certain number of ROS including free radicals are naturally produced by the body due to cell metabolism. For instance, the synthesis of some hormones involves the generation of free radicals while polymorphonuclear leukocytes use the production of free radicals as a form of "chemical warfare" to kill bacteria, thereby guarding the body against infections. Other free radicals, such as Nitric Oxide (NO) are fundamental for the homeostasis of the body, because they act as chemical messengers to modulate important functions, including vascular tone, platelet aggregation, cell adhesion, and so on.

**[0010]** Free radicals are potentially dangerous because they spontaneously tend to fill their unfilled external orbital with a second electron. The presence of two electrons in the same orbital is the condition of maximal stability—minimum energy. Therefore, when a free radical collides with a "target molecule", having one or more "available" electrons, such as the molecule of an unsaturated fatty acid (e.g., arachidonic acid), it immediately "extracts" an electron from the target molecule. Due to this effect—"oxidation"—the original free radical loses its potential dangerousness whilst the newly generated molecule is "oxidized" and, in turn, may become a new free radical, thus perpetuating the reaction, if no antioxidants are available to damp it. The reaction can continue to other molecules, including carbohydrates, lipids, amino acids, peptides, proteins, nucleotides, nucleic acids and so on ("chain reaction effect"). Such action by free radicals can result in varying degrees of tissue damage and can cause (or conversely result from) inflammatory responses. An initial or primary site of ROS release may be an appropriate response to an invading microorganism, but the invader is not destroyed or if redox homeostasis is not restored following destruction of the invader, the redox state may spread and the continuing secondary oxidative redox state may result in a chronic, damaging pathology with collateral tissue damage. An example would be traumatic brain injury (TBI) which leads to localized inflammation and oxidative redox status in the brain. If homeostasis is not reestablished, chronic oxidative redox status may result leading to long term tissue damage and chronic traumatic encephalopathy (CTE).

**[0011]** There thus exists a need in the art for compositions and methods for controlling cellular redox levels.

## SUMMARY

**[0012]** The methods and compositions disclosed herein are not limited to specific advantages or functionality.

**[0013]** In a first aspect, the present disclosure provides a use of a toll-like receptor (TLR) agonist composition in the manufacture of a medicament for regulating redox status in a

subject, the composition comprising: (a) a TLR agonist comprising at least one lysate and/or lysate fraction of a bacterium, wherein the TLR agonist activates at least one or more TLRs or NLRs; (b) an optional promoter for enhancing absorption of the composition; and (c) an optional carrier for increasing a volume of the composition, wherein an effective amount of the composition is to be administered to the subject, wherein administration of the composition measurably reduces oxidative stress levels in the subject.

**[0013a]** In another aspect, the present disclosure provides a pharmaceutical formulation comprising the combination of: (a) a lysate composition comprising (i) a bacterial lysate and/or lysate fraction capable of activating at least one or more toll-like receptors (TLRs) or Nod-like receptors (NLRs); (ii) an optional promoter for enhancing absorption of the composition; and (iii) an optional carrier for increasing a volume of the composition; and (b) one or more pharmaceutical agent.

**[0013b]** In one example, the disclosure provides toll-like receptor (TLR) agonist compositions for regulating redox status in a subject, the composition comprising: (a) a TLR

agonist comprising at least one lysate and/or lysate fraction of a bacterium, wherein the TLR agonist activates at least one or more TLRs or NLRs; (b) an optional promoter for enhancing absorption of the composition; and (c) an optional carrier for increasing a volume of the composition, wherein administration of an effective amount of the composition to the subject measurably reduces oxidative stress levels in the subject.

**[0014]** In another example, the disclosure provides methods of regulating redox status in a subject, the method comprising administering a therapeutically effective amount of a lysate composition according to the disclosure to a subject in need thereof. In some examples, redox status regulation is assessed by measuring changes in isoprostane concentration in the subject.

**[0015]** In another example, the disclosure provides methods of regulating redox status in a subject, the method comprising the steps of: (a) repeatedly administering to a subject in need thereof doses spaced apart in time and consisting of a composition comprising: (i) a toll-like receptor (TLR) agonist comprising at least one lysate and/or lysate fraction of a bacterium, wherein the agonist activates at least one or more different TLRs or NLRs; (ii) an optional promoter for enhancing absorption of the composition; and (iii) an optional carrier for increasing a volume of the composition; and (b) making measurements of a bodily fluid of the subject to detect changes in oxidative stress levels.

**[0016]** In another example, the disclosure provides methods of decreasing the amount of isoprostane in the urine or blood of a subject, the method comprising the steps of: (a) determining the level of isoprostane in the urine or blood of the subject; (b) administering to the subject an effective amount of a composition comprising: (i) a toll-like receptor (TLR) agonist comprising at least one bacterial lysate and/or lysate fraction from a bacterium, wherein the TLR agonist activates at least one or more different TLRs or NLRs; and (ii) an optional promoter for enhancing absorption of the composition; and (c) continuing administration of the composition until the level of isoprostane in the urine or blood of the subject is decreased.

**[0017]** In another example, the disclosure provides compositions comprising: (a) a bacterial lysate and/or lysate fraction capable of activating at least one or more toll-like receptors (TLRs) or Nod-like receptors (NLRs); (b) an optional promoter for enhancing absorption of the composition; and (c) an optional carrier for increasing a volume of the composition.

**[0018]** In another example, the disclosure provides pharmaceutical formulations comprising lysate compositions according to the disclosure, wherein the pharmaceutical formulation is

formulated for buccal or sublingual administration. In some examples, the pharmaceutical formulations are formulated to dissolve in not less than 1 minute after administration.

**[0019]** In another example, the disclosure provides methods of producing a bacterial lysate comprising the steps of: (a) fermenting a bacterium in a growth medium to the stationary growth phase to produce a fermentation broth; (b) harvesting bacteria from the fermentation broth; (c) pasteurizing the harvested bacteria; and (d) lysing the pasteurized bacteria with a lysozyme to produce a bacterial lysate. In some examples, the bacteria are harvested in the mid-logarithmic phase, the late-logarithmic phase, the early stationary phase, the mid-stationary phase, or the late stationary phase.

**[0020]** In another example, the disclosure provides bacterial lysates produced according to methods comprising the steps of: (a) fermenting a bacterium in a growth medium to the stationary growth phase to produce a fermentation broth; (b) harvesting bacteria from the fermentation broth; (c) pasteurizing the harvested bacteria; and (d) lysing the pasteurized bacteria with a lysozyme to produce a bacterial lysate. In some examples, the bacteria are harvested in the mid-logarithmic phase, the late-logarithmic phase, the early stationary phase, the mid-stationary phase, or the late stationary phase.

**[0021]** In another example, the disclosure provides methods for alleviating one or more oxidative stress-related side effects associated with administration of a pharmaceutical agent, the method comprising administering in combination with the pharmaceutical agent a therapeutically effective amount of a lysate composition comprising: (a) a lysate and/or lysate fraction of a bacterium; (b) an optional promoter for enhancing absorption of the composition; and (c) an optional carrier for increasing a volume of the composition; wherein the pharmaceutical agent and lysate composition are administered simultaneously or in any order, and through the same or different routes of administration.

**[0022]** In another example, the disclosure provides methods for treating oxidative stress-related diseases or conditions in a subject, the method comprising administering to the subject a therapeutically effective amount of a composition comprising: (a) a bacterial lysate and/or lysate fraction capable of activating at least one or more toll-like receptors (TLRs) or Nod-like receptors (NLRs); (b) an optional promoter for enhancing absorption of the composition; and (c) an optional carrier for increasing a volume of the composition.

**[0023]** In another example, the disclosure provides methods for reducing oxidative stress in a subject, the method comprising: (a) determining the level of oxidative stress in the subject by measuring the amount of isoprostane in the urine or blood of the subject; (b) administering to the subject an effective amount of a composition comprising: (i) a toll-like receptor (TLR)

agonist comprising at least one lysate and/or lysate fraction from a bacterium, wherein the TLR agonist activates at least one or more TLRs or NLRs; and (ii) an optional promoter for enhancing absorption of the composition; and (c) continuing administration of the composition until the level of oxidative stress is reduced, as determined by a decreased amount of isoprostane in the urine of the subject.

**[0024]** In another example, the disclosure provides therapeutic combinations comprising: (a) a lysate composition comprising (i) a bacterial lysate and/or lysate fraction capable of activating at least one or more toll-like receptors (TLRs) or Nod-like receptors (NLRs); (ii) an optional promoter for enhancing absorption of the composition; and (iii) an optional carrier for increasing a volume of the composition; and (b) one or more pharmaceutical agents; wherein the lysate composition and the one or more pharmaceutical agents are administered simultaneously or in any order, and wherein the lysate composition and the one or more pharmaceutical agents are administered via the same or different routes of administration.

**[0025]** In another example, the disclosure provides pharmaceutical formulations comprising the combination of: (a) a lysate composition comprising (i) a bacterial lysate and/or lysate fraction capable of activating at least one or more toll-like receptors (TLRs) or Nod-like receptors (NLRs); (ii) an optional promoter for enhancing absorption of the composition; and (iii) an optional carrier for increasing a volume of the composition; and (b) one or more pharmaceutical agents. In some examples, the one or more pharmaceutical agents are selected from the group consisting of: an antispasmodic, a motility stimulant, an H2-Receptor antagonist, antimuscarinic; a chelate, a prostaglandin analog, an aminosalicylate, a corticosteroid, an drug affecting immune response, a stimulant laxative, a drug affecting biliary composition and flow, a bile acids sequestrant, a dopamine antagonist, a proton pump inhibitor, an opioid, an opioid receptor antagonist, an analgesic, a sleep drug, a cardiac glycoside, a phosphodiesterase inhibitor, a thiazide, a diuretic, a potassium sparing diuretic, an aldosterone antagonist, an osmotic diuretic, a drug for arrhythmia, a beta adrenoreceptor blocking drug, a hypertension drug, a drug affecting the renin-angiotensin system, a nitrate, a calcium blocker, an antianginal drug, a peripheral vasodilator, a sympathomimetic, an anticoagulant, a protamine, an antiplatelet drug, a fibrinolytic drug, an antifibrinolytic drug, a lipid regulating drug, an omega three fatty acid compound, a CNS drug, an anti-infective, or another drug selected from the group consisting of Benztropine, procyclidine, biperiden, Amantadine, Bromocriptine, Pergolide, Entacapone, Tolcapone, Selegeline, Pramipexole, budesonide, formoterol, quetiapine fumarate, olanzapine, pioglitazone, montelukast,

Zoledromic Acid, valsartan, latanoprost, Irbesartan, Clopidogrel, Atomoxetine, Dexamfetamine, Methylphenidate, Modafinil, Bleomycin, Dactinomycin, Daunorubicin, Idarubicin, Mitomycin, Mitoxantrone, Azacitidine, Capecitabine, Cladribine, Clofarabine, Cytarabine, Fludarabine, Flourouracil, Gemcitabine, mercaptopurine, methotrexate, Nelarabine, Pemetrexed, Raltitrexed, Thioguanine, Apomorphine, Betamethasone, Cortisone, Deflazacort, Dexamethosone, Hydrocortisone, Methylprednisolone, Prednisolone, Triamcinolone, Ciclosporine, Sirolimus, Tacrolimus, Interferon Alpha, and Interferon Beta.

**[0026]** In another example, the disclosure provides formulations comprising (a) a lysate composition comprising (i) a bacterial lysate and/or lysate fraction capable of activating at least one or more toll-like receptors (TLRs) or Nod-like receptors (NLRs); (ii) an optional promoter for enhancing absorption of the composition; and (iii) an optional carrier for increasing a volume of the composition; and (b) an isolated human anti-TNFalpha antibody or antigen-binding fragment thereof or TNF inhibitor. In some examples, the human anti-TNFalpha antibody or antigen-binding fragment thereof is adalimumab. In another example, the disclosure provides uses of such formulations in the manufacture of a medicament for the treatment of rheumatoid arthritis (RA), late-onset RA, or psoriatic arthritis in a subject. In another example, the disclosure provides methods for the treatment of rheumatoid arthritis (RA), late-onset RA, or psoriatic arthritis in a subject, the method comprising administering to the subject a therapeutically effective amount of such formulations.

**[0027]** In some examples of any of the methods or compositions disclosed herein, the bacterium is a Gram-positive or Gram-negative bacterium. In some examples of any of the methods or compositions disclosed herein, the Gram-positive bacterium is selected from the group consisting of a bacterium of *Lactobacillaceae* family, a bacterium of *Streptococcaceae* family, a bacterium of *Bifidobacteriaceae* family, and a bacterium of *Bacillaceae* family. In some examples, the Gram-positive bacterium is selected from the group consisting of *Bacillus coagulans*, *Lactobacillus sporogenes*, *Streptococcus thermophilus*, *Bifidobacterium animalis*, *Bifidobacterium. animalis*, subspecies *animalis*, *Bifidobacterium infantis*, *Bifidobacterium longum*, *Bifidobacterium breve*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus delbrueckii*, *Lactobacillus delbrueckii* subspecies *bulgaricus*, *Lactococcus lactis*, *Lactococcus lactis* subspecies *lactis*, *Streptococcus lactis*, *Streptococcus thermophilus*, *Bifidobacterium lactis*, *Bifidobacterium breve*, *Pediococcus acidilactici*, and *Lactobacillus helveticus*.

**[0028]** In some examples of any of the methods or compositions disclosed herein, the Gram-negative bacterium is selected from the group consisting of a bacterium of

*Pseudomonas* genus, *Klebsiella* genus, *Xanthomonas* genus, *Shigella* genus, and *Enterobacter* genus. In some examples, the Gram-negative bacterium is selected from the group consisting of *Klebsiella oxytocia, Shigella flexneri, Xanthomonas campestris,* and *Pseudomonas flourescens.*

**[0029]** In some examples of any of the methods or compositions disclosed herein, the TLR agonist, lysate, lysate fraction, or cell wall fraction activates at least one or more of TLR 2, TLR 3, TLR 4, TLR 5, TLR 7, TLR 8, TLR 9, NOD1, and NOD2. In some examples, the TLR agonist, lysate, lysate fraction, or cell wall fraction activates two or more of TLR 2, TLR 3, TLR 4, TLR 5, TLR 7, TLR 8, TLR 9, NOD1, and NOD2. In some examples, the TLR agonist, lysate, lysate fraction, or cell wall fraction activates TLR 2 and TLR 4. In some examples, the TLR agonist, lysate, lysate fraction, or cell wall fraction activates three or more of TLR 2, TLR 3, TLR 4, TLR 5, TLR 7, TLR 8, TLR 9, NOD1, and NOD2.

**[0030]** In some examples of any of the methods or compositions disclosed herein, the promoter is selected from the group consisting of amino acids, amino sugars, and sugars. In some examples, the carrier is selected from the group consisting of a binder, a gum base, and combinations thereof. In some examples, the gum base comprises at least one hydrophobic polymer and at least one hydrophilic polymer. In some examples, the binder is selected from the group consisting of a sugar, a sugar alcohol, and combinations thereof. In some examples, the sugar alcohol is selected from the group consisting of mannitol, sorbitol, xylitol, and combinations thereof.

**[0031]** In some examples, the compositions are manufactured as a dosage form selected from the group consisting of a lozenge, a chewing gum, a chewable tablet, a candy, and a dissolving tablet. In some examples, the dosage form delivers the TLR agonist to an oral mucosa. In some examples, the oral mucosa is selected from the group consisting of the sublingual mucosa, buccal mucosa, and a combination thereof.

**[0032]** In some examples of any of the methods and compositions disclosed herein, the compositions are formulated for oral mucosal delivery; in some examples, the compositions are formulated for sublingual or buccal delivery. In some examples, the compositions are formulated to dissolve in not less than 1 minute after administration.

**[0033]** These as well as other examples, advantages, and alternatives, will become apparent to those of ordinary skill in the art by reading the following detailed description, with reference where appropriate to the accompanying drawings, and taken together with the accompanying claims.