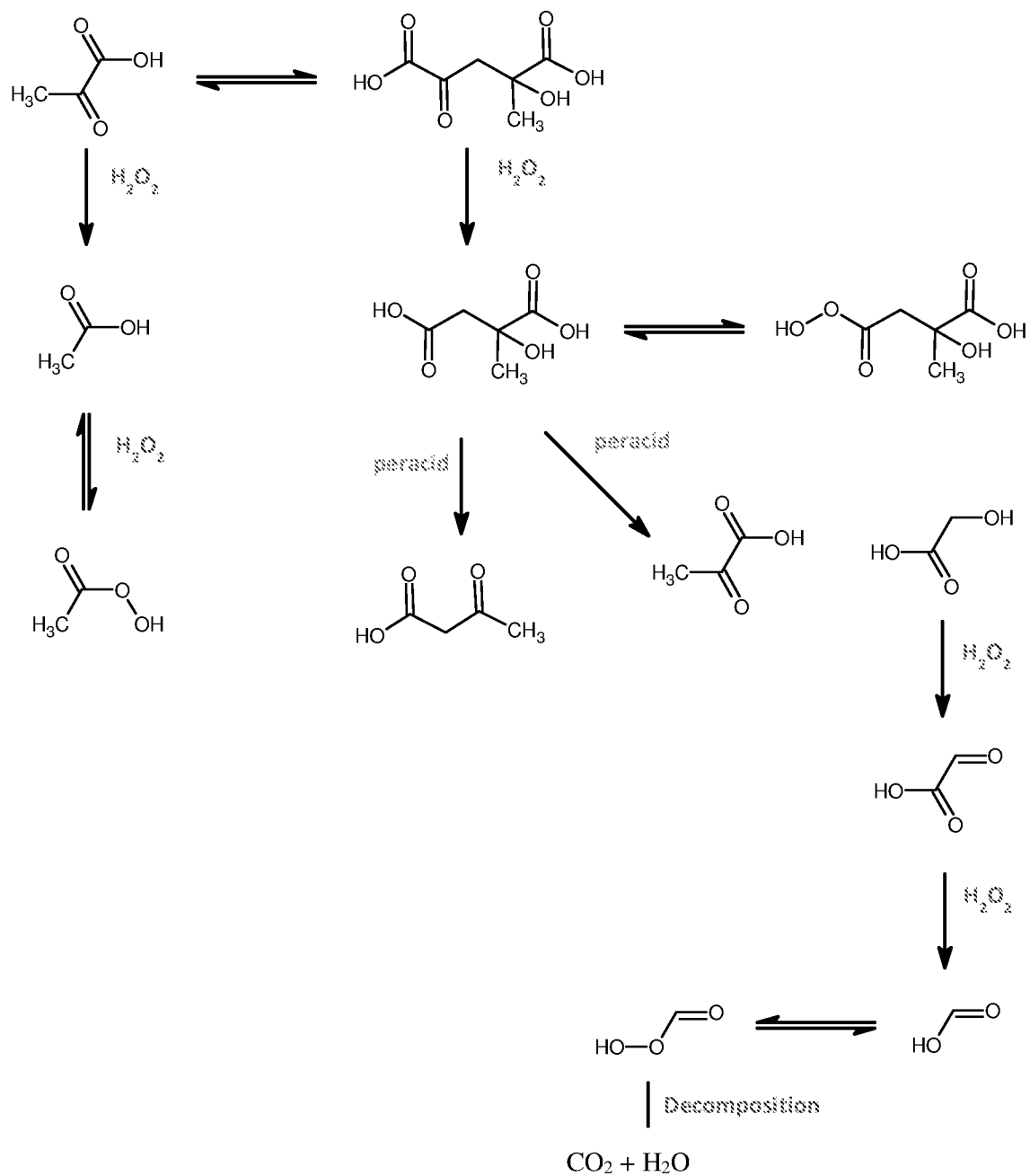


FIG. 3

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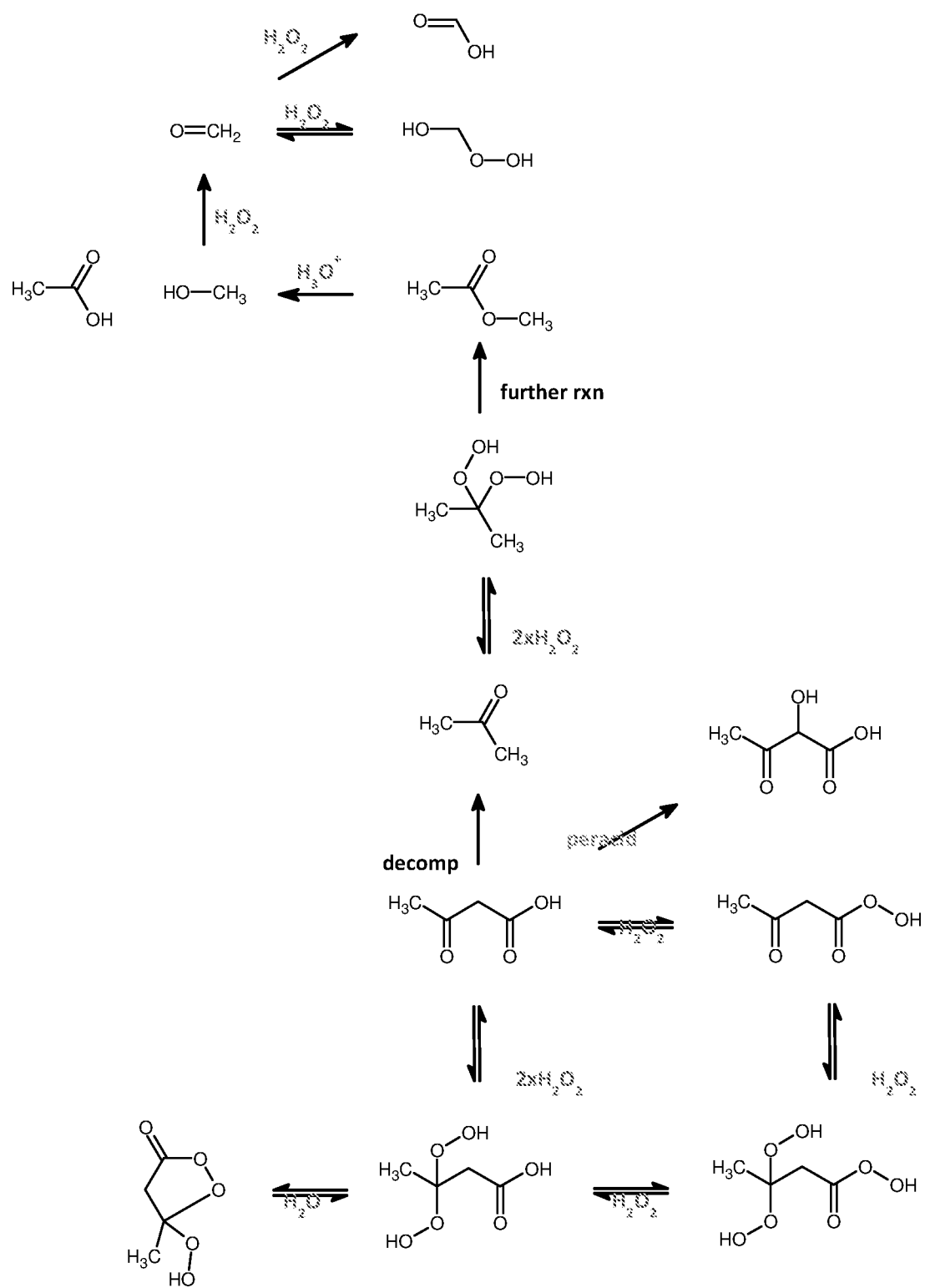


FIG. 4

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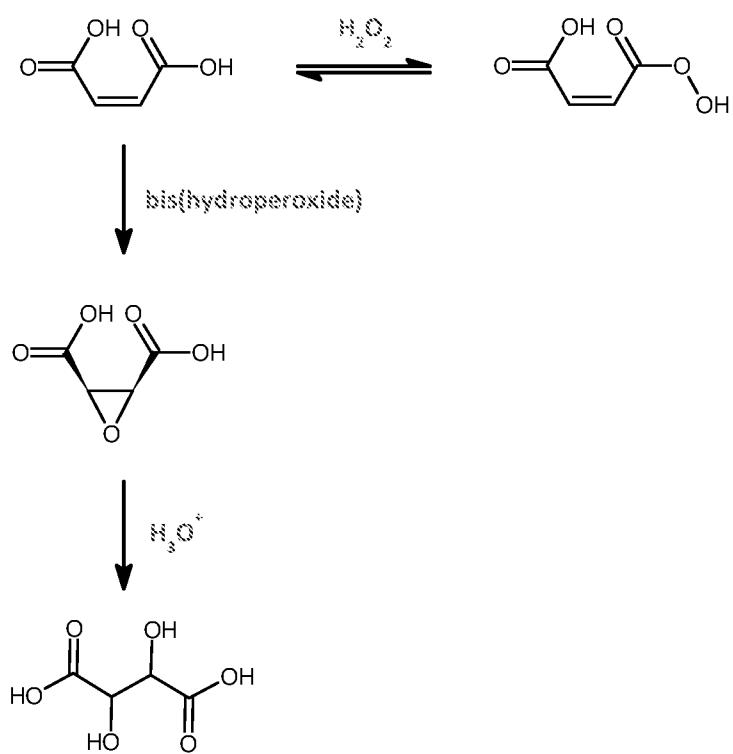


FIG. 5

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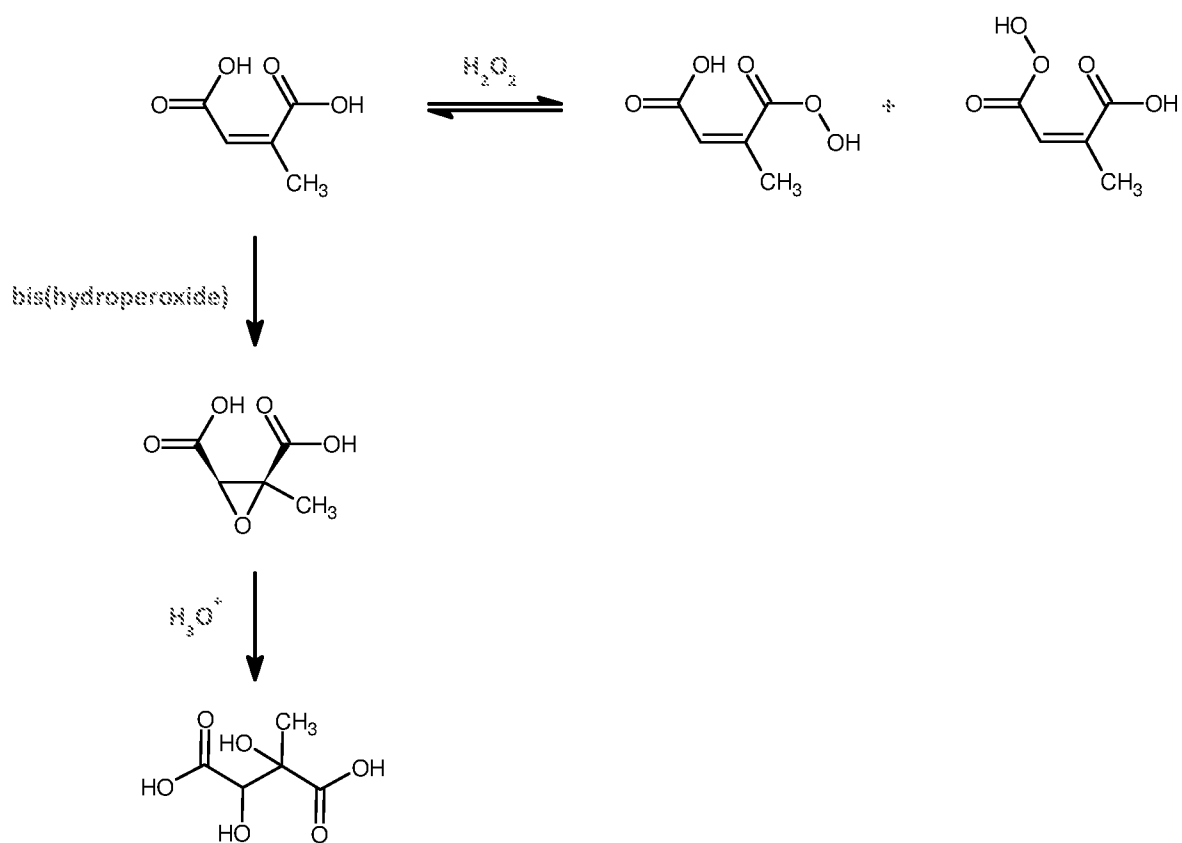


FIG. 6

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2018/041163

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A01N 37/42; A61K 31/327; A61K 33/40; A61K 45/06; C07C 409/24 (2018.01)

CPC - A01N 37/42; A01N 59/00; A01N 2300/00; A61K 31/327; A61K 33/40; A61K 45/06; C07C 409/24 (2018.08)

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

USPC - 424/616; 514/557; 562/2 (keyword delimited)

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.
X	US 2017/0118991 A1 (CHD BIOSCIENCE INC) 04 May 2017 (04.05.2017) entire document	1, 2, 4-6, 10-15, 18, 19, 22, 29-32

Y		3, 8, 9, 16, 17, 20, 21, 27, 28
Y	US 2005/0197397 A1 (MARTIN) 08 September 2005 (08.09.2005) entire document	3, 8, 9, 20, 21
Y	US 2017/0100335 A1 (THE REGENTS OF THE UNIVERSITY OF MICHIGAN) 13 April 2017 (13.04.2017) entire document	16, 17, 27, 28
A	US 2013/0330397 A1 (CHD BIOSCIENCE INC) 12 December 2013 (12.12.2013) entire document	1-32
A	US 2012/0213835 A1 (NEAS et al) 23 August 2012 (23.08.2012) entire document	1-32
P, X	US 2017/0303538 A1 (CHD BIOSCIENCE INC) 26 October 2017 (26.10.2017) entire document	1-32

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search

21 August 2018

Date of mailing of the international search report

24 SEP 2018

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