GASEOUS NITRIC OXIDE-SEQUESTERING PRODUCTS AND PROCESSES OF PREPARING SAME

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Abstract
A process of preparing articles having gaseous NO sequestered therein is disclosed, as well as articles prepared thereby. Also disclosed are articles having gaseous NO sequestered therewithin and having reduced amount of oxygen-containing and/or nitrogen-containing reactive species. Also disclosed are processes of preparing packaged articles having a non-gas permeable package that comprises gaseous NO therein and packaged articles made therefrom. Also disclosed are charging devices which can be utilized in the above-described processes. The articles prepared by the above-described processes are preferably medical devices such as indwelling catheters, intubation devices and tampons. Tampons having sequestered therein gaseous NO, uses thereof and processes of preparing same are also disclosed.
FIG. 6

FIG. 7
FIG. 8

FIG. 9
Nitrite release from tracheal tubes

- Mallinckrodt: Hi-Lo Tracheal tube 6.5 mm ID ref# 86110
- Mallinckrodt, Hi-Contour Tracheal tube 4.5 Oral/Nasal 6.2, 11 mm ref# 107-45

FIG. 16
FIG. 20

FIG. 21
FIG. 23

FIG. 24
GASEOUS NITRIC OXIDE-SEQUESTERING PRODUCTS AND PROCESSES OF PREPARING SAME

FIELD AND BACKGROUND OF THE INVENTION

[0001] The present invention, in some embodiments thereof, relates to medical products and, more particularly, but not exclusively, to products having gaseous nitric oxide (NO) sequestered therein, to processes and systems for producing such products and to uses thereof.

[0002] Infection is a constant risk to any healthy person, and poses even a higher risk to hospitalized patients. Infections which are a result of treatment in a hospital or a healthcare service unit are often termed nosocomial infections. The risk of infection is further increased when the natural infection barriers of skin or other epithelial surfaces are breached during a surgical procedure, and/or otherwise in cases where bacteria normally present on the skin or in the air is allowed to access the interior surfaces of the body.

[0003] Nosocomial infections occur even when good hygiene and sterile technique is followed, although the incidence is reduced. Despite even the most rigorous aseptic procedures, people cannot be completely isolated from infectious agents, and the use of prophylactic antibiotics, while reducing some pathogens, may allow other pathogens (e.g., resistant pathogens) to emerge.

[0004] Medical devices used in the care or treatment of people are sterilized before use. While medical devices that comprise metal, glass or other heat-stable materials can be treated with steam (autoclave) or hot air, the use of various polymers and plastics in medical devices may render heat-dependent sterilization unsuitable. Heat-sensitive medical devices are typically sterilized using gamma irradiation or sterilant gas technologies employing ethylene oxide, chlorine dioxide, hydrogen peroxide, and the like, which generally do not require heat application.

[0005] However, regardless of the method of sterilization, the medical device is only sterile until it is exposed to a non-sterile environment. Once the medical device is exposed to a non-sterile environment, which, for example, may occur after a package containing the medical device is opened in a non-sterile environment, microbial contaminants in the air or in the subject's body (e.g., skin or intestinal flora) can contact the device. Under the generally warm and moist conditions found in a subject's body, the microbial contaminants can establish an infection. Contamination may even occur in a surgical environment.

[0006] Catheter-associated bacteriuria (CAB) is the most common nosocomial infection worldwide. It accounts for up to 40% of hospital-acquired infections in the US each year. CAB carries with it a 2.8-fold increased risk of death and results in bacteremia in approximately 3% of patients, constituting a serious complication. Bacteriuria is typically caused by a single organism, with Escherichia coli (E. Coli) being the most frequently isolated species. Together, Escherichia coli and Pseudomonas aeruginosa account for over 39% of cases. As a result of the widespread use of urinary catheterization, CAB results in considerable antimicrobial use. According to some studies, the daily rate of bacteriuria in catheterized patients ranges from 3 to 8%; and the incidence of bacteriuria is directly related to the duration of catheterization.

[0007] Catheter-associated urinary tract infection (CAUTI) is the most prevalent form of CAB. The risk of acquiring CAUTI depends on the method and duration of catheterization, the quality of catheter care, and host susceptibility.

[0008] CAB is difficult to treat with current antimicrobial strategies because of the antibiotic resistance biofilm that forms from free-floating bacteria that adhere to the surface of catheters and colonize them. These biofilm bacteria are highly differentiated and extremely resistant to antibiotics. Biofilms are especially relevant in catheterization, as indwelling urinary catheters provide a surface for the attachment of microbial host cell binding receptors that are recognized by bacterial adhesins. This in turn facilitates microbial adherence and subsequent colonization with uropathogens. Catheterization also disrupts the uroepithelial mucosa, exposing new binding sites for bacterial adhesions. Once biofilms are established they shed cells that seed other sections of the catheter and bladder. They also protect pathogenic bacteria from antibiotics and the host immune response.

[0009] Various approaches have been developed to prevent biofilm formation, including the application of antiseptic lubricating gels at the catheter insertion point, the use of antireflux valves, and the application of a tape seal to the catheter drainage tubing junction [Stickler, Neurourology and Urodynamics 27: 748, 2008]. In addition to these measures, catheter coatings are also being investigated to determine whether they can inhibit the formation of biofilms. Some studies with anti-adherence agents (e.g., heparin) have shown promise [Stickler, Symposium series (Society for Applied Microbiology) 163S-70S, 2002; Tenke et al. Int J Antimicrob Agents 23 Suppl 1: 567-574, 2004]. Other studies have antibiotic coating to eradicate bacteria. In addition, gendine-coated catheters, silver alloy-coated and nitric oxide-coated catheters have demonstrated inhibition of biofilm formation in some recent studies [Regev-Shoshani et al. Antimicrob agents and chemother 54: 273-279, 2010]; Hachem et al. Antimicrob agents and chemother 53: 5145-5149, 2009].

[0010] Silver is a very effective antibacterial substance and silver alloy coated-catheters have been utilized in recent years in an effort to reduce infection rates. However, silver oxide catheters were not associated with a statistically significant reduction in CAB, and other meta-analyses have similarly concluded that silver oxide coated catheters are ineffective. Recent Infectious Diseases Society of America (IDSA) guidelines (2009) described the treatment effect observed with silver alloy-coated catheters as being significantly smaller in more recent studies than in earlier research. Bjarnsholt et al. [APMIS 115: 921-928, 2007] demonstrated that up to one hundred times more silver would be needed to achieve efficacy against biofilm organisms as compared to planktonic organisms.

[0011] Antibiotic-coated catheters may reduce the risk of CAB by preventing or delaying onset in hospitalized patients [Darouiche et al., J infect diseases 176: 1109-1112, 1997; Stickler, 2008, supra], and have demonstrated antimicrobial effects against bacteriuria pathogens in several in vitro studies [Johnson et al. Antimicrobial agents and chemother 43: 2990-2995, 1999; Darouiche et al., 1997, supra], and in vivo studies also report positive effects [Jacobson et al. Clinical Microbiol Reviews 21: 26-59, 2008].

[0012] Ciprofloxacin, gentamicin, norfloxacin, nitrofurazone, and combinations of compounds such as chlorhexidine and protamine sulfate have been successfully incorporated into catheter coatings [Jacobson et al., 2008, supra]. Nitro-
furans have proven to be effective against a wide spectrum of gram-positive and gram negative bacteria, including a variety of strains of the common urinary pathogens. In vitro studies indicate that nitrofuran zathigers might have a stronger antibacterial effect than silver hydrogel catheters [Barnes et al. 2007, supra]. However, nitrofurans have been demonstrated to be ineffective against most strains of Pseudomonas aeruginosa, and they do not inhibit viruses or fungi. Nitrofurazone catheters are also very expensive.

[0013] Bacterial vaginitis (BV) is the most common vaginal infection in women of childbearing age, with a prevalence of approximately 30% (Allsworth & Peipert, 2007). BV has gained increasing attention as many epidemiological studies have established its association with a wide array of infectious morbidities, such as increased susceptibility to sexually transmitted infections (including HIV and genital herpes), as well as unfavorable pregnancy outcomes, in particular pre-term birth [Nyrjersy, P. 2008. Infect Dis Clin N Am 22: pp. 637-652].


[0015] VVC affects primarily healthy women, with risk factors for infection including frequent sexual intercourse, receptive oral sex, high-oestrogen oral contraceptives, condoms, and spermicides, hormone replacement therapy, uncontrolled diabetes mellitus, conditions with high reproductive hormone levels, genetic predispositions pregnancy, and antibiotic usage [Achkar and Fries, 2010, supra].

[0016] C. albicans is the most common species identified in VVC cases, with an estimated incidence of 76 to 89%. Candida albicans is an opportunistic fungus capable of colonizing the vaginal mucosa, which is present in the vaginal tracts of up to 70% of non-pregnant women [Bauters et al., 2002, supra; Beigi et al., 2004, Obstet Gynecol. November; 104 (5 Pt 1):pp. 926-30]. In the absence of immunosuppression or compromised mucosa, individuals with vaginal colonization of Candida are asymptomatic. Upon disturbance in the balance between colonization and the host, Candida can cause vulvovaginal candidiasis (VVC), resulting in clinical signs and symptoms of inflammation [Achkar and Fries, 2010, supra]. The transition from asymptomatic colonization to symptomatic candidiasis may also result from factors that enhance fungus virulence (Cassone et al., 2007).

[0017] An estimated 80 to 90% of uncomplicated VVC cases may be successfully treated with short-course or single-dose therapy with azoles, with fluconazole being the most widely used oralazole; treatment duration ranges from 1 to 7 days, depending on the product [Sobel, 2007, supra]. Studies of vaginal yeast isolates suggest that resistance of C. albicans to fluconazole ranges from 3.7% to 68.2% [Bauters et al., 2002, supra]. While topical azoles are considered to be safe and generally well tolerated, 5 to 10% of patients experience a burning sensation with use [Sobel, 2007, supra; Nyrjersey, 2008, supra]. Studies also suggest that most patients prefer oral azole administration for its convenience and lack of local side effects and messiness [Tooley, 1985, Practitioner 229: pp. 655-660]. However, orally-administered azoles such as fluconazole may be associated with side effects, such as GI intolerance and headache, and sometimes rash and liver toxicity [Nyrjersey, 2008, supra].

[0018] While VVC is commonly managed with intravaginal azole regimens of varying duration, there is a growing trend toward shorter therapies, including single-dose treatments. However, women with chronic or persistent yeast infections (RVVC) are less likely to respond to shorter treatment regimens, and symptomatic relapse occurs in more than half of RVVC cases within a short time of treatment cessation.

[0019] There is also concern that repeated azole treatments may induce drug resistance, shifting the spectrum of causative Candida species toward the azole resistant non-C. albicans.

[0020] Multiple-day treatment regimens are more effective in women prone to RVVC and in certain populations, but the inconvenience of these regimens may negatively impact treatment compliance and satisfaction, symptom control, and quality of life. Furthermore, single-dose oral or intravaginal therapy is limited in its efficacy to the treatment of mild to moderate infections. Oral azoles are also contraindicated during pregnancy, are ineffective in vaginitis caused by C. glabrata, and may be associated with side effects such as GI intolerance, headache, and more rarely, rash and liver toxicity.

[0021] Bacterial vaginoses (BV) is recognized as being the most common cause of vaginal discharge. BV typically results from anaerobic bacterial infections from species such as Gardnerella vaginalis, Mycoplasma hominis, and Ureaplasma urealyticum, although aerobic bacteria have also been observed as causative agents.

[0022] To date, treatment of BV includes both intravaginal and oral formulations of the antibiotics clindamycin and metronidazole [Chen et al. J Womens Health (Larchmt). 18(12): 1997-2004], although a cure rate of only 61% following metronidazole treatment has recently been reported [Milikic and Budak, 2010. Arch Gynecol Obstet 282:43-47]. Furthermore, reported recurrence rates following antibiotic therapy range from 3 to 38%. High rates of recurrence and lack of symptom resolution indicate that the primary treatment of BV with antibiotics may not be completely effective. In addition, antibiotic resistance becomes a concern during continued antibiotic treatment of BV, as well as possible changes to the composition of the normal flora of the vagina.

[0023] Forty-two percent of women with recurrent vaginal infections have been reported to resort to alternative therapies, such as probiotics or homeopathy, dietary restriction of sugars and inclusion of yogurt, and hormonal manipulation with depot medroxyprogesterone and desensitization therapy, to prevent recurrences, yet, support for the efficacy of these methodologies are sparse and often lack methodological quality and/or sufficient data.

[0024] The feasibility of utilizing tampons as drug delivery systems for prolonged intravaginal drug administration of metronidazole has been studied using different commercially available tampon brands [Chen et al. J Pharm. Sci. 71(7): 767-771].

[0025] Nitric oxide (NO) is a small, naturally produced, hydrophobic, free-radical gas that has a major role in innate immunity. NO exhibits broad reactivity and rapid diffusive properties through biological liquids and lipid membranes, with a short half-life in a physiological milieu [Subczynski
and Wisniewska. 2000. Acta Biochim. Pol. 47:613-625. Overproduction of NO induced by the enzymatic activity of inducible nitric oxide synthase (iNOS) in various cell types has been shown to play a vital role in several inflammatory and immunoregulatory processes. NO has been shown to play important roles in vasodilatation, neurotransmission, angiogenesis, modulation of wound healing, and non-specific responses to infection.

NO has been shown to be bacteriostatic and bactericidal. Miller et al. [Nitric Oxide 20: 16-23, 2009] demonstrated that multiple 30 minute treatments of 160 ppm nitric oxide resulted in over a 5 log 10 colony forming unit per milliliter (CFU/ml) decrease in the bacterial load of Staphylococcus aureus, E. coli, and Pseudomonas aeruginosa.

Nitric oxide is also known as an antimicrobial agent. Gaseous nitric oxide at a concentration of about 200 ppm has been demonstrated to clear pneumonia caused by pathogens such as Pseudomonas aeruginosa or S. aureus [McMullin et al., 2005, Respir. Care 50:1451-1456]. Topical applications of gaseous nitric oxide at about 200 ppm has been demonstrated to inhibit or prevent growth of a variety of microbial pathogens including P. aeruginosa, S. aureus, E. coli, Streptococcus spp. and Candida albicans [Ghaflari et al., 2005, Nitric Oxide 14:21-29]. Nitric oxide has also been demonstrated to inhibit replication of a variety of viral pathogens including influenza virus, retroviruses, flaviviruses, e.g. vesicular stomatitis virus, flavivirus e.g. Japanese Encephalitis virus [Rimmelzwaan et al., 1999, J. Virol 73(10): 8880-8883 and references cited therein].

At very low concentrations (up to 0.1 parts per million in air), gaseous nitric oxide (gNO) may be administered to humans having breathing problems since it was found to have beneficial effects due to its bronchodilatory and vasodilatory activity. However, nitric oxide is rather complicated for use as a gas. Moreover, colorless gaseous NO may react with oxygen under certain conditions, yielding nitrogen dioxide (NO₂), a reddish-brown gas with much higher toxicity than NO.


Additional background art includes U.S. Pat. Nos. 5,374,710, 5,155,137, 6,951,902, 6,949,530, 6,911,478 and 6,110,453.


Regar-Shoshani et al. (2010, supra) disclose studies conducted for investigating the potential of NO to prevent biofilm formation. Catheters impregnated with gaseous nitric oxide (gNO) demonstrated slow-release of nitric oxide over a 14-day period, were rendered antiseptic, and were able to prevent bacterial colonization and biofilm formation on their luminal and exterior surfaces. NO impregnated catheters inhibited the growth of Escherichia coli within the surrounding media and eradicated bacterial concentrations of up to 10⁴ CFU/ml.

SUMMARY OF THE INVENTION

According to an aspect of some embodiments of the present invention there is provided a process of preparing an article having gaseous nitric oxide sequestered therewithin, the process comprising: placing an article within a chamber; generating a reduced pressure in the chamber; and filling the chamber with a gaseous nitric oxide-containing environment, thereby preparing the article having gaseous nitric oxide sequestered therewithin.

According to some embodiments of the invention, generating the reduced pressure comprises reducing the pressure by from ~1 psi to ~50 psi.

According to some embodiments of the invention, reducing the pressure is such that a humidity in the article is reduced by at least 50%.

According to some embodiments of the invention, reducing the pressure is such that an amount of oxygen in the article is reduced by at least 50%.

According to some embodiments of the invention, filling the chamber with the gaseous nitric oxide-containing environment is effected for a time period that ranges from 30 minutes to 24 hours.

According to some embodiments of the invention, the article includes at least a portion of a surface configured to sequester nitric oxide.

According to some embodiments of the invention, the article includes at least a portion of a surface configured to sequester at least 1 ppm nitric oxide per cm².

According to some embodiments of the invention, the article includes at least a portion of a surface configured to sequester at least 200 ppm nitric oxide per cm².

According to some embodiments of the invention, at least a portion of the article comprises a plurality of voids for sequestering nitric oxide.

According to some embodiments of the invention, the article is a medical device.

According to some embodiments of the invention, the medical device is selected from the group consisting of a catheter, an endotracheal tube, a tampon, a tubing, a prosthesis, a medical implant, an artificial joint, an artificial valve, a needle, an intravenous access device, a cannula, a biliary stent, a nephrostomy tube, a vascular graft, an infusion pump, an adhesive patch, a suture, a fabric, a mesh, a polymeric surgical tool or instrument, an intubation device, prosthethics, artificial joints, artificial valves, adhesive patches, sutures, fabrics, a cardiovascular stent, a cardiac surgery device, an
orthopedic surgery device, an orthodontic or periodontic device, a dental surgery device, a veterinary surgery device, a bone scaffold, a hemodialysis tubing or equipment, a blood exchanging device, an implantable prostheses, a bandage, a heart valve, an ophthalmic device and a breast implant.

According to some embodiments of the invention, the medical device is an implantable device.

According to some embodiments of the invention, the medical device is selected from the group consisting of an indwelling catheter and a tracheal tube.

According to some embodiments of the invention, the catheter is selected from the group consisting of umbilical catheter, central venous catheter, biliary vascular catheter, pulmonary artery catheter, peripheral venous catheter, arterial line, central venous catheter, peritoneal catheter, epidural catheter and central nervous system catheter.

According to some embodiments of the invention, the medical device is a tampon.

According to some embodiments of the invention, the medical device comprises a polymeric material.

According to some embodiments of the invention, the polymeric material is a hydrophilic polymeric material.

According to some embodiments of the invention, the polymeric material is selected from the group consisting of silicone, polyacetal, polyurethane, polyester, polytetrafluoroethylene, polyethylene, polymethylmethacrylate, polyhydroxethyl methacrylate, polyvinyl alcohol, polypropylene, polymethylpentene, polyetherketone, polyphenylene oxide, polyvinyl chloride, polycarbonate, polysulfone, acrylonitrile-butadiene-styrene, polyetherimide, polyvinylidine fluoride, polysiloxane, fluorinated polysiloxane, polyvinylethylene, ethylene vinyl acetate, methacrylic acid, ethylene oxide, propylene oxide, polystyrene, ethylene-propylene rubber, fluoroelastomer, silastic elastomer, polyethylene tetraphthalate, colloidion, carbothane, poly(1-lactide), poly(DL-lactide), poly(DL-lactide-co-glycolide), poly(e-caprolactone), poly(paradioxanone), polytrimethylene carbonate, collagen, silk, elastin, chitin, coral, hyaluronic acid, bone, rayon, cotton, cellulose polymer, copolymers of any of foregoing and any combination of the foregoing.

According to some embodiments of the invention, the polymeric material is a hydrophilic or amphiphilic polymeric material.

According to some embodiments of the invention, the polymeric material is selected from the group consisting of rayon, cotton and cellulose polymer (as in the case of, for example, tampons).

According to some embodiments of the invention, the article is selected from the group consisting of a packaged article and a bare (unpackaged) article.

According to some embodiments of the invention, the packaged article comprises a gas-permeable package.

According to some embodiments of the invention, placing the article in the chamber comprises positioning the article within an enclosure.

According to some embodiments of the invention, the enclosure is a non-gas permeable enclosure.

According to some embodiments of the invention, the process further comprises, subsequent to the filling, sealing the enclosure.

According to some embodiments of the invention, the process further comprises disposing a desiccant within the enclosure.

According to some embodiments of the invention, the process further comprises disposing a nitric oxide indicator within the enclosure.

According to some embodiments of the present invention there is provided an article having a gaseous nitric oxide sequestered therewithin, prepared by the process described hereinabove.

According to some embodiments of the invention, the article further comprises an enclosure.

According to as aspect of some embodiments of the invention there is provided an article having sequestered therewithin at least 1 ppm nitric oxide per cm³ and comprising less than 1 ppm per cm³ nitrogen-containing and/or oxygen containing reactive species.

According to some embodiments of the invention, the article has sequestered therein from 1 ppm to 200 ppm per cm³ nitric oxide.

According to some embodiments of the invention, the sequestered nitric oxide is releasable in an aqueous solution during a time period that ranges from 1 hour to 1 month.

According to some embodiments of the invention, any of the articles described herein further comprises an enclosure.

According to some embodiments of the invention, the enclosure comprises a gaseous nitric oxide-containing environment.

According to some embodiments of the invention, the environment is an ambient environment.

According to some embodiments of the invention, the enclosure is a non-gas permeable enclosure.

According to some embodiments of the invention, any of the articles described herein further comprises a desiccant disposed within the enclosure.

According to some embodiments of the invention, any of the articles described herein further comprises a nitric oxide indicator disposed within the enclosure.

According to some embodiments of the invention, any of the articles described herein comprises at least 1 ppm per cm³ nitric oxide sequestered therewithin.

According to some embodiments of the invention, any of the articles described herein comprises at least 200 ppm per cm³ nitric oxide sequestered therewithin.

According to some embodiments of the invention, the sequestered nitric oxide is releasable in an aqueous solution during a time period that ranges from 1 hour to 1 month.

According to some embodiments of the invention, any of the articles described herein is being substantially devoid of humidity.

According to some embodiments of the invention, any of the articles described herein is being substantially devoid of oxygen.

According to some embodiments of the invention, an amount of nitrogen-containing and/or oxygen containing reactive species in the article is lower than 1 ppm per cm³.

According to some embodiments of the invention, an amount of nitrogen-containing and/or oxygen containing reactive species in the article is lower than 1 ppb per cm³.

According to some embodiments of the invention, any of the articles described herein is a medical device, as described herein.

According to an aspect of some embodiments of the present invention there is provided a tampon having sequestered therein gaseous nitric oxide.
According to some embodiments of the invention, an amount of the nitric oxide ranges from 1 ppm to 200 ppm per cm³.

According to some embodiments of the invention, an amount of the nitric oxide is at least 200 ppm per cm³.

According to an aspect of some embodiments of the present invention there is provided a process of preparing a tampon having sequestered therein nitric oxide, the process comprising: exposing a tampon to gaseous nitric oxide-containing environment, thereby preparing the tampon having sequestered therein nitric oxide.

According to some embodiments of the invention, the exposing comprises: placing the tampon in a chamber; and filling the chamber with the nitric oxide-containing environment.

According to some embodiments of the invention, the process further comprises, prior to the filling, generating a reduced pressure in the chamber.

According to some embodiments of the invention, generating the reduced pressure is such that a humidity in the enclosure is reduced by at least 50%.

According to some embodiments of the invention, reducing the pressure is such that an amount of oxygen in the enclosure is reduced by at least 50%.

According to some embodiments of the invention, filling is effected by flowing nitric oxide into the chamber.

According to some embodiments of the invention, the process further comprises, prior to the filling, absorbing humidity from the package.

According to some embodiments of the invention, filling is effected such that an ambient environment is provided within the package.

According to some embodiments of the invention, the nitric oxide-containing environment comprises at least 0.01% nitric oxide.

According to some embodiments of the invention, the packaged article has gaseous nitric oxide sequestered within the article.

According to an aspect of some embodiments of there invention there is provided a process of preparing a packaged article, wherein the packaged article comprises a non-gas permeable enclosure, the process comprising: positioning an intact article within the non-gas permeable enclosure, to thereby obtain a non gas-permeable enclosure having the article disposed therewithin; exposing the enclosure with a gaseous nitric oxide-containing environment, so as to introduce into the enclosure the nitric oxide-containing environment; and sealing the enclosure, thereby preparing the packaged article.

According to some embodiments of the invention, the exposing comprises: placing the enclosure in a chamber; and filling the chamber with the gaseous nitric oxide-containing environment.

According to some embodiments of the invention, the process further comprises, prior to the filling, sealing the chamber.

According to some embodiments of the invention, reducing the pressure is such that a humidity in the enclosure is reduced by at least 50%.

According to some embodiments of the invention, reducing the pressure is such that an amount of oxygen in the enclosure is reduced by at least 50%.

According to some embodiments of the invention, the filling is effected by flowing nitric oxide into the chamber.

According to some embodiments of the invention, the process further comprises, prior to the filling, absorbing humidity from the enclosure.

According to some embodiments of the invention, the filling is effected such that an ambient environment is provided within the enclosure.

According to some embodiments of the invention, the nitric oxide-containing environment comprises at least 0.02% nitric oxide.

According to some embodiments of the invention, the packaged article has gaseous nitric oxide sequestered within the article.
According to an aspect of some embodiments of the invention there is provided a packaged article prepared by the process described herein.

According to an aspect of some embodiments of the invention there is provided a package comprising: a material configured to form an enclosure; a article disposed within the enclosure; and a gaseous nitric oxide-containing environment within the enclosure.

According to some embodiments of the invention, the package is a non-gas permeable package.

According to some embodiments of the invention, the enclosure is a sealed enclosure.

According to some embodiments of the invention, the environment is an ambient environment.

According to some embodiments of the invention, the environment comprises gaseous nitric oxide in an amount sufficient to sterilize an interior of the enclosure and the article.

According to some embodiments of the invention, the amount of gaseous nitric oxide is at least 200 ppm.

According to some embodiments of the invention, the article has gaseous nitric oxide sequestered therewith.

According to some embodiments of the invention, the amount of the gaseous nitric oxide sequestered in the article is at least 1 ppm.

According to some embodiments of the invention, the gaseous nitric oxide sequestered in the article is releasable in an aqueous solution during at least 1 minute.

According to some embodiments of the invention, the package further comprises a desiccant.

According to some embodiments of the invention, the package further comprises a nitric oxide indicator.

According to some embodiments of the invention, the article is a medical device, as described herein.

According to an aspect of some embodiments of the present invention there is provided a charging device comprising: a chamber comprising an inlet for receiving a gaseous nitric-oxide containing environment and an outlet for releasing the gaseous nitric-oxide containing environment; and an article disposed within the chamber.

According to some embodiments of the invention, the device further comprises a desiccant configured to absorb humidity from the gaseous environment.

According to some embodiments of the invention, the device further comprises a nitric oxide indicator configured to undergo a color change suitable for visual assessment of whether the article has been exposed to the nitric oxide.

According to some embodiments of the invention, the device further comprises an outlet for generating a reduced pressure in the chamber.

According to some embodiments of the invention, the device further comprises a package enclosing the article.

According to some embodiments of the invention, the package is a non-gas permeable package.

According to some embodiments of the invention, the article includes at least a portion of a surface configured to sequester the nitric oxide.

According to some embodiments of the invention, the surface is configured to sequester at least 1 ppm nitric oxide.

According to some embodiments of the invention, the surface is configured to sequester about 1 ppm nitric oxide to about 20,000 ppm nitric oxide.

According to an aspect of some embodiments of the invention there is provided a charging device comprising: a sealed chamber having a reduced pressure there within; and an article disposed within the chamber.

According to some embodiments of the invention, the chamber further comprises an outlet for generating the negative pressure within the chamber.

According to some embodiments of the invention, the device further comprises an inlet configured for receiving a gaseous nitric oxide-containing environment and an outlet for releasing the gaseous nitric oxide-containing environment.

According to some embodiments of the invention, the device further comprises a package enclosing the article.

According to some embodiments of the invention, the package is selected from the group consisting of a gas permeable package and a non-gas-permeable package.

Unless otherwise defined, all technical and/or scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of the invention, exemplary methods and/or materials are described below. In case of conflict, the patent specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be necessarily limiting.

BRIEF DESCRIPTION OF THE DRAWINGS

Some embodiments of the invention are herein described, by way of example only, with reference to the accompanying drawings. With specific reference now to the drawings in detail, it is stressed that the particulars shown are by way of example and for purposes of illustrative discussion of embodiments of the invention. In this regard, the description taken with the drawings makes apparent to those skilled in the art how embodiments of the invention may be practiced.

In the drawings:

FIG. 1 is a bar graph demonstrating the antimicrobial effect of an exemplary polymeric medical device sample (a catheter section) dosed with nitric oxide for 1, 5, 15, 30, 60 or 120 minutes, upon incubation in a bacterial suspension of 10^7; 10^8; or 10^9 CFU/ml for 8 hours at 37°C, while showing the number of CFU/ml remaining in the suspension with respect to dose time and starting culture CFU/ml.

FIG. 2 is a chart presenting the total accumulation of nitrites and nitrates, produced from an exemplary medical device sample (a catheter section) dosed with nitric oxide and immersed in water, and showing a time-dependent nitric oxide dosing.

FIGS. 3A-B present a micrograph (FIG. 3A) and bar graphs (FIG. 3B) demonstrating the effect of catheter storage conditions on the antimicrobial activity of control and nitric oxide-eluting samples after storage and upon incubation with suspensions of 10^7 CFU/ml E. Coli for 1 minute followed by 24 incubation in PBS. FIG. 3A presents images of representative three-compartment petri plates of control and nitric oxide-eluting samples after both sets were stored for 7 days in 50 ml sterile air (I) or 5 ml sterile water (II); FIG. 3B presents the viable counts of the triplicate CFU with the hatched bars are data from the control, and the checkered bars are data from the NO-impregnated catheters. The error bars indicate standard deviations.
FIGS. 4A-B present a bar graph (FIG. 4A) and a micrograph (FIG. 4B) showing the biofilm formation and biofilm-embedded bacteria on the inner lumen of control and NO-dosed catheter samples subjected to 24 hours of urine flow. FIG. 4A presents biofilm formation as the absorbance at 595 nm of the crystal violet attached to the catheter pieces after extraction with ethanol. The hatched bars are data from the control, while the checked bars are data from the impregnated catheters. FIG. 4B presents representative images of biofilm-embedded bacteria determined in 1 μl (bottom) and 10 μl (top) of water surrounding a selected catheter piece and incubated for 24 hours.

FIGS. 5A-B present a photograph showing the effect of nitric oxide gas on biofilms of Escherichia coli after exposure for 5, 10, 30, 60 or 120 minutes, relative to controls (FIG. 5A); and a photograph showing the effect of nitric oxide gas on biofilms of Acinetobacter baumannii after exposure for 5, 10, 30, 60 or 120 minutes, relative to controls (FIG. 5B).

FIG. 6 presents a bar graph showing the antimicrobial activity of nitric oxide-dosed catheter sections on the bacterial strains Enterococcus faecalis #29212 (E.f. #29212), Staphylococcus saprophyticus #15305 (S.s. #15305), Staphylococcus epidermidis #35984 (S.e. #35984), Escherichia coli #25922 (E.c. #25922), Pseudomonas aeruginosa #14210 (P.a. #14210), Acinetobacter baumannii #BAA-747 (A.b. #BAA-747) and Candida albicans (C.a. #14053).

FIG. 7 is a bar graph showing the antimicrobial activity of nitric oxide-dosed catheters the bacterial clinical isolates Enterococcus faecalis (E.f.), Staphylococcus aureus (S.a.), E. coli (E.c.), P. aeruginosa (P.a.), and Stenotrophomonas maltophilia (S.m.).

FIG. 8 is a bar graph presenting the relative biofilm formation on luminal surfaces of nitric oxide-sequestering catheter sections following 72 hours incubation in urine inoculated with 10^6 CFU/ml of Enterococcus faecalis #29212 (E.f. #29212), Staphylococcus saprophyticus #15305 (S.s. #15305), Staphylococcus epidermidis #35984 (S.e. #35984), Escherichia coli #25922 (E.c. #25922), Pseudomonas aeruginosa #14210 (P.a. #14210), Acinetobacter baumannii #BAA-747 (A.b. #BAA-747), and Candida albicans (C.a. #14053).

FIG. 9 presents a bar graph showing the data obtained for the growth of biofilm-embedded bacteria on nitric oxide-sequestering catheter sections following 72 hours incubation in urine inoculated with 10^6 CFU/ml of Enterococcus faecalis #29212 (E.f. #29212), Staphylococcus saprophyticus #15305 (S.s. #15305), Staphylococcus epidermidis #35984 (S.e. #35984), Escherichia coli #25922 (E.c. #25922), Pseudomonas aeruginosa #14210 (P.a. #14210), Acinetobacter baumannii #BAA-747 (A.b. #BAA-747), and Candida albicans (C.a. #14053).

FIGS. 10A-C present scanning electron micrographs of Staphylococcus epidermidis (ATCC #35984) biofilms on Untreated (control) catheter sections, magnification 2.5 k (inset=20 k) (FIG. 10A); Untreated (control), magnification 2.5 k (right inset=15 k, left inset=20 k) (FIG. 10B); and NO-sequestering catheter sections, magnification 1.5 k (FIG. 10C).

FIGS. 11A-C present scanning electron micrographs of A. baumannii (ATCC #BAA-747) biofilms on Untreated (control) catheter sections, magnification 2.5 k (inset=15 k) (FIG. 10A); Untreated (control), magnification 1.0 k (inset=5 k) (FIG. 10B); and NO-sequestering catheter sections, magnification 1.0 k (FIG. 10C).

FIG. 12 is a bar graph presenting comparative data of E. coli growth in media containing pieces of an NO-sequestering catheter (NOX), a silver-alkoy coated catheter (AC) and an antibiotic-coated catheter (NFC) versus media from control catheter, after immersion of the catheters for 24 hours in suspension comprising 10^6 CFU/ml and incubated for 24 hours at 37° C.

FIG. 13 presents images of representative three compartment LB agar petri plates showing E. coli growth in urine after 72 hours exposure to pieces of NO-sequestering catheter (NOX; bottom left), a silver-alkoy coated catheter (AG; top right) and an antibiotic-coated catheter (NFC; bottom right) and control catheters (top left). Within each three compartment LB agar petri plate 1, 10, and 100 μl of each sample were plated and incubated overnight at 37° C.

FIG. 14 presents images of representative three compartment LB agar petri plates showing E. coli colonization on NO-sequestering catheter (NOX; bottom left), a silver-alkoy coated catheter (AG; top right) and an antibiotic-coated catheter (NFC; bottom right) and control catheters (top left), after immersion of catheters for 24 hours in suspension containing 10^6 CFU/ml of E. coli. In each LB Petri dish a catheter was rolled over the surface and then incubated at 37° C overnight.

FIGS. 15A-B present bar graphs showing comparative data of colonized biofilm formation on NO-sequestering catheter (NOX), a silver-alkoy coated catheter (AG) and an antibiotic-coated catheter (NFC) versus control after 72 hours of incubation, demonstrated by absorbance at 595 nm (FIG. 15A) and the bacterial growth from the biofilms from the different catheters (FIG. 15B).

FIG. 16 presents comparative plots showing nitrite release over time from the NO-sequestering Tracheal tubes Mallincrodt: Hi-Lo Tracheal tube 6.5 mm ID ref No. 86110 (triangles) and Mallincrodt: Hi-Contour Tracheal tube 4.5 oral/nasal 6.2, 11 mm ID ref No. 107-145.

FIG. 17 is a bar graph showing the amount of nitric oxide released after 30 minutes from four exemplary NO-sequestering tampons (see, Table 19), as measured by the respective total amount of nitrites released from the tested tampons Nitrites were measured using Griess reagent. The nitrites released were calculated per 1 tampon.

FIG. 18 presents comparative plots showing the accumulation profiles of nitric oxide production during the first 5 hours for four exemplary NO-impregnated tampons, as measured by the respective total accumulation of nitrites in water produced from the tested tampons. Nitrites were measured using Griess reagent. The nitrites released were calculated per 1 tampon.

FIGS. 19A-D demonstrate the antimicrobial effect of four exemplary NO-sequestering tampons A (FIG. 19A), B (FIG. 19B), C (FIG. 19C) and D (FIG. 19D), as identified in Table 19 herein, by showing images of representative petri plates in which NO-treated tampons inoculated with C. albicans culture for 4 hours at 30° C. were rolled and upon incubating the plates overnight at 30° C.

FIG. 20 is a bar graph demonstrating the anti-infective activity of four exemplary NO-sequestering tampons A-D, as identified in Table 19 herein, by showing the growth of C. albicans in media after immersion of the tampons for 6 hours in suspension comprising 10^6 CFU/ml of C. albicans (dark bars) compared with negative control tampons (white bars). Numbers represent viable counts of the triplicate CFUs. Error bars represent standard deviation.
FIG. 21 is a bar graph demonstrating the anti-infective activity of four exemplary NO-sequestering tampons A-D, as identified in Table 19 herein, by showing the growth of *C. albicans* in media after immersion of the tampons for 4 hours in suspension comprising 10^6 CFU/ml of *C. albicans* (dark bars) compared with negative control tampons (white bars). Numbers represent viable counts of the triplicate CFUs. Error bars represent standard deviation.

FIG. 22 presents representative photos demonstrating the zone of inhibition resulting from NO-treated tampons. NO-treated tampon B (top right), NO-treated tampon C (bottom right) and untreated tampons B and C (top and bottom left, respectively) were cut lengthwise and placed onto a LB agar petri plate that had been inoculated with 200 μl of *E. coli* at 10^6 CFU/ml, then incubated overnight at 37°C.

FIG. 23 presents a schematic illustration of the experimental setup used to test the zone of microbial inhibition in a vaginal model.

Fig. 24 presents representative photos demonstrating the zone of inhibition in a vaginal model. A solution containing LB and 2% (w/v) gelatin was inoculated with *E. coli* (10^6 CFU/ml), placed in a 250 ml Erlenmeyer flask and tampon B (see, Table 19) was suspended therein. The Erlenmeyer flasks were incubated at 37°C overnight. Shown is a picture of NO-treated (right) and untreated (left) tampon B placed inside the inoculated solution after incubation.

FIG. 25 shows total accumulation of nitrates, produced in water during 4 hours, from tampons impregnated with nitric oxide outside their wrappers. Nitrates were measured using Griess reagent. The nitrates released were calculated per 1 tampon.

FIG. 26 presents a schematic illustration of an exemplary charging device 300, according to some embodiments of the present invention, which is fitted with an inlet 345 for receiving gNO within chamber 337 to form gaseous nitric oxide-containing environment 306, valve 315 for closing outlet 345, an outlet 326 for releasing ambient environment or gaseous nitric oxide-containing environment from chamber 337, valve 336 for closing outlet 326, an article 301 disposed within chamber 337, and an optional moisture scavenger in the form of desiccant 302 disposed within chamber 337 and configured to absorb an amount of humidity from the within chamber 337.

FIG. 27 is a schematic illustration of an exemplary charging device 300, as presented in FIG. 26, wherein chamber 337 also includes a surface coating 370 which protects the material of chamber 337 from chemically interacting with gNO-containing environment 306, a gNO indicator 371 configured to undergo a color change when article 301 has been exposed to gNO in environment 306, and humidity indicator 374 for signaling exposure of article 301 to humidity.

FIG. 28 is a schematic illustration of an exemplary charging device 400, according to some embodiments of the present invention, which is fitted with an inlet 445 for receiving gNO within chamber 447 to form gaseous nitric oxide-containing environment 406, valve 415 for closing outlet 445, an outlet 426 for releasing ambient environment or gaseous nitric oxide-containing environment from chamber 447, valve 436 for closing outlet 426, and a non-gas permeable package 473 disposed within chamber 447 and housing article 401.

FIG. 29 is a schematic illustration of an exemplary charging device 400, as presented in FIG. 28, wherein chamber 437 includes surface coating 470 which protects the material of chamber 437 from chemically interacting with gNO-containing environment 406, a gNO indicator 471 configured to undergo a color change when article 401, having gNO-sequestering surface 472 and housed within non-gas permeable package 473 equipped with seal 490, has been exposed to gNO in environment 406, and humidity indicator 474 for signaling exposure of article 401 to humidity.

FIG. 30 presents a schematic illustration of an exemplary charging device 500 having a chamber 137, inlet 125 with valve 11, inlet 135 with valve 2, and inlet 145 with valve 15, inlets which may share a common tube 23 with valve 19 that connects to chamber 137, outlet 126 with valve 36 and connect to purge flow rotomoter 46, outlet 136 with valve 41 and connect to purge flow rotomoter 45, outlets which may share a common tube 33 that connects to chamber 137 and.

FIGS. 31A-B present schematic illustrations of a package for charging and containing gNO-sequestering medical device, according to some embodiments of the present invention, such as a package 200, comprising non-gas permeable package 203 configured to be impermeable to gNO and optionally comprises seal 205 (FIG. 31B), medical device 201 disposed within non-gas permeable package 203, gNO-containing environment 206 engulfing medical device 201 within non-gas permeable package 203 and comprising a predetermined concentration of gNO capable of sterilizing the interior of non-gas permeable package 203 and medical device 201, and further optionally comprising nitric oxide indicator 204 (FIG. 31B), desiccant 202 disposed within non-gas permeable package and configured to absorb humidity from gNO-containing environment 206, and humidity indicator 208 (FIG. 31B) configured to indicate moisture saturation of the desiccant 202.

DESCRIPTION OF SPECIFIC EMBODIMENTS OF THE INVENTION

The present invention, in some embodiments thereof, relates to medical products and, more particularly, but not exclusively, to products having gaseous nitric oxide (NO) sequestered therein, to processes and systems for producing such products and to uses thereof.

Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not necessarily limited in its application to the details of construction and the arrangement of the components and/or methods set forth in the following description and/or illustrated in the drawings and/or the Examples. The invention is capable of other embodiments or of being practiced or carried out in various ways.

As discussed hereinabove, impregnation of gaseous nitric oxide (gNO) in medical devices is regarded highly beneficial for sterilizing articles such as medical devices, by imparting the medical devices with the gNO capabilities to reduce or prevent growth of a variety of pathogenic microorganisms, as well as biofilm formation.

As demonstrated in the Examples section that follows, gaseous nitric oxide sequestered in the solid substance of medical devices, can be used to kill bacteria in an established biofilm and in the surrounding environment; be used as an effective sterilizing agent of such devices; and prevents adherence and growth of various strains or species of bacteria and fungi over an extended period of time following sterilization.
[0181] It has further been demonstrated that medical devices comprising one or more polymers and sequestering gaseous nitric oxide are self-sterilizing at a sufficient level so as to prevent microbial adherence and growth on the medical device or in the liquid media in its immediate vicinity for an extended period of time.

[0182] Furthermore, it has been demonstrated that the physical properties of polymeric material composing the device, as exemplified by silicone, or silicone-coated medical devices, are not substantially altered by treatment with nitric oxide, however, in some cases, a color change is observed that may be useful as an indicator of NO load of the device.

[0183] Overall, impregnation of gNO in medical devices has been shown to effectively provide the device with self-sterilization capacity.

[0184] As further demonstrated in the Examples section that follows, and is referred to in more detail hereinafter, the present inventors have further practiced NO impregnation with medical devices such as tampons, and have shown that (i) tampons can be effectively charged with gNO so as to result in gNO-sequestering tampons; and (ii) tampons having gNO sequestered therewith efficiently reduce microbial growth and thus can be used to treat or prevent various common vaginal infections.

[0185] In view of the proven beneficial properties of medical devices having gNO sequestered therewith, the present inventors have searched for an improved process for impregnating gNO within medical devices. The present inventors have thus designed and successfully practiced a process which involves exposing the article to be impregnated (e.g., a medical device) to reduced pressure, so as to substantially evacuate ambient oxygen and humidity prior to exposure to gNO.

[0186] Without being bound by any particular theory, the present inventors have contemplated that by removing ambient atmosphere from the article to be loaded, and thereby evacuating voids within the article, the gNO sequestering efficiency of the article would be improved due to enhanced free void volume to be occupied by gNO. The enhancement in void volume can be translated in some cases to enhanced affinity of NO, as is discussed in detail hereinafter. The present inventors have also contemplated that removal of ambient atmosphere would prevent gNO degradation and would thus substantially reduce the production of undesired reactive nitric oxide species associated with gNO degradation.

[0187] While reducing the present invention to practice it was found that the above-described process substantially improved previous methodologies by improving the efficiency by which gNO is sequestered from a nitric oxide-containing environment (gNO-containing environment), namely by improving the gNO intake by an article which has been pre-exposed to reduced pressure prior to exposure to a gNO-containing environment. By enhancing the relative amount of gNO taken from the gNO-containing environment, less gNO was spent during the loading process, and shorter exposure time was spent in order to achieve the same gNO loading levels compared to some previously described processes. This process has therefore been shown to be more efficient in terms of both time and cost, and to be safer, as compared to processes that involve loading articles with gaseous nitric oxide without pre-exposure to reduced pressure.

[0188] This process has further been shown efficient by substantially reducing the formation of reactive species (e.g., reactive oxygen species) in the article, which bears a particular importance for implantable medical devices, as is discussed in further detail hereinafter.

[0189] The present inventors have further contemplated a process for producing sterilized and even self-sterilizing packages, which takes advantage of the sterilizing effect imparted by gaseous NO. Thus, the present inventors have designed and successfully practiced a process in which medical devices are packaged in a non-gas permeable enclosure which is loaded with gaseous NO. Such a packaging process allows, for example, storing and/or transporting packaged articles while maintaining the packaged articles sterilized.

[0190] Hence, according to an aspect of embodiments of the present invention, there is provided a process of preparing an article having gaseous nitric oxide (gNO) sequestered therewith. The process, according to this aspect of embodiments of the invention, is effected by:

[0191] placing an article within a chamber;

[0192] generating a reduced pressure in the chamber; and

[0193] filling the chamber with a gaseous NO-containing environment.

[0194] For clarity, it is noted that the term “sequestering” in the context of gaseous nitric oxide (gNO), and the terms “impregnated”, “charged”, “loaded”, “dosed” and “treated” are used interchangeably hereinbelow and throughout to denote an article releasably sequestering gNO therein.

[0195] As used herein, the term “sequestering” and any inflections thereof refer to a state of an article having a foreign substance, such as a gas, incorporated therein; a state which exists substantially from the time the substance is introduced into the article from an external source to the time the substance leaves the article. According to some embodiments of the present invention, in the chemical sense the sequestered substance being released from the article is essentially the same substance that was charged into the article.

[0196] The term “sequestering” therefore encompasses the phrase “releasably sequestering”.

[0197] Hence, “releasably sequestering”, as used herein, is meant to define an article having gNO absorbed therein in a reversible manner, wherein the gNO can be released to the ambient environment from the article under certain conditions. According to some embodiments of the present invention, gNO is released from the article releasably sequestering gNO at an essentially controllable manner.

[0198] The phrase “sequestered therewith” refers to gaseous NO sequestered within the article, namely, in or on at least a portion of the article or in or on any surface thereof. The terms “therein” and “therein” are used herein interchangeably.

[0199] In the context of embodiments of the invention, gNO is releasably sequestered in an article by charging the article with gNO.

[0200] As discussed in detail hereinbelow, the amount of sequestered gNO depends, among other factors, on the size, volume, surface area and composition of the article. The article may be composed and assembled from various parts, layers and compositions, each having a different capacity to sequester gNO according to chemical composition, accessibility, surface area and the likes. In some embodiments, gNO is releasably sequestered within at least a portion of the surface of the article.

[0201] For simplicity, a part of the article which can sequester gNO is referred to herein as a “Surface”, however it is to be understood that the term “surface” as used herein is not lim-
ited to the exterior or upper boundary of the article, not to an external part or a layer of the article, and not to its outwardly expressed manifestations of the article, but rather to all of these features as well as all other internal and external features of the article which can sequester gNO, according to some embodiments of the present invention.

[0202] In cases of articles having components which are inaccessible to the gNO-containing environment, the article can be disassembled to expose these components, or be assembled from raw materials and/or components that were charged with gNO according to some embodiments of the present invention.

[0203] It is noted herein that the article to be charged with gNO in the process presented herein preferably does not sequester gNO in advance, prior to effecting the process. Hence, according to some embodiments of the present invention, the article is untreated with gNO at the time it is first placed in the chamber of the gNO charging device.

[0204] Thus, in the context of embodiments of the invention, the article which is exposed to gaseous nitric oxide-containing environment can be seen as an intact article, whereby the phrase “intact article” is meant to describe an article that has not been exposed to gNO-containing environment.

[0205] The process according to embodiments of this aspect of the present invention starts by placing an article in a chamber. The article placed in the chamber can therefore be regarded as an intact article.

[0206] The chamber can be any tank, canister, vat, barrel, cask, hogshead, drum, case, wrapper, sheath, bag, compartment, vessel, container or receptacle which can serve as an enclosure for the article in terms of size (internal size) and capacity to contain gases, and particularly gNO containing environment. By capacity to contain, it is meant that the chamber is sealed to an extent that allows charging the article, and mechanically fit to sustain both negative and positive pressure. Such requirements typically translate to mechanical integrity for maintaining impermeability to gases, rigidity and/or durability to maintain negative and positive pressure, and the ability to be fitted with inlets and outlets without losing containment of gases, while maintaining the integrity of the article disposed therein.

[0207] The chamber forms a part of a charging device, which is designed to carry out the process presented herein and includes inlets and outlets (a single or a plurality of each); tubes for connecting the inlets and outlets to the chamber, to external sources, pumps and exhausts and therewith; various inlets and/or outlet valves; various optional inlet and/or outlet gauges for monitoring inflow and outflow of gases; and various optional absorbers and scavengers of undesirable contaminants, as well as gauges and indicators for monitoring the environment within the chamber at various steps of the process, as is further detailed hereunder.

[0208] The process involves placing an article of interest (an article to be impregnated) into the chamber with the intention of loading the article with gNO.

[0209] The article disposed within the chamber can be any article-of-manufacture, any part of an article-of-manufacture or a stock or raw material for manufacturing an article-of-manufacture or manufacturing a part of an article-of-manufacture. For example, in cases the article is a medical device having various surfaces, coating, appendages and tubing, the process may be carried out by placing the entire medical device in the chamber, placing some of the parts of the medical device, such as tubing or surface-forming parts of the medical device in the chamber, or placing in the chamber the pre-processed, pre-shaped, pre-formed or pre-cut raw materials constituting parts of the medical device.

[0210] Once the article in disposed within the chamber, the process involves generating a reduced pressure in the chamber. The term “reduced pressure”, is used synonymously with the terms “negative pressure” “under pressure” and/or “vacuum”. Evacuating the chamber from the ambient environment substantially evacuates (removes) gaseous substances and/or volatile substances found in the chamber as well as gaseous substances and/or volatile substances found in the article, at least to the extent of parts of the article which are exposed to the negative pressure.

[0211] In the context of embodiments of the invention, the term “gaseous” refers to a state of a substance being a gas under certain conditions of pressure and temperature. For example, the melting point of nitric oxide at atmospheric pressure is −164°C and the boiling point of nitric oxide is −152°C, hence nitric oxide is a gaseous substance at ambient conditions of pressure and temperature (i.e., room temperature). Oxygen, nitrogen and CO₂ present in an ambient atmosphere, are also gaseous substances. Humidity and moisture forming-water is essentially a mixture of vapor (gaseous water) and liquid water at ambient conditions of pressure and temperature, however, under reduced pressure the equilibrium of gas-liquid of water would essentially shift towards the gaseous state. Hence, reducing the pressure in the chamber, according to some embodiments of the present invention, facilitates the removal of moisture and humidity from the chamber prior to introducing gNO therein.

[0212] Generating a reduced pressure in the chamber can be effected by connecting any inlet or outlet of the charging device to an external source of reduced pressure, such as a vacuum pump, or a vacuum reservoir. A vacuum reservoir can be in the form of, for example, a container which has been evacuated from its content to possess a volume under reduced pressure which is substantially larger than the volume of the chamber, thereby being capable of taking-in (sucking in) at least a part of the ambient atmosphere of the chamber.

[0213] In the context of embodiments of the invention, the level of the reduced pressure which is reached in the chamber can be at any level of vacuum which is reasonably attainable in the charging device resented herein. According to some embodiments of the present invention, the depth of the vacuum can range from low vacuum levels (about 100 kPa to 3 kPa) to high vacuum levels (about 100 mPa to 100 nPa). In the context of embodiments of the invention, vacuum levels can be expressed as a negative value, namely by the value representing the difference in pressure relative to atmospheric pressure (which is 760 Tor, 101.32 kPa, 14.7 psi or 1 atmosphere). Hence, according to some embodiments of the present invention, the reduced/negative pressure attained in the chamber can range from about −50 psi to −0.5 psi. The unit “psi” is used in the context of some embodiments of the present invention, to denote a pressure difference, typically as recorded by a gage or device, from a reference pressure, typically atmospheric pressure. “0 psi” therefore denotes atmospheric pressure (e.g., ambient pressure), whereby “−X psi” is used to express negative pressure (under pressure).

[0214] In some embodiments of this aspect of the present invention, generating the reduced pressure in the chamber is effected for a time period of at least 1 minute, or for a time period that ranges from 1 minute to 60 minutes, or from 1
minute to 30 minutes, or from 1 minute to 20 minutes, or from 1 minute to 15 minutes, e.g., for 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 minutes, or for 30, 40, 50 or 60 minutes. During this period, the article is exposed to reduced pressure and in essence undergoes a purging process during which gases and volatile substances are substantially removed therefrom. The generation of reduced pressure in the chamber can thus be regarded as a degassing process or as a gas-evacuation process.

[0215] It is noted herein that water and gNO tend to interact such that the capacity of a moist article to sequester gNO is reduced. It is further noted herein that gNO is a relatively hydrophobic substance and hence the presence of water (moisture of humidity) can adversely interfere with sequestering gNO. Hence, it is desirable to reduce the humidity in the article prior to exposing the article to gNO-containing environment.

[0216] Oxygen and gNO also interact to produce reactive species such as nitrogen dioxide (nitrate), according to the reaction 2NO+O₂→2NO₂, which is a toxic brownish gas. Water, oxygen and gNO react to produce nitrite according to the reaction 4NO+O₂+2H₂O→4HNO₂. Nitrates and nitrites are known to be capable of participating in various reactions in vivo, in which toxic reactive oxygen species are formed. These reactions and subsequent reactions involving products of these reactions are commonly referred to herein as gNO degradation, and the process presented herein attempts to minimize this gNO degradation, by minimizing the presence of reactive species other than gNO.

[0217] Nitrates, nitrites, and any other species that are formed directly or indirectly by a reaction of nitric oxide with oxygen and/or water or humidity are referred to herein as "reactive species other than nitric oxide" or simply as "reactive species" and are meant to include nitrogen and/or oxygen-containing reactive species.

[0218] In some embodiments, generating reduced pressure in the chamber is effected so as to reduce the humidity level in the article/chamber by at least 50% of its original (ambient) level. According to some embodiments of the present invention, generating reduced pressure is effected so as to effect a decrease in the humidity level in the article by more than 50%, more than 60%, 70%, 80%, 90% and up to 100% reduction in humidity, which is essentially desiccating the article to a level of humidity of almost zero or essentially zero humidity. Preferably, the amount of humidity (relative humidity) in the ambient environment prior to introducing gNO into the chamber is reduced such that the relative humidity in the chamber is less than 50% of the original ambient level. According to some embodiments of the present invention, generating reduced pressure in the chamber is effected so as to effect reduction of oxygen level in the article/chamber by at least 50% of its original (ambient) level. According to some embodiments of the present invention, generating reduced pressure in the chamber is effected so as to effect reduction of both oxygen and humidity levels to below 50%, or below 40%, 30%, 20%, 10% and down to essentially 0% of the ambient levels of each of oxygen and humidity before the exposure to gNO-containing environment.

[0221] In some embodiments, once the invention is placed within the chamber, the chamber is sealed so as to allow the application of reduced pressure therein for any desired length of time. After sealing and generating a reduced pressure in the sealed chamber, the ambient environment in the chamber can be replaced with a gNO-containing environment as described hereinbelow.

[0222] Once most of the ambient environment, which may contain undesired levels of water and oxygen which may react with gNO, has been substantially removed by generating reduced pressure from within the chamber and the article, the chamber is filled with a replacement environment which comprises gaseous nitric oxide. In some embodiments, the gNO-containing environment is an ambient environment comprising nitric oxide. The phrase "ambient environment comprising nitric oxide" and the phrase "gaseous nitric-oxide containing environment" refer equally to pure gNO or to any mixture of gNO and a carrier gas. A carrier gas can be any inert or otherwise biologically and chemically compatible gas such as, but not limited to, helium, argon, nitrogen gas and any combination thereof.

[0223] It is noted herein that filling the chamber with a gNO-containing environment can be performed by allowing a gNO-containing environment to flow into the chamber, namely, by simply connecting the chamber, in which reduced pressure was generated, to a source of the gNO-containing environment. This gaseous environment will flow into the chamber due to pressure differences. In some embodiments, filling the chamber with a gNO-containing environment can be performed by pushing the gNO-containing environment into the chamber at an elevated pressure, or allowing the gNO-containing environment to suck into the chamber by force of the vacuum present therein.

[0224] Filling the chamber substantially charges the article with gNO. The gNO-containing environment which is introduced into the chamber contains a predetermined concentration of gNO which is capable of exerting the desired effect of NO on the article, as described herein. The pre-determined concentration of gNO in the provided gNO-containing environment may range from about 0.05% to about 10%, or any amount therebetween, for example about 0.05%, 0.1%, 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23% or 24%.

[0219] In the context of oxygen found in the article and/or the interior of the chamber, generating reduced pressure in the chamber is effected so as to effect reduction of oxygen level in the article/chamber by at least 50% of its original (ambient) level. According to some embodiments of the present invention, generating reduced pressure may decrease the oxygen level in the article by more than 50%, more than 60%, 70%, 80%, 90% and up to 100% reduction in oxygen level, which renders the article essentially devoid of oxygen.

[0220] According to some embodiments of the present invention, generating reduced pressure in the chamber is effected so as to effect reduction of both oxygen and humidity levels to below 50%, or below 40%, 30%, 20%, 10% and down to essentially 0% of the ambient levels of each of oxygen and humidity before the exposure to gNO-containing environment.

[0225] The gNO-containing environment may be provided (maintained, supplied as a continuous flow or as a single disbursement) for a pre-determined amount of time that ranges from about 1 minute to about 24 hours, namely from about 1 minute to about 24 hours, or any period therebetween, for example about 1, 2, 5, 10, 15, 20, 30, 40, 50 or 60 minutes,
A continuous flow of gNO-containing environment can be effected at a flow rate that ranges from about 1 cubic centimeter or milliliter per minute (cc/min) to about 2,000 cc/min or any rate therebetween, for example 1, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 250, 500, 750, 1,000 or 2,000 cc/min, or any rate therewithin. According to some embodiments, the flow rate ranges from about 250 cc/minute to 1,700 cc/minute.

The ability to control the flow rate, concentration of gNO in the gNO-containing environment and the duration of exposure serve as three finely tuned means to adjust the amount of gNO sequestered in the article. For example, a higher concentration of gNO in the carrier gas will result in an article sequestering a higher amount of gNO. Maintaining the article exposed to the gNO-containing environment for a longer time will result in an article sequestering a higher amount of gNO. Similarly, increasing the flow rate of the gNO-containing environment through the chamber corresponds to increasing the amount of gNO which the article is exposed to, resulting in an article sequestering a higher amount of gNO. In some cases the article cannot sustain high vacuum levels to long or any duration of time, and in these cases controlling the amount of sequestered gNO will be achieved by concentration and flow rate of the gNO-containing environment.

It is noted herein that the goal level of sequestered gNO in the article can be achieved in more than one way, using all possible variables presented herein (such as, for example, gNO concentration, duration of exposure, flow of gNO-containing environment, degassing by vacuum, desiccation and oxygen purging).

It is, however, noted herein that the mode by which the desired amount of sequestered gNO is achieved in the article depends on the nature and the composition of the article. Articles of a higher volume and/or bulky articles and/or articles made of a material with lower affinity to NO and/or articles that require particularly high level of sequestered NO (due to the intended use thereof) typically require higher loading time and/or higher duration and/or extent of reduced pressure and/or higher flowing rate of gaseous NO.

Undersewing the optimal parameters of the aforementioned variables will result in an article with sub-effective level of sequestered gNO, and overshooting may damage the article or some of its components and/or the chamber.

According to some embodiments of the present invention, the concentration of gNO in the gNO-containing environment ranges from 150 ppm to 60,000 ppm.

According to some embodiments of the present invention, the concentration of gNO in the gNO-containing environment ranges from 800 ppm to 20,000 ppm; the period of time of exposure to the environment in the chamber ranges from 1 to 600 minutes or overnight (10-24 hours); and/or the flow rate at which the environment is introduced into the chamber ranges from 0.5 liter/minute to 5 liter/minute.

During the step of dosing (filling the chamber and maintaining the aforementioned amount of gNO in the chamber for the aforementioned period of time), the article or at least a portion thereof may sequester gNO at a range of 1 ppm to about 20,000 ppm. Hence, the result of the process presented herein is essentially an article (releasably) sequestering gNO in at least a portion thereof, as discussed hereinbelow. In some embodiments, the process results in an article sequestering from 1 ppm to 200 ppm gNO per cm² of the article. In some embodiments, the process results in an article sequestering from 1 ppm to 200 ppm gNO as the total amount of gNO. However, higher amounts of gNO are also contemplated, as well as any value between the 1 ppm and 20,000 ppm.

In general, it is noted herein that the amount of gNO sequestered in an article impregnated upon generating a reduced pressure is higher by at least 5%, 10%, 20%, 30%, 40% and even 50% compared to the gNO sequestered in an article without prior generation of reduced pressure.

Once exposure to gNO-containing environment is completed, the process, according to some embodiments of the present invention, involves evacuating the residual gNO-containing environment from the chamber, in preparation to opening the chamber and retrieval of the treated article from the charging device. Such a step can be effected by purging the chamber by flowing a carrier gas or ambient atmosphere into the chamber, by pumping the gNO-containing environment from the chamber with a vacuum pump or by a combination of pumping and purging.

Depending on the material(s) making the article, the amount of gNO in the environment, the duration of exposure to the environment during dosing and on various other factors as described hereinabove, the resulting gNO-sequestering article or at least portions thereof, are dosed with a predetermined concentration of gNO which is sufficient to sustain release of an effective amount of gNO (e.g., for maintaining sterility) at storage conditions for a time period ranging from about 1 day to about 5 years, or from about 1, 2, 3, 4, 5, 6, 7 days, 2 weeks, 3 weeks, 4 weeks, about 1, 2, 3, 4, 5, 6, 12, 18, 24, 30, 36, 42, 48, 54 or about 60 months, or any period therebetween.

By “storage conditions”, it is meant that the article is kept under conditions which maximize the length of time that gNO can be released therefrom at an effective amount once it is removed from storage conditions. The term “storage conditions” encompasses any form of maintaining a gNO-containing environment in the immediate vicinity of the article, either in the charging device, the sealed chamber or in a non-gas permeable container or package, as these are discussed hereinbelow. Storage conditions also include maintaining an environment which is low in oxygen, moisture, heat or other factors which may reduce the levels of gNO sequestered in the article.

As mentioned hereinabove, the amount of sequestered gNO depends, among other factors, on the size, volume, surface area and composition of the article.

As known in the art, different substances have different solubility in different media, and this general principle holds also in the case of the inherent capacity of a substance to sequester gNO at any given conditions. Articles can be selected to be made from materials having a high intrinsic capacity to sequester gNO, or alternatively, one can select parts of the article which are made from materials having a high intrinsic capacity to sequester gNO, to be assembled into a complete article.

Materials having a high intrinsic capacity to sequester gNO, according to some embodiments of the present invention, include polymers, co-polymers and resins of various chemical compositions and other mechanical and morphological properties.

Articles are made primarily from materials which are most suitable to serve the intended use of the article, such
as, for example, medical devices, which are generally made from polymeric materials that are compatible with the internal or external surfaces of tissues and organs of a living subject (biocompatible).

[0242] In some embodiments, the article includes at least a portion of a surface configured to sequester nitric oxide, preferably a surface configured to sequester at least 1 ppm nitric oxide per cm², and optionally, a surface configured to sequester at least 200 ppm nitric oxide per cm², depending on the intended use of the article.

[0243] In some embodiments, at least a portion of the article comprises a plurality of voids for accepting and sequestering nitric oxide. Articles that comprise voids may be comprised of polymeric materials that have a certain degree of porosity due to e.g., chemical composition and manufacturing parameters of the polymeric material (e.g., cross-linked polymers) and/or due to physical parameters such as articles made of condensed layers or fibrous structures of a (e.g., polymeric) material or a plurality of materials.

[0244] In general, articles that have at least a portion thereof which is gas-permeable are suitable for use in the context of these embodiments of the present invention.

[0245] In the context of embodiments of the present invention, polymeric materials, polymers, co-polymers and resins which are both suitable to serve the intended use of the article they comprise, and have a sufficient intrinsic capacity to sequester gNO are commonly referred to herein as “suitable polymeric materials”.

[0246] Examples of suitable polymeric materials for forming polymeric material-based medical devices include resins which may exhibit microporous structure, and which are generally gas-permeable, and are able to sequester gNO. Upon contact with moisture exemplified by water, saline or other irrigation fluids, and/or bodily fluids such as urine, blood, mucus, and the like, the sequestered gNO can be released controllably from these compatible polymers over an extended period of time, thereby creating a localized microbially-free environment surrounding the medical device, or a portion thereof, thus rendering the medical device self-sterilizing.

[0247] Since any gNO-permeable material wrapping or encasing the article would undergo gNO dosing at the same time as the article, or at least allow gNO to pass-through to impregnate the article therein, the term “article”, as used herein, is meant to encompass an entire article and any appendage or supplemental part attached or connected thereto, including, without limitation, any gNO-permeable wrapping, coating and/or encapsulation material completely engulfing or partially encasing the article, and in particular any gNO-permeable wrapping or encasement which can also sequester gNO.

[0248] Chemical composition is one of the characteristics that influence a material’s intrinsic capacity to sequester gNO. For example, suitable polymeric materials are permeable to gNO by virtue of their composition, degree of crosslinking, crystallinity and the likes. In addition, since gNO is substantially non-polar, it can diffuse more readily into non-polar media, such as, without limitation, hydrophobic polymers and in general, polymers composed of non-polar constituents.

[0249] Hydrophobic polymers have a high intrinsic capacity to sequester higher amounts of gNO and further to sequester gNO for extended periods of time, due to higher affinity of gNO to hydrophobic substances. Hence, hydrophobic polymers are suitable in applications where prolonged release of gNO is beneficial.

[0250] It is noted herein that suitable polymeric materials in the context of embodiments of the present invention are not limited to hydrophobic polymers, and hydrophilic or amphiphilic polymeric material can also be impregnated with gNO effectively, according to some embodiments of the present invention.

[0251] Less hydrophobic and hydrophilic polymers and other substances may still be charged with gNO using the aforementioned factors of reduced pressure, gNO concentration in the environment, time of exposure to the environment and filled voids occurring in the impregnated substance.

[0252] It is noted that less hydrophobic, amphiphilic and hydrophilic polymers typically require exposure to gNO-containing environment for longer time periods and/or at higher flow rates and/or upon generating reduced pressure for longer time periods.

[0253] In general, a hydrophobic material is characterized by an oil-in-water partition coefficient (Log P) greater than 0, greater than 1, greater than 2, greater than 3, or greater than 4.

[0254] Non-limiting examples of suitable polymeric materials useful in making gNO-charged articles, such as for example medical devices, include silicone, polycetal, polyurethane, polyether, polytetrafluoroethylene, polyethylene, polyvinyl methyl ether, polyvinyl alcohol, polypropylene, polyvinylidene, polyvinyl acetate, polystyrene, polyvinylidene fluoride, copolymers, polyoxymethylene, polyethylene, polystyrene, ethylene-propylene rubber, fluoroelastomer, silastic elastomers, polyethylene tetrafluoroethylene, colloidion, carboxylic acid, and combinations thereof.

[0255] Other examples of suitable polymeric materials include biodegradable and bioresorbable polymers exemplified by polyglycolic acid (PGA), polyactic acid (PLA), poly(l-lactide), poly(DL-lactide), poly(DL-lactide-co-glycolide), polyglycolic acid, poly(glycolic acid), polyglycolic acid, polyethylene, polyvinyl chloride, polyethylene, polyvinyl alcohol, polyvinylidene fluoride, copolymers, polyoxymethylene, polyethylene, ethylene-propylene rubber, fluoroelastomer, silastic elastomers, polyethylene tetrafluoroethylene, colloidion, carboxylic acid, and combinations thereof.

[0256] Additionally, suitable polymeric materials can be used as coating on an article such as a medical device, or serve as enasement of the medical device, whereby the coating or enasement provides gNO-sequestering capacity to the medical device. For example, an implantable pump which forms a part of an implantable drug delivery system can be enasened within one or more layers comprising one or more of the suitable polymeric materials exemplified herein.

[0257] Mechanical and morphological properties also influence a material’s intrinsic capacity to sequester gNO. For example, suitable polymeric materials can be prepared such that they possess a porous microstructure, ranging from solid compositions having microscopic voids dispersed sparsely therein to low density foams having more voids than substance. Hence, suitable polymeric materials may range
widely on the scale of degree of porosity. In the context of embodiments of the present invention, polymers and resins having a porous microstructure to any degree of porosity, referred to herein as “porous substances”, can sequester gNO by virtue of having gNO fill the voids during the charging process. It is noted herein that such voids act as receptacles for gNO, and hence in the context of a substance having voids the term “sequestering” is regarded also as “accepting” or “containing” gNO in said voids.

The texture of the polymeric material can range from rigid to soft, and be elastic, plastic, pliant and resilient. The degree of rigidity, elasticity, plasticity, pliability or resilience of suitable polymeric materials can vary with the specific design and application of an article, such as a medical device. The choice of characteristics for a selected medical device will be apparent to and within the ability of a one of ordinary skill in the art. As the aforementioned properties correlate to certain chemical properties such as crosslinking, such mechanical properties may also correlate with the capacity of a polymeric material to sequester or accept gNO.

According to some embodiments of the present invention, the article includes at least a portion of a surface configured to sequester gNO.

As the portion of the article can be defined by its volume, the amount of gNO sequestered in that portion can be quantified by ppm of gNO per volume unit. Hence, according to some embodiments of the present invention, the article includes at least a portion of a surface configured to sequester at least 1 ppm nitric oxide per cm².

FIG. 27 presents an illustration of charging device wherein article further includes at least a portion thereof made from a suitable polymeric material, represented by surface 372, and configured to sequester gNO readily and effectively.

As discussed hereinabove, the methodology described herein is highly beneficial for preparing a medical device having gNO sequestered therewithin.

Thus, in some embodiments, the article to be charged with gNO is a medical device. Non-limiting examples of medical devices which can be impregnated with gNO beneficially include catheters, tubing, endotracheal tubing, tampons, prosthetic devices, medical implants, artificial joints, artificial valves, needles, intravenous devices, cannula, stents, biliary stents, cardiovascular stents, cardiac surgery devices, nephrostomy tubes, vascular grafts, infusion pumps, adhesive patches, sutures, fabrics, meshes, polymeric surgical tools or instruments, intubation devices, orthopedic surgery devices, pacemakers, endoscope components, dental surgery devices, veterinary surgery devices, bone scaffolds, hemodialysis tubing or equipment, blood exchanging and transfusion devices, implantable prostheses, bandages, ophthalmic devices and breast implants.

According to some embodiments of the invention, the medical device is an implantable medical device, including medical devices that are implanted transiently or permanently. Examples include an indwelling catheter or an intubation device such as a tracheal tube. Non-limiting examples of indwelling catheters include urinary catheters, central venous catheters, biliary vascular catheters, pulmonary artery catheters, peripheral venous catheters, arterial lines, central venous catheters, peritoneal catheters, epidural catheters and central nervous system catheters.

As discussed hereinabove, while CAB is one of the major causes of nosocomial infections, sterilization of urinary catheters is highly desired. Preparing urinary catheters the releasably sequester gNO thus achieves this goal (amongst other goals that are met by the described methodology).

In some embodiments, the medical device is such that is made of any of the suitable polymeric materials described hereinabove (e.g., at least a portion of the medical device comprises a hydrophobic polymeric material with an intrinsic capacity to sequester gNO).

According to some embodiments of the invention, the medical device is a tampon. As discussed hereinabove, gNO has been shown to be bacteriostatic and bactericidal, and has been shown herein to be effective in eradicating microorganisms causing bacterial vaginitis. Apart from self-sterilization, gNO-charged tampons can serve also as a therapeutic medical device by delivering a medication (gNO) to an infected bodily site (vagina).

Since tampons are made essentially from fibers of less hydrophilic and/or even hydrophilic polymers and other substances, impregnating tampons with gNO can advantageously be effected under reduced pressure and/or using extended gNO exposure times and/or at higher gNO concentrations compared to an article made from hydrophobic polymers. However, as tampons are designed for absorbing liquids, their microstructure is essentially rich in voids and crevices which can be charged and filled with gNO, sequester and contain gNO, and effectively and controllably release sequestered gNO therefrom. Additional features related to gNO-impregnated tampons are discussed in further detail hereinbelow.

As discussed hereinabove, the article used in the methodology described in these embodiments of present invention encompasses bare (unwrapped) articles, coated articles, and wrapped articles, as long as the coating and/or wrapping does not impair the process of gNO loading to an extent that is not mitigated by means within the process (namely by process-controllable factors that can increase gNO uptake).

This definition therefore makes the distinction between gas-permeable and non-gas permeable (gas-impermeable) packaging and wrapping, since the latter impairs the gNO charging process when the package is sealed and gNO cannot diffuse therethrough. An unsealed gas-impermeable package having an article housed therein does not impair gNO penetration to the article and hence is regarded as an open or unsealed package and not as an effective gNO barrier.

According to some embodiments of the present invention, the process presented herein can be effected with a packaged article as well as with a bare (unpackaged) article.

In some embodiments, the package is a gas-permeable package.

The term “gas-permeable” refers to a chemical attribute of a material, which allows gas molecules to pass therethrough by flux, conveyance, diffusion or transportation. “Gas-permeability” also refers to a mechanical attribute of an article made from any material in the sense that the material can be shaped, designed, manufactured and processed to be impermeable, permeable and anywhere between the extremes (semi-permeable). A material can be made porous or caulked, shaped thin or thick, processes glassy or rough and the likes, all of which are examples of mechanical properties that affect gas-permeability. In the context of embodiments of the present invention, semi-permeability in the sense of rate of permeation is regarded as gas-permeable, and semi-perme-
ability in the sense of selectivity towards one gas species to another is regarded as gas-permeable if it can permeate gNO, and gas-impermeable if it does not allow gNO to permeate therethrough.

Non-limiting examples of gas-permeable materials include low density polyethylene (LDPE), high density polyethylene (HDPE), medical grade paper, polycarbonate (PC), polyester, polyvinyl chloride (PVC), polyvinylidene chloride, perfluoroalkoxy (PFA), acrylonitrile-styrene, polypropylene (PP) polytetrafluoroethylene (PTFE), polycarbonate, acrylic, polycarbonate, polyacrylonitrile-butadiene-styrene, polymethylpentene (PMP), polyacetal, polystyrene (PS) or the like.

Hence, according to some embodiments of the present invention, the article can be housed in (e.g., enclosed by) a gas-permeable package when disposed in the chamber during the process of generating a reduced pressure and charging the article with gNO; wherein the presence of the gas-permeable package may or may not affect one or more parameters of the process. The result end of the process would be a gNO-charged article that includes a gNO-charged gas-permeable package.

In some embodiments, the process described herein is used for providing an article as described herein, with or without a gas-permeable package, within a non-gas permeable enclosure, in order to maintain the gNO-containing environment within the package for prolonged time and under various conditions.

The term “non-gas permeable” or the equivalent “gas-impermeable”, as used herein, refers to an attribute of a substance which is capable of preventing the passage of gas molecule therethrough by flux, conveyance, diffusion or transportation. In some embodiments of the invention, these terms refer to impermeability of gNO, however, this term is also used to indicate impermeability of the non-gas permeable to other gases such as water vapor and oxygen.

Without being bound by a particular theory, it is assumed that since gNO has a lower diffusion cross-section than water vapor or oxygen, a gNO impermeable substance forming the non-gas permeable container or package will also be impermeable to water vapor and oxygen, and that a substance that is impermeable to water vapor and oxygen may still be permeable to gNO.

Examples of materials suitable for making gas-impermeable packaging include, without limitation, glass, glassy ceramics, metals, metallic foils, metallic-plastic composite foils and the likes and a combination thereof as composites or as parts in a complete gas-impermeable package. The gas-impermeable material for packaging can also comprise more than one layer of a polymer, a metal, a resin or a plastic, and in some examples the packaging can comprise a plastic-backed metallic foil, such as used in many air-tight packaging. A gas-permeable material can be combined with a gas-impermeable material to form a composite gas-impermeable package.

In the context of embodiments of the present invention, a gas-impermeable package is also referred to interchangeably as a non-gas permeable enclosure or gas-impermeable enclosure.

According to some embodiments of the present invention, the article is housed in an unsealed (unsealed, open) sealable gas-impermeable package (a non-gas permeable enclosure that can be closed and sealed) when disposed in the chamber and during the generation of reduced pressure and charging it with gNO. According to such embodiments, the process is carried out essentially as described herein-above, and at the end of the process the non-gas permeable enclosure is closed and sealed so as to constitute an intact and complete gNO barrier with respect to the gNO in the gNO-containing environment and the gNO-sequestering article enclosed therein, insulating the article from ambient environment.

The sealable gas-impermeable package for insulating a gNO-sequestering article enclosed therein can further include a desiccant, a humidity indicator and a gNO indicator, as these are described herein, for monitoring the gNO-charging process and to indicate post-sealing integrity of the package and post-sealing level of gNO enclosed therein after manufacturing. These optional attachments may be placed inside the package and behind a transparent portion of the package so as to allow a visual signal generated therein to be detected without opening the sealed package. Alternatively, the indicators may be designed to form a part of the gas-impermeable package such that the inner side is in communication with the inner environment and can interact therewith while the outer side of the indicator maintains gas-impermeability towards the outside ambient environment.

Additional details of a process involving preparing an article packaged in a non-gas-permeable enclosure are provided hereinafter.

Utilizing the process described herein, articles having gNO sequestered therein are provided. Such articles are advantageously characterized as comprising at least 1 ppm per cm³ nitric oxide sequestered therewithin, optionally at least 200 ppm per cm³ nitric oxide sequestered there within, and further optionally from 1 ppm to 20,000 ppm per cm³.

In some embodiments, the gaseous nitric oxide sequestered in the article is resealable in an aqueous solution during a time period that ranges from 1 hour to 1 month.

The rate of release of NO depends on a variety of parameters, including, for example, the affinity of NO to the material composing the article (the material in which gNO is sequestered), the amount of sequestered gNO, the position of gNO within the article (being an external or internal surface), as well as the conditions at which gNO is released.

As discussed herein, while considering the nature of the article (its structure and chemical composition, and more particularly, but not exclusively, its affinity to NO, its size, volume and bulkiness, etc.) and its intended use, the amount of gNO charged into the article can be pre-determined, so as to impart a desired release rate of gaseous NO therefrom.

For example, urine catheters are typically transiently implanted in patients for a period of from a few hours to a few days. Such articles would therefore be loaded by a process that utilizes conditions for high loading of NO (e.g., longer duration of exposure to NO-containing environment).

Tracheal tubes are typically utilized for a few hours, yet require high load of gNO since they are typically used during surgery or other emergency cases.

Tampons are intended to be replaced every few hours.

The amount of gNO sequestered in the articles described herein can be readily measured by measuring the amount of nitrates and/or nitrites, as gNO degradation products, in an aqueous solution in which the gNO-sequestering article is placed. Determining the amount of nitrates and nitrites is exemplified in Examples section that follows.
Other methods for quantitatively determining nitrites and nitrates will be recognized by a person skilled in the art and are also contemplated herein.

[0291] The article disclosed herein is further characterized as being substantially devoid of humidity and/or oxygen.

[0292] In some embodiments, the article described herein is characterized by no more than 25% humidity, no more than 15% humidity, no more than 10%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.1%, 0.05%, 0.01%, 0.005%, 0.001% humidity, and preferably by 0% humidity. The “%” represents weight percentages from the gaseous environment within the article.

[0293] Alternatively, or in addition, in some embodiments, the article described herein is characterized by no more than 20% of oxygen, no more than 15% oxygen, no more than 10%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.1%, 0.05%, 0.01%, 0.005%, 0.001% oxygen, and preferably by 0% oxygen. The “%” represents weight percentages from the gaseous environment within the article.

[0294] In some embodiments, the article comprises no more than 1 ppm of oxygen and/or humidity.

[0295] In some embodiments, the article comprises no more than 1 ppb of oxygen and/or humidity.

[0296] In some embodiments, an amount of reactive species other than nitric oxide, as defined herein, in the article is lower than 1 ppm per cm³ of the article, preferably lower than 1 ppb (part per billion) per cm³ of the article.

[0297] In some embodiments, an amount of reactive species other than nitric oxide, as defined herein, in the article, is lower than 1 ppm of the entire article, preferably lower than 1 ppb (part per billion) of the entire article.

[0298] Depending on the process particulars and the article loaded with gNO, the article may further comprise an enclosure (e.g., a non gas-permeable enclosure).

[0299] In some embodiments, the enclosure comprises a gaseous nitric oxide-containing environment.

[0300] In some embodiments, the environment is an ambient environment (e.g., has an ambient pressure).

[0301] In some embodiments, the enclosure contains a gaseous NO-containing environment with a concentration of NO therein that is sufficient to sterilize the interior of the package and/or the article, as is further discussed hereunder.

[0302] The article and/or enclosure can further comprise a desiccant disposed within the enclosure, and/or a nitric oxide indicator disposed within the enclosure, as is further discussed in detail hereunder.

[0303] Hence, according to as aspect of some embodiments of the present invention there is provided an article having sequestered therewithin at least 1 ppm gaseous nitric oxide, which is advantageously characterized as comprising less than 1 ppm, or less than 1 ppb, per cm³ of the article, reactive oxygen-containing and/or nitrogen-containing species other than nitric oxide.

[0304] The article can be further characterized by any of the features described hereinafore.

[0305] In some embodiments, the article is a medical device, as described herein.

[0306] In some embodiments, the article is a tampon.

[0307] The term “tampon”, as used herein, refers to a medical device in the form of a plug made from a mass of absorbent materials which is inserted into a wound or a body site to absorb exuded fluids, such as blood. One of the most common types of tampons in daily household use is designed to be inserted into the vagina during menstruation to absorb the flow of menstrual fluid. Such tampons are regarded officially as medical devices in many courtiers around the world, and according to the United States Food and Drug Administration, tampons are a Class II medical device.

[0308] In some embodiments, the term “tampon” describes tampons designed to be inserted into the vagina during menstruation to absorb the flow of menstrual fluid. The tampon can be a commercially available tampon of any type, composition, absorption rate, size and/or blend. Alternatively, one or more of the polymeric materials used for forming a tampon have a gaseous NO sequestered therewithin, and the tampon is made from these raw materials.

[0309] Tampons are typically made from cotton, rayon and blends thereof, and are available in different sizes for various conditions and absorbing rates. Tampons may include an applicator, which is a polymeric tube sheathing the absorbent plug for facilitating its insertion into the vagina.

[0310] Both cotton and rayon, comprising the major and absorbing part of a tampon, are cellulose fibers, which are regarded in the context of embodiments of the present invention as hydrophilic polymers.

[0311] As discussed hereinafore, tampons releasably sequestering gNO are highly advantageous, particularly in the context of prevention and/or treatment of vaginal medical conditions such as vaginal infections.

[0312] As demonstrated in the Examples section that follows, the present inventors have successfully prepared tampons having sequestered therein gaseous NO and have shown that such tampons exhibit both an antimicrobial effect and a self-sterilization effect (by e.g., preventing bacterial growth, biofilm formation and/or bacterial growth within a biofilm).

[0313] Due to the hydrophilic nature of the material(s) composing tampons, discussed hereinafore, the ability to have gaseous nitric oxide sequestered therewithin is surprising.

[0314] Thus, according to an aspect of some embodiments of the invention there is provided a tampon having sequestered therein gaseous nitric oxide.

[0315] According to some embodiments, an amount of the sequestered gaseous nitric oxide ranges from 1 ppm to 200 ppm per cm² of the tampon. Alternatively, an amount of the sequestered gaseous nitric oxide ranges from 1 ppm to 200 ppb of the tampon.

[0316] According to some embodiments, an amount of sequestered gaseous nitric oxide is 200 ppm per cm². Alternatively, an amount of the sequestered gaseous nitric oxide is 200 ppm of the tampon. Higher load of gaseous nitric oxide is also contemplated (e.g., 300 ppm, 400 ppm, 500 ppm, 1,000 ppm and up to 20,000 ppm).

[0317] According to an aspect of some embodiments of the present invention, there is provided a process for preparing a tampon having sequestered therein gNO, which is generally effected by exposing the tampon to gNO-containing environment.

[0318] As described hereinafore in the context of charging a general article in a gNO-charging device, in some embodiments, the process of preparing a gNO-sequestering tampon includes exposing the tampon to gNO-containing environment by placing the tampon in a chamber and filling the chamber with the gNO-containing environment, wherein the exposure is subject to variable exposure time periods and concentration of gNO in the gNO-containing environment for obtaining a predetermined gNO-load in the gNO-charged tampon.
According to some embodiments of the present invention, the process of preparing a gNO-sequestering tampon may further include, subsequent to placing the tampon in the chamber, sealing the chamber and generating a reduced pressure (vacuum) in the chamber, prior to filling the chamber with the gNO-containing environment.

As described hereinabove, the depth of the vacuum and the duration of the time of maintaining the chamber under reduced pressure serve as additional means to control the amount of gNO sequestered in the tampon.

Optionally, the process of preparing a gNO-sequestering tampon is effected without generating reduced pressure. Such a process may comprise, prior to filling the chamber with NO, reducing the humidity within the tampon and/or within the chamber.

Since tampons comprise hydrophilic polymers, they require longer exposure to higher concentrations of gNO and optionally more negative pressure applied prior to exposure to gNO, compared to articles of similar mass and shape comprising hydrophobic polymers. However, the principles of the process for impregnating tampons with gNO follow the same guidelines as the principles of impregnating other packaged or unpackaged articles with gNO, as described in detail herein.

When subjected to exposure to gNO-containing environment, the tampon can be either wrapped or unwrapped.

Apart of optionally being individually wrapped in a gas-permeable wrapping material, tampons may also include an applicator which can be made from hydrophobic polymers as well as other polymers, and can be regarded as a “gNO-sequestering surface” or a “surface”, as the term is defined hereinabove, and serves as an additional gNO-sequestering component in the gNO-sequestering tampon. In the context of embodiments of the invention, the term “tampon” also encompasses tampons sheathed in an applicator.

As described hereinabove, tampons can be charged with gNO as bare tampons or packaged tampons wrapped with a gas-permeable package. Both forms of tampons can also be charged with gNO while housed in a gas-impermeable enclosure, as described hereinabove for charging a general article with gNO. The end result of such a process can be either a gNO-sequestering tampon, a gNO-sequestering tampon packed in a gas-permeable package, and/or a wrapped or bare gNO-sequestering tampon packaged in a gas-impermeable packaging material, either individually or as a plurality of gNO-sequestering tampons.

The gNO-sequestering tampon is self-sterilizing, which adds a notable benefit for this medical device for insertion into a bodily site, however, a gNO-sequestering tampon may also serve for medicinal therapy of various illnesses associated with the vagina. Hence, according to an aspect of embodiments of the present invention, there is provided a gNO-sequestering tampon which is identified for use in treating or preventing (or preventing the recurrence of) a vaginal medical condition.

Accordingly, there is provided a method of treating a vaginal medical condition, the method being effected by placing in the vagina of the subject the gNO-sequestering tampon as presented herein.

Such a method comprises intravaginal administration of gaseous NO.

Accordingly, according to an aspect of some embodiments of the present invention there is provided a method of treating a vaginal medical condition, which is effected by intravaginal administration of gaseous NO. In some embodiments, intravaginal administration of NO is effected by a tampon having (releasably) sequestered therein gaseous NO, as described herein.

According to an aspect of some embodiments of the invention there is provided a tampon having gaseous NO sequestered therein, for use in a method of treating a vaginal medical condition, by intravaginal administration of gaseous NO.

According to another aspect of some embodiments of the invention there is provided a method of delivering gaseous nitric oxide into the vagina, which is effected by placing in a vagina of a subject in need thereof a tampon having gaseous NO sequestered therewithin, as described herein.

Exemplary vaginal medical condition include, without limitation, bacterial vaginitis (BV), toxic shock syndrome (TSS), toxic shock-like syndrome (TSLS), streptococcal toxic shock syndrome (STSS), vulvovaginal candidiasis (VVC), chronic or persistent yeast infections (RVVC), a sexual dysfunction, a female reproductive system related disorder and a post-surgery vaginal related condition.

According to another aspect of embodiments of the present invention there is provided a use of gaseous nitric oxide in the treatment or prevention of a vaginal medical condition. In some embodiments, the gaseous nitric oxide is used in the form of a gNO-sequestering tampon, such as described herein.

As discussed hereinabove, the present inventors have further contemplated using a methodology of impregnating gaseous NO for providing sterilized and self-sterilizing packages of articles.

Accordingly, to an aspect of some embodiments of the present invention there is provided a process of preparing a packaged article, wherein the packaged article comprises a gas-permeable package, the process comprising exposing a packaged medical device to a gaseous nitric oxide-containing environment, as described herein.

In some embodiments, exposing is effected by placing the packaged article in a chamber, and filling the chamber with a nitric oxide-containing environment.

In some embodiments, the process is effected by generating a reduced pressure in the chamber, prior to filling the gNO-containing environment, as described in detail hereinabove.

In some embodiments, filling is effected by flowing nitric oxide into the chamber, as described hereinabove.

In some embodiments, typically in cases where generation of reduced pressure is not effected, the process further comprises, prior to filling the chamber, absorbing humidity from the package and/or chamber. In some embodiments, absorbing humidity is effected so as to reduce humidity by at least 50%, as described hereinabove.

In some embodiments, filling is effected such that an ambient environment (e.g., ambient pressure) is provided within the package.

Such a process results in a packaged article having gaseous nitric oxide sequestered within the article and within the package.
Such a process can be advantageously utilized for sterilizing articles such as medical devices which are packaged in a gas-permeable package, without the need to open the sealed package. The package can comprise a plurality of articles, individually packaged articles, and a package that comprises individually packaged articles.

In some embodiments, the article is a medical device, as described herein.

As discussed hereinabove, it is advantageous to provide NO-sequestering articles in a non-gas permeable package. The present inventors have thus devised and successfully prepared and practiced a process of preparing such packaged articles.

Thus, according to an aspect of some embodiments of the present invention there is provided a process of preparing a packaged article, wherein the packaged article comprises a non-gas permeable enclosure.

The process according to this aspect of embodiments of the invention is effected by positioning an article (e.g., an intact article) within a non-gas permeable enclosure, to thereby obtain a non-gas-permeable enclosure having the article disposed therewithin;

exposing the enclosure to a gaseous nitric oxide-containing environment, so as to introduce into the enclosure the nitric oxide-containing environment; and

sealing the enclosure.

The (intact) article placed within the enclosure can be packaged with a gas-permeable package or be un Packaged, as described hereinabove.

Exposing the enclosure to a gaseous nitric oxide-containing environment is effected, according to some embodiments, following the guidelines described herein, with or without generating reduced pressure in the chamber.

In some embodiments, the nitric oxide-containing environment comprises at least 0.02% nitric oxide.

Such a process results in an article packaged in a non-gas-permeable package, wherein the article has gaseous nitric oxide sequestered therewithin and/or the package comprises a nitric oxide-containing environment.

In some embodiments, the package contains nitric oxide in an amount sufficient to sterilize an interior of the enclosure as well as to sterilize the article.

Such an amount is at least 1 ppm nitric oxide, and range from 1 ppm to 200 ppm nitric oxide. In some embodiments, the amount is higher than 200 ppm, as described hereinabove.

By “sterilize” it is meant to eliminate substantially all living microorganisms.

Substantially encompasses a majority of a population of microorganisms, namely, at least 80%, 85%, 90% 95%, 98%, 995, 99.9% and optionally 100% of the microorganism population.

According to some embodiments of the present invention, the process presented herein can be carried out so as to manufacture an article impregnated with gNO and encased in a non-gas permeable package as follows:

placing an article housed in an open and scalable non-gas permeable package within a chamber, wherein the package may include any one or more of the optional humidity indicator, gNO indicator or desiccant;

optionally generating a reduced pressure in the chamber;

filling the chamber with a gNO-containing environment (exposure conditions); and then either

sealing the package under the exposure conditions; and

purging the chamber, opening the chamber and retrieving the sealed package encasing the article now sequestering gNO;

or

purging the chamber, opening the chamber and retrieving the open package encasing the article now sequestering gNO; and

sealing the package under ambient conditions,

thereby preparing the article having gNO sequestered therewithin packaged in a gas-impermeable enclosure.

Alternatively, the process is carried out essentially as described hereinabove with an article which is not housed in a non-gas permeable package, and the process further includes, subsequent to opening the chamber and retrieving the gNO-charged article, a step of placing the gNO-charged article in a non-gas permeable package and sealing the package.

In all the above embodiments a bare article and an article in a gas-permeable wrapping, coating and/or packaging is regarded essentially the same in the context of the gNO-charging process, since a gas-permeable wrapping, coating and/or packaging does not substantially preclude or interfere with the process.

Thus, according to an aspect of some embodiments of the present invention there is provided a package which comprises a material configured to form an enclosure; an article disposed within the enclosure; and a gaseous nitric oxide-containing environment within the enclosure.

In some embodiments, the package is a non-gas permeable package.

In some embodiments, the enclosure is a sealed enclosure.

In some embodiments, the environment within the enclosure is an ambient environment, as described herein.

In some embodiments, the article in the package has gaseous nitric oxide sequestered therein.

The package as described herein can further include desiccants, nitric oxide indicators and other components, as described herein.

The article is preferably a medical device, such as an indwelling catheter, an intubation device or a tampon, as described herein.

Turning now to the figures, FIGS. 31A-B present schematic illustrations of a package according to some embodiments of the invention, such as a package 200, comprising non-gas permeable package 203 configured to be impermeable to gNO and optionally comprising seal 205 (FIG. 31B), medical device 201 disposed within non-gas permeable package 203, gNO-containing environment 206 engulging medical device 201 within non-gas permeable package 203 and comprising a predetermined concentration of gNO capable of sterilizing the interior of non-gas permeable package 203 and medical device 201, and further optionally comprising nitric oxide indicator 204 (FIG. 31B), desiccant 202 disposed within non-gas permeable package and configured to absorb humidity from gNO-containing environment 206, and humidity indicator 208 (FIG. 31B) configured to indicate moisture saturation of the desiccant 202.

According to an aspect of some embodiments of the invention, there is provided charging device (or system), which comprises:
a chamber comprising an inlet for receiving a gaseous nitric-oxide containing environment and an outlet for releasing the gaseous nitric-oxide containing environment; and

a article, as described herein, disposed within the chamber.

Such a chamber can be utilized for practicing any of the processes described herein.

The chamber may be coated with within a protective surface coating suitable for preventing gNO from reentering with the chamber’s material. Such protective coating may include, as non-limiting examples, glass, chromium, stainless steel and other gNO-resistant substances and mixtures thereof.

The device may further comprise a nitric oxide indicator configured to undergo a color change suitable for visual assessment of whether the article has been exposed to the nitric oxide.

In some embodiments, a gNO indicator comprises one or more gNO-sensitive substances that can produce a detectable signal when exposed to a certain or any level of gNO in an environment. For example, the detectable signal may be a color change of the gNO-sensitive substance which occurs upon exposure to gNO. Such a gNO indicator is suitable for a visual confirmation for sufficient exposure of the article to gNO. Exemplary gNO-sensitive substances include dyes such as, for example, 4-amino-5-methylamino-2,7-di-fluorofluorescein (DAF-FM). It is to be understood that other gNO-indicators are contemplated in the context of embodiments of the invention, such as other chemical indicators, electronic indicators, off-line indicators (a sample for measuring the exposure thereof to gNO at a site unrelated to the exposure site) and the likes.

In some embodiments, a desiccant can be placed in the chamber to absorb humidity, as illustrated in FIGS. 26-30 (desiccants 202, 302, 402). The desiccant can be disposed within the chamber and be configured to absorb humidity from the ambient environment.

Suitable desiccants include, but are not limited to, Drierite®, silica gel, calcium sulfate, calcium chloride, montmorillonite clay, molecular sieves, etc. The desiccant can reduce the amount of humidity in the ambient environment by about 75% to about 100%.

The charging device may also include at least one humidity indicator (see, indicators 208, 374 and 474 in FIGS. 32, 27 and 29 respectively) configured to indicate moisture saturation of the desiccant or humidity level in the chamber. A non-limiting example of a humidity indicator for indicating desiccant saturation include cobalt chloride (CoCl₂), which undergoes a color change from blue (anhydrous state) to purple (CoCl₂·2H₂O) to pink (Co(H₂O)₆Cl₂) as the absorption of water increases.

Devices which are configured for practicing any of processes as described herein while generating reduced pressure in the chamber further comprise an outlet for generating a reduced pressure in the chamber.

In some embodiments, a package enclosing (or housing) the article is further included within the charging device. In some embodiments, the package is as non-gas permeable enclosure, utilized according to embodiments of the invention to provide a packaged gNO-sequestering article as described herein.

FIG. 26 is a schematic illustration of an exemplary charging device 300, according to some embodiments of the present invention, which is fitted with an inlet 345 for receiving gNO within chamber 337 to form gaseous nitric oxide-containing environment 306, valve 315 for closing outlet 345, an outlet 326 for releasing ambient environment or gaseous nitric oxide-containing environment from chamber 337, valve 336 for closing outlet 326, an article 301 disposed within chamber 337, and an optional moisture scavenger in the form of desiccant 302 disposed within chamber 337 and configured to absorb an amount of humidity from the within chamber 337. According to some embodiments of the present invention, inlet 345 can be used to connect to an external source of negative pressure, e.g. a vacuum pump (not shown in FIG. 26), for generating a reduced pressure in chamber 337.

FIGS. 27-29 present schematic illustrations of exemplary charging devices similar with respect to the chamber, the environment, the inlet, the outlet, the valves and the article, to the charging device presented in FIG. 26.

FIG. 27 is a schematic illustration of an exemplary charging device 300, as presented in FIG. 26, wherein chamber 337 also includes a surface coating 370 which protects the material of chamber 337 from chemically interacting with gNO-containing environment 306. A gNO indicator 371 configured to undergo a color change when article 301 has been exposed to gNO in environment 306, and humidity indicator 374 for signaling exposure of article 301 to humidity.

FIG. 28 is a schematic illustration of an exemplary charging device 400, according to some embodiments of the present invention, which is fitted with an inlet 445 for receiving gNO within chamber 447 to form gaseous nitric oxide-containing environment 406, valve 415 for closing outlet 445, an outlet 426 for releasing ambient environment or gaseous nitric oxide-containing environment from chamber 447, valve 436 for closing outlet 426, and a non-gas permeable package 473 disposed within chamber 447 and housing article 401. According to some embodiments of the present invention, inlet 445 or outlet 426 can be used to connect to an external source of negative pressure, e.g. a vacuum pump (not shown), for generating a reduced pressure in chamber 447.

FIG. 29 is a schematic illustration of an exemplary charging device 400, as presented in FIG. 28, wherein chamber 437 includes surface coating 470 which protects the material of chamber 437 from chemically interacting with gNO-containing environment 406, a gNO indicator 471 configured to undergo a color change when article 401, having gNO-sequestering surface 472 and housed within non-gas permeable package 473 equipped with seal 490, has been exposed to gNO in environment 406, and humidity indicator 474 for signaling exposure of article 401 to humidity.

While FIGS. 26-29 present exemplary charging devices fitted with only a single inlet and a single outlet, the charging device may include a plurality of inlets and a plurality of outlets, as illustrated in FIG. 30.

FIG. 30 presents a schematic illustration of an exemplary charging device 500 having a chamber 137, inlet 125 with valve 11, inlet 135 with valve 2, and inlet 145 with valve 15, inlets which may share a common tube 23 with valve 19 that connects to the chamber 137; outlet 126 with valve 36 and connect to purge flow rotomoter 46, outlet 136 with valve 41 and connect to purge flow rotomoter 45, outlets which may share a common tube 33 which connects to the chamber 137. Separate inlets 125, 135, 145 and outlets 126, 136 allow for gasses (e.g. gNO, carrier gases) to enter and exit the chamber 137 at separate locations. Each of inlets 125,
135, 145 may also allow a vacuum creating mechanism (not shown) to have separate access to the chamber 137 for generating reduced pressure therein.

[0397] Such a setup makes it convenient for an operator of the charging device 137 to operate device 137 by removing the need to rehook vacuum, gNO and carrier gas sources to a single inlet at different stages of the process. Using the configuration presented in FIG. 30 allows each inlet 125, 135, 145 to be connected to a different source of gases or vacuum. For example, inlet 145 may serve as a vacuum port, inlet 125 may serve as an inlet for gNO or nitrogen gas, and inlet 135 may serve as a purging gas inlet for a carrier gas such as nitrogen gas, helium, argon, or any combination thereof.

[0398] The vacuum port 145 in FIG. 30 may be used to create a vacuum within chamber 137 so that the gNO introduced to chamber 137 does not react with any gases or moisture already contained within chamber 137 or within the article disposed therein (not shown). Chamber 137 may be pressurized to any suitable pressure, for example, once the article has been disposed therein and chamber 137 has been sealed and evacuated substantially from the ambient environment therein, chamber 137 may be pressurized to 50 psig of gNO or of gNO mixed with a carrier gas such as helium, argon, nitrogen gas, and any combination thereof, helping to stabilize gNO.

[0399] Separate inlets 126, 136 in FIG. 30 may be connected by tubes to two rotometer kits 45, 46 which are flow meters that indicate flow rate and can be operated in parallel.

[0400] The gaseous nitric oxide (gNO) sequestering articles presented herein can be prepared (charged with gNO) and be ready for use well in advance and kept in storage, or be prepared just prior to use, depending on the use, conditions and other preferences. In some cases, the article can be kept for extended lengths of time in a sealed chamber which is designed for charging its content with gNO, and then, prior to use, the device and article can be charged with gNO.

[0401] According to an aspect of embodiments of the invention, there is provided a charging device which includes a sealed chamber having a reduced pressure therewith; and an article, as described herein (e.g., a medical device), disposed within the chamber.

[0402] Such a chamber can be utilized, for example, for charging the article with gNO, as described herein, and thus can further comprise an inlet configured for receiving a gaseous nitric oxide-containing environment and an outlet for releasing said gaseous nitric oxide-containing environment.

[0403] In some embodiments, such a chamber further comprises an outlet used for generating the negative pressure within the chamber.

[0404] Such a chamber can further comprise a non-gas permeable enclosure in which the article is positioned, as described herein. The device can further comprise desiccants and NO-indicators as described herein.

[0405] It is expected that during the life of a patent maturing from this application many relevant nitric oxide sequestering articles will be developed and the scope of the phrase “nitric oxide sequestering article” is intended to include all such new technologies a priori.

[0406] As used herein the term “about” refers to ±10%.

[0407] The terms “comprises”, “comprising”, “includes”, “including”, “having”, and their conjugates mean “including but not limited to”.

[0408] The term “consisting of” means “including and limited to”.

[0409] The term “consisting essentially of” means that the composition, method or structure may include additional ingredients, steps and/or parts, but only if the additional ingredients, steps and/or parts do not materially alter the basic and novel characteristics of the claimed composition, method or structure.

[0410] The word “exemplary” is used herein to mean “serving as an example, instance or illustration”. Any embodiment described as “exemplary” is not necessarily to be construed as preferred or advantageous over other embodiments and or to exclude the incorporation of features from other embodiments.

[0411] The word “optionally” is used herein to mean “is provided in some embodiments and not provided in other embodiments”. Any particular embodiment of the invention may include a plurality of “optional” features unless such features conflict.

[0412] As used herein, the singular form “a”, “an” and “the” include plural references unless the context clearly dictates otherwise. For example, the term “a compound” or “at least one compound” may include a plurality of compounds, including mixtures thereof.

[0413] Throughout this application, various embodiments of this invention may be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

[0414] Whenever a numerical range is indicated herein, it is meant to include any cited numeral (fractional or integral) within the indicated range. The phrases “ranging/ranges between” a first indicate number and a second indicate number and “ranging/ranges from” a first indicate number “to” a second indicate number are used herein interchangeably and are meant to include the first and second indicated numbers and all the fractional and integral numerals therebetween.

[0415] As used herein the term “method” refers to manners, means, techniques and procedures for accomplishing a given task including, but not limited to, those manners, means, techniques and procedures either known to, or readily developed from known manners, means, techniques and procedures by practitioners of the chemical, pharmacological, biological, biochemical and medical arts.

[0416] As used herein, the term “treating” includes abrogating, substantially inhibiting, slowing or reversing the progression of a condition, substantially ameliorating clinical or aesthetical symptoms of a condition or substantially preventing the appearance of clinical or aesthetical symptoms of a condition.

[0417] It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable subcombination or as suitable in any other described
embodiment of the invention. Certain features described in the context of various embodiments are not to be considered essential features of those embodiments, unless the embodiment is inoperative without those elements.

Various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below find experimental support in the following examples.

EXAMPLES

Reference is now made to the following examples, which together with the above descriptions illustrate some embodiments of the invention in a non limiting fashion.

Materials and General Experimental Methods

The preparation of various articles, objects and devices, releasably sequestering gaseous nitric oxide, according to some embodiments of the present invention, is presented below. For clarity, it is noted that the terms “impregnated”, “charged”, “dosed” and “treated” are used interchangeably hereinbelow and throughout to denote an article releasably sequestering gaseous nitric oxide therein.

Nitric Oxide Impregnation—General Procedure I

Articles are exposed to nitric oxide (Aigas Specialty Gases, Chicago, Ill.) in nitrogen (N₂) or argon (Ar) as a carrier gas (at a concentration ranging from 800 ppm to 20,000 ppm, unless otherwise indicated) for a period of 1-600 minutes or overnight (10-24 hours) in a chamber, such as a stainless steel chamber. Articles are placed in a chamber and nitric oxide is introduced into the chamber at a flow rate of from 0.5 liter/minute to 5 liter/minute (unless otherwise indicated), depending on the parameters and intended use of the device to be impregnated.

NO impregnation of articles within a gas-permeable package is generally effected by placing the packaged article in the chamber and exposing the packaged article to NO, as described herein.

Preparation of gNO-impregnated packaged articles with a non-gas permeable package is generally effected by placing the article within an ensacement made from the non-gas permeable packaging material while keeping the encaement open, exposing the article and the encaement to gNO as described herein and thereafter sealing the encaement containing the device within.

Nitric Oxide Impregnation—General Procedure II

Articles are exposed to nitric oxide (Aigas Specialty Gases, Chicago, Ill.) in nitrogen (N₂) or argon (Ar) as a carrier gas for a period of 1-600 minutes or overnight (10-24 hours) in a sealed chamber, such as a stainless steel chamber. Articles are placed in the chamber and the chamber is sealed. Prior to filling the chamber with nitric oxide, a reduced (negative) pressure (e.g., of ~10 PSI; about ~0.7 atmospheres) is maintained by a vacuum pump connected to the outlet of the chamber for a period of e.g., 1-15 minutes. When the desired negative pressure is reached, the pump is stopped and the outlet valve closed to maintain the negative pressure inside the chamber. Thereafter, the inlet valve connected to a nitric oxide-containing cylinder is opened to allow the nitric oxide in the carrier gas to flow in and fill the chamber. The gas inflow is stopped when a pressure of zero PSI is reached. After the exposure time, the gas inside the chamber is purged with air for at least 5 minutes through nitric oxide absorbing filters. Thereafter the chamber is opened and the exposed article is kept at room temperature.

In a typical procedure, an article is placed in a stainless steel chamber as described herein and the chamber is sealed. Prior to filling the chamber with nitric oxide, a reduced (negative) pressure of ~10 PSI (about ~0.7 atmospheres) is maintained by a vacuum pump connected to the outlet of the chamber for a period of 1-15 minutes. When the negative pressure is reached, the pump is stopped and the outlet valve closed to maintain the negative pressure inside the chamber. Thereafter, the inlet valve connected to a nitric oxide-containing cylinder is opened to allow the nitric oxide (Aigas Specialty Gases, Chicago, Ill.) in nitrogen (N₂) or argon (Ar) as a carrier gas (0.05-10 percent nitric oxide) to flow and fill the chamber. The gas inflow is stopped when a pressure of zero PSI is reached. After the exposure time, the gas inside the chamber is purged with air for at least 5 minutes through nitric oxide absorbing filters. Thereafter the chamber is opened and the exposed article is kept at room temperature.

Nitric Oxide Impregnation General Procedure II

After the exposure time, the gas inside the chamber is purged with air for at least 5 minutes through nitric oxide absorbing filters. Thereafter the chamber is opened and the exposed article is kept at room temperature.

Nitric oxide has a half-life of a few seconds; therefore stable metabolites of nitric oxide (nitrates and nitrates) were measured using the Griess test [Green et al., Anal Biochem 126:131-138] to determine nitric oxide concentration in an aqueous medium.

Absorbance was measured at 543 nm and was translated to nitrate concentration using a standard curve prepared using samples with known nitrate concentrations. Nitrite production was converted into parts per million of nitric oxide as follows: nitric oxide ppm = (46x[N₂O₃]x0.65x10⁻³). Each μM (μmol/liter) was multiplied by the molecular mass of nitrite (46 grams/mol). This value was converted to ppm of nitric oxide, taking into account the difference in molecular weight (MW) between nitrates and nitric oxide (1:0.65 ratio), and the multiplication factor between grams and milligrams (10⁶).

Bacterial and Fungal Culture Preparation:

The clinical strains Enterococcus faecalis, Staphylococcus aureus, E. coli, Pseudomonas aeruginosa, and Sienotrophomonas malthophilia, or strains from the American Type Culture Collection (ATCC)—E. faecalis #29212, Staphylococcus saprophyticus #15305, Staphylococcus epidermidis #35984, E. Coli #25922, P. aeruginosa #14230, Acinetobacter baumannii #BAA-747, Candida albicans #14053 were used for the described studies.

Bacteria were grown to 0.5 McFarland standard, and 1-ml aliquots of these preparations each containing approximately 2.5x10⁷ CFU/ml were stored in vials at ~70°C. On the day of the experiments, the fresh stock was removed from the freezer, thawed, and 2 ml of media was added (Luria Broth (LB) for E. coli, P. aeruginosa, A. baumanii; S. aureus, Dextrose Broth for C. albicans; Brain Heart Infusion (BHI) for E. faecalis, S. saprophyticus, S. epidermidis). Cultures were further diluted with some media to 10⁷ CFU/ml in volumes appropriate to the experimental conditions.

Biofilm Formation Assay:

Biofilm formation assays were performed as described in O’Toole et al., 1998, Mol. Microbiol., 30:295-304. Briefly, an object sample was cut lengthwise and placed in vial with 4 ml of 1 percent weight per volume crystal violet for 15 minutes. The vial was washed, and the solution was replaced with 4 ml of 95 percent ethanol. The extracted color was measured by absorbance at 595 nm.
Discoloration Measurements:

Color measurements were obtained using a LabScan® XE spectrophotometer (Hunter Labs; LabScan® XE is a registered trademark of Hunter Associates Laboratory, Inc. 11495 Sunset Hills Road Reston Va. 22090), and analyzed with the easymatch QC software (version 3.72) following manufacturers’ protocols and the method disclosed by Engberger et al., 2006. Food Nutr Bull 27(4):281-91. Briefly, three nitric oxide-dosed (“treated”), or control (“untreated”) samples were put into a small petri dish lengthwise and side-by-side with no space between them, and measurements of each plate were done from four different angles (2 vertical and 2 horizontal), in triplicates. The color values obtained included: L*—the lightness of the color (0 yields black and 100 indicates diffuse white); a*—position between red/magenta and green (negative values indicate green while positive values indicate magenta; on a numeric scale range from -100 to +100); and b*—position between yellow and blue (negative values indicate blue and positive values indicate yellow; on a numeric scale range from -100 to +100).

Hardness and Springiness Measurements:

Hardness and springiness measurements were obtained using a TA.XT2 Texture Analyzer (Texture Technologies Corp.), and then analyzed with the Exponent 32 software (version 3.6.0.0). Treated and untreated 3-cm samples of the studies object were stretched at a constant distance using the texture analyzer, following manufacturer’s protocols. The maximum force that was used to stretch a specific piece was measured, using a “Miniature tissile grip” probe, and total area under the curve of force as a function of time was thereby determined.

Tensile Strength Measurements:

Tensile strength measurements were conducted using a TA.XTPLUS Texture analyzer, (Texture Technologies Corp.), and then analyzed with the Exponent 32 software (version 3.6.0.0). Treated and untreated 3-cm samples of the studies object were stretched at a constant distance using the texture analyzer, following manufacturer’s protocols. The maximum force that was used to stretch a specific piece was measured, using a “Miniature tissile grip” probe, and total area under the curve of force as a function of time was thereby determined.

Example 1

NO-Impregnated Silicone Catheters

Six-mm diameter Folyss™ silicone Foley catheters, (catalog no. AA6118; Coloplast® Corp, Minneapolis, Minn., USA) (Folyss™ and Coloplast® are registered trademarks of Coloplast A/S, Humlebaek, Denmark) were aseptically cut into 2-cm sections and incubated by immersion in 2 ml of one of 10⁵ CFU/ml, 10⁶ CFU/ml, or 10⁷ CFU/ml E. coli. inocula for 30 minutes. Incubated catheter sections were subsequently dosed with 20,000 parts per million of nitric oxide (at a flow rate of 30 cc/minutes) during 1, 5, 15, 30, 60 or 120 minutes, using the general procedure I described hereinabove. Control catheter sections were not impregnated with nitric oxide, but were stored in sterile sealed vials.

The nitric oxide-treated and untreated catheter segments were then immersed separately in 2 ml media and then incubated for 8 hours at 37° C. Aliquots of the incubation media were plated and numbers of CFU counted. Bacterial loads were calculated as CFU per ml.

FIG. 1 shows the results of dosing the medical device samples with nitric oxide for 1, 5, 15, 30, 60 or 120 minutes, following incubation in a bacterial suspension of 10⁵, 10⁶ or 10⁷ CFU/ml for 8 hours at 37° C. The CFU/ml remaining in the suspension are shown with respect to dose time and starting culture CFU/ml.

Table 1 presents the colony formation following exposure of catheter segments to nitric oxide at various durations, wherein TNTC denotes “too numerous to count” or over 10⁶ CFU/ml.

<table>
<thead>
<tr>
<th>Control</th>
<th>1 minute</th>
<th>5 minutes</th>
<th>15 minutes</th>
<th>30 minutes</th>
<th>60 minutes</th>
<th>120 minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFU/ml</td>
<td>10⁵</td>
<td>8 x 10⁵</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CFU/ml</td>
<td>10⁶</td>
<td>1 x 10⁶</td>
<td>6 x 10⁵</td>
<td>40</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CFU/ml</td>
<td>10⁷</td>
<td>TNTC</td>
<td>TNTC</td>
<td>TNTC</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

As can be seen in Table 1 and FIG. 1, 15 minutes of dosing with 30 cc/minutes of 20,000 ppm nitric oxide (2 percent nitric oxide in nitrogen) was sufficient time to impregnate the catheter segment with sufficient nitric oxide to kill all bacteria up to a 10⁷ inoculation with E. coli. At lower CFU, e.g., 10⁵ CFU/ml inoculation, one minute was sufficient time to dose the catheter segment with sufficient nitric oxide to kill all bacteria. With a bacterial culture of 10⁵ CFU/ml, 1 to 5 minutes were sufficient times to dose the catheter segment with sufficient nitric oxide to provide antimicrobial effects as measured by reductions in CFU. Sterilization was observed with 15 or more minutes dosing times. These data demonstrate that nitric oxide exhibited an antimicrobial effect at all three concentrations of bacterial stocks tested, and sterilized the catheter segments after 1 minute (10⁵) and 15 minutes (10⁶ and 10⁷) of treatment.
Example 2

NO-Impregnated Silicone Catheters

[0447] A commercially-available catheter, such as a six-mm diameter Folsil® silicone

[0448] Foley catheters, (catalog no. AA6118; Coloplast® Corp. Minneapolis, Minn., USA) is aseptically cut into 2-cm sections and inoculated by immersion in 2 ml of one of 10^5 CFU/ml, 10^6 CFU/ml, or 10^7 CFU/ml E. coli. inocula for 30 minutes. Inoculated catheter sections are subsequently dosed with nitric oxide using the general procedure I described hereinabove. Control catheter sections are not impregnated with nitric oxide, but are stored in sterile sealed vials.

[0449] The nitric oxide-treated and untreated catheter segments are then immersed separately in 2 ml media and incubated for 8 hours at 37° C. Aliquots of the incubation media are plated and numbers of CFU counted. Bacterial loads are calculated as CFU/ml.

[0450] Catheters impregnated with NO using general procedure II are found to eradicate bacteria at all of the tested bacteria concentrations. Sterilization is thus achieved by NO-impregnation using general procedure II.

Example 3

Nitric Oxide Loading in NO-Impregnated Catheters

[0451] Six-mm diameter Folsil® silicone Foley catheters were aseptically cut into 2-cm sections and exposed to nitric oxide for 1, 5, 10, 20, 40, 60, 120, 180 or 240 minutes, using general procedure I as described hereinabove. The samples were then immersed in doubly-distilled water and after 1 hour were assayed for nitrates and nitrates, as described hereinabove. All calculations were done per 1 cm of catheter.

[0452] FIG. 2 presents a plot of nitrite in μmol per cm of catheter relative to dosing time.

[0453] Table 2 presents the raw data of release of nitrates and nitrates from nitric oxide dosed catheter sample over varying dose times, wherein "Abs." denotes absorbance, and "Abs average" denotes the average absorbance measured from duplicate sampling.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Abs.</th>
<th>Abs.</th>
<th>Abs. average</th>
<th>conc. NO_2 (μM)</th>
<th>NO_2 μmol in solution</th>
<th>NO_2 μmol/cm</th>
<th>NO ppm/cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.066</td>
<td>0.069</td>
<td>0.07</td>
<td>25.96</td>
<td>0.08</td>
<td>0.04</td>
<td>0.39</td>
</tr>
<tr>
<td>5</td>
<td>0.275</td>
<td>0.274</td>
<td>0.27</td>
<td>105.58</td>
<td>0.33</td>
<td>0.16</td>
<td>1.58</td>
</tr>
<tr>
<td>10</td>
<td>0.398</td>
<td>0.364</td>
<td>0.38</td>
<td>146.54</td>
<td>0.44</td>
<td>0.22</td>
<td>2.19</td>
</tr>
<tr>
<td>20</td>
<td>0.491</td>
<td>0.53</td>
<td>0.51</td>
<td>196.35</td>
<td>0.59</td>
<td>0.29</td>
<td>2.94</td>
</tr>
<tr>
<td>40</td>
<td>0.531</td>
<td>0.574</td>
<td>0.55</td>
<td>212.50</td>
<td>0.64</td>
<td>0.32</td>
<td>3.18</td>
</tr>
<tr>
<td>60</td>
<td>0.808</td>
<td>0.912</td>
<td>0.81</td>
<td>348.08</td>
<td>1.04</td>
<td>0.52</td>
<td>5.20</td>
</tr>
<tr>
<td>120</td>
<td>1.265</td>
<td>1.312</td>
<td>1.29</td>
<td>495.88</td>
<td>1.49</td>
<td>0.74</td>
<td>7.40</td>
</tr>
<tr>
<td>180</td>
<td>1.689</td>
<td>1.557</td>
<td>1.62</td>
<td>624.23</td>
<td>1.87</td>
<td>0.94</td>
<td>9.33</td>
</tr>
<tr>
<td>240</td>
<td>2.096</td>
<td>1.618</td>
<td>1.86</td>
<td>714.23</td>
<td>2.14</td>
<td>1.07</td>
<td>10.68</td>
</tr>
</tbody>
</table>

[0454] As can be seen in Table 2 and FIG. 2, the amount of sequestered nitric oxide, reflected by the amount of nitrates, correlates to the time of exposure, showing a tendency for saturation at exposure periods over 240 minutes.

Example 4

Nitric Oxide Loading in NO-Impregnated Catheters

[0455] In order to compare nitric oxide change using general procedures I and II as described hereinabove, 2-cm sections of commercially available catheters as described herein are placed in a chamber as described herein and are exposed to 20,000 ppm nitric during 240 minutes, according to general procedure I described hereinabove.

[0456] In a separate assay, 2-cm sections of commercially available catheters as described herein are placed in a chamber as described herein, the chamber is sealed and reduced pressure is generated in the chamber, and nitric oxide is then allowed to fill the chamber, according to general procedure II described hereinabove.

[0457] The samples of each assay are then immersed in doubly-distilled water and after 1 hour are assayed for nitrates and nitrates, as described hereinabove, using the Griess reagent, and nitrates and nitrates concentrations are determined.

[0458] Calculations are done per 1 cm of catheter and show NO charge that is higher by at least 20%, or at least 50%, compared to catheters impregnated with nitric oxide using general procedure I as described hereinabove.

Example 5

Effects of Storage of NO-Impregnated Catheters on Antimicrobial Activity

[0459] To assess the effect of storage on antimicrobial activity after dosing articles with nitric oxide, dosed sections of silicone catheters were stored in sealed containers containing air or water for one week.

[0460] Six-mm diameter Folsil® silicone Foley catheters were aseptically cut into 2-cm sections and dosed with nitric oxide overnight (10-24 hours) as described in general procedure I described hereinabove.

[0461] After storage, the catheter sections were immersed in suspensions of 10^5 CFU/ml E. coli for 1 minute. After transfer to PBS and incubation for 3 hours or 24 hours, no bacterial growth was observed in the aliquots prepared from nitric oxide sequestering catheter sections. However, significant bacterial growth (10^4 CFU/ml) occurred in the aliquots prepared from the control catheter.

[0462] FIGS. 3A-B show images of representative plates of control and nitric oxide-eluting samples (FIG. 3A); and viable counts of CFU in triplicate (FIG. 3B), wherein the hatched bars represent data taken from control experiments, and the checker bars represent data taken from the nitric oxide sequestering samples.

[0463] As can be seen in FIGS. 3A-B, after a week of storage in air or water, catheter section samples sequestering nitric oxide had absorbed sufficient nitric oxide to subse-
quently elute sufficient nitric oxide to demonstrate antimicrobial and sterilizing activity when exposed to bacterial cultures.

These results demonstrate the capacity of nitric oxide-dosed medical devices to elute nitric oxide levels sufficient for self-sterilization after being stored for 1 week in either air or water.

Example 6

Effects of Storage of NO-Impregnated Catheters on Antimicrobial Activity

Commercially available catheters, such as six-mm diameter Foley silicone Foley catheters are aseptically cut into 2-cm sections and dosed with nitric oxide as described in general procedure II described hereinabove.

After a week of storage in air or water storage, the catheter sections are immersed in suspensions of $10^3$ CFU/ml of E. coli for 1 minute. After transfer to PBS and incubation for 3 hours or 24 hours, no bacterial growth is observed in the aliquots prepared from nitric oxide sequestering catheter sections.

Example 7

Effects of NO-Impregnated Catheters on Urinal Bacterial Flora

Six-mm diameter Foley silicone Foley catheters were dosed with 20,000 ppm nitric oxide at a flow rate of 30 cm/minute for 24 hours, according to the general procedure I described hereinabove.

Using a dynamic urine flow model system, urine (2 liters) was collected from male volunteers and placed in two liter sterile plastic containers. Two ml of bacterial culture ($10^2$ CFU/ml) were inoculated in each container.

One catheter was circulated through nitric oxide-dosed catheter tubes and the control container was circulated using untreated catheter tubes. Urine samples from each of the containers were re-circulated in a separate closed system using a flow rate of 1.5 ml/minute (Rabbit-Plus peristaltic pump; Rainin) through the catheters, for 24 hours at $37^\circ$ C. Bacterial levels in the control and treated containers reached $10^6$ and $10^3$ CFU/ml after 24 hours, respectively.

The catheters were then aseptically removed, washed with sterile water, cut into 3-cm pieces, and processed as follows:

A biofilm formation assay was performed as described hereinabove with each catheter piece. FIG. 4A shows the absorbance at 595 nm of the crystal violet that was attached to the catheter pieces after extraction with ethanol. The hatched bars are data from the control, while the checkered bars are data from the impregnated catheters. The error bars indicate standard deviations.

As can be seen in FIG. 4A, more than twice the amount of biofilm matrix was formed on the luminal surface of the control catheter compared with the impregnated catheter when using crystal violet.

In order to measure biofilm-embedded bacteria, each piece was cut lengthwise, washed and put into 4 ml sterile water. Following sonication for 30 seconds, 1 l and 10 l of water surrounding a selected catheter piece were plated on LB plates and incubated at $37^\circ$ C for 24 hours.

FIG. 4B presents images demonstrating biofilm-embedded bacteria within the biofilms on control and impregnated catheters, grown in LB plates from the 1 l (bottom) and 10 l (top) of water surrounding a selected catheter piece.

As can be seen in FIG. 4B, after the removal of matrix-bound bacteria by sonication, there were no bacteria on the impregnated catheter surface, and there were an average of 1.210 CFU/ml on the control piece.

Example 8

Effects of NO-Impregnated Catheters on Urinal Bacterial Flora

Commercially available catheters, such as six-mm diameter Foley silicone Foley catheters are dosed with nitric oxide, according to either general procedure I or general procedure II described hereinabove.

Using a dynamic urine flow model system, urine (2 liters) is collected from male volunteers and placed in two liter sterile plastic containers. Two ml of bacterial culture ($10^2$ CFU/ml) are inoculated into each container.

One catheter is circulated through catheter tubes dosed by nitric oxide using general procedure I, and one catheter is circulated through catheter tubes dosed by nitric oxide using general procedure II, and the control container is circulated through untreated catheter tubes. Urine samples from each of the containers are re-circulated in a separate closed system using a flow rate of 1.5 ml/minute (Rabbit-Plus peristaltic pump; Rainin) through the catheters, for 24 hours at $37^\circ$ C. Bacterial levels in the control and treated containers are then measured.

The catheters are then aseptically removed, washed with sterile water, cut into 3-cm pieces, and processed as follows:

A biofilm formation assay is performed as described hereinabove with each catheter piece.

Absorbance measurement show that NO-treated catheters exhibit a substantially lower amount of biofilm matrix formed on the luminal surface thereof compared with control catheters. Catheters impregnated with NO using general procedure II exhibited an amount of biofilm matrix formed on the luminal surface which is lower by 20-50% compared to catheters impregnated with NO using general procedure I.

In order to measure biofilm-embedded bacteria, each piece is cut lengthwise, washed and put into 4 ml sterile water. Following sonication for 30 seconds, 1 l and 10 l of water surrounding a selected catheter piece are plated on LB plates and incubated at $37^\circ$ C for 24 hours.

After the removal of matrix-bound bacteria by sonication, there are no bacteria on the NO-impregnated catheter surface.

Example 9

Preparation and Characterization of unpackaged NO-Impregnated Catheters

Six-mm diameter Foley silicone Foley catheters were cut into 3 cm sections, referred to as "samples" or "medical device samples", and were dosed with nitric oxide for 24 hours at a flow rate of 50 ml/minute as described in general procedure I hereinabove. The physical properties of the samples were tested immediately upon NO dosing and
after one-week storage either in water or in air (in a sealed plastic vial at room temperature (about 20° C.).

[0486] Measurements after Dosing:

[0487] Color measurements were conducted as described above, using three 3-cm nitric-oxide treated catheter sections and three untreated catheter sections as control.

[0488] Table 3 presents the results of the discoloration assessment of NO-treated samples after 24-hour nitric oxide dosing, and of untreated control samples.

[0489] For all the following tables, T-test of (−) denotes 'no statistical difference when P>0.05', and (+) denotes 'statistical difference when P<0.05'.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>untreated</th>
<th>NO-treated</th>
<th>T-test</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>vertical</td>
<td>25.9 ± 1.4</td>
<td>25.5 ± 0.46</td>
<td>−</td>
<td>0.56</td>
</tr>
<tr>
<td>average L*</td>
<td>1.29 ± 0.42</td>
<td>1.06 ± 0.17</td>
<td>−</td>
<td>0.24</td>
</tr>
<tr>
<td>average a*</td>
<td>0.69 ± 0.13</td>
<td>0.71 ± 0.18</td>
<td>−</td>
<td>0.89</td>
</tr>
<tr>
<td>horizontal</td>
<td>25.7 ± 1.02</td>
<td>26.1 ± 0.47</td>
<td>−</td>
<td>0.52</td>
</tr>
<tr>
<td>average L*</td>
<td>1.36 ± 0.4</td>
<td>1.09 ± 0.19</td>
<td>−</td>
<td>0.15</td>
</tr>
<tr>
<td>average a*</td>
<td>0.86 ± 0.07</td>
<td>1.50 ± 0.16</td>
<td>+</td>
<td>0.006</td>
</tr>
</tbody>
</table>

[0490] As can be seen in Table 3, no significant change in color was observed during storage; however, the NO-treated samples were indistinguishable from the untreated samples in a visual inspection thereof.

[0491] Table 4 presents the results obtained in the tensile strength measurements of NO-treated and untreated samples.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>untreated</th>
<th>NO-treated</th>
<th>T-test</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Max force (g)</td>
<td>3.17 ± 0.1</td>
<td>3.13 ± 0.06</td>
<td>−</td>
<td>0.38</td>
</tr>
<tr>
<td>Area</td>
<td>14.79 ± 0.79</td>
<td>14.61 ± 0.42</td>
<td>−</td>
<td>0.64</td>
</tr>
</tbody>
</table>

[0492] Table 5 presents the results obtained in the hardness and springiness measurements of NO-treated and untreated samples.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>untreated</th>
<th>NO-treated</th>
<th>T-test</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Max force-1 (g)</td>
<td>23.8 ± 0.43</td>
<td>23.8 ± 0.42</td>
<td>−</td>
<td>0.95</td>
</tr>
<tr>
<td>Max force-2 (g)</td>
<td>21.1 ± 0.34</td>
<td>21.2 ± 0.39</td>
<td>−</td>
<td>0.53</td>
</tr>
<tr>
<td>Area</td>
<td>32.0 ± 0.78</td>
<td>32.2 ± 0.38</td>
<td>−</td>
<td>0.5</td>
</tr>
<tr>
<td>L2/L1</td>
<td>0.94 ± 0.02</td>
<td>0.95 ± 0.01</td>
<td>−</td>
<td>0.673</td>
</tr>
</tbody>
</table>

[0493] As can be seen in Table 4 and Table 5, no significant differences were observed in tensile strength, hardness or springiness of the NO-treated medical device samples compared to the untreated control samples, following 24 hour of dosing with nitric oxide as described.

[0494] Measurements after Dosing and Wet Storage:

[0495] Table 6 presents the results of the discoloration assessment of NO-treated samples after 24-hour nitric oxide dosing and one week of storage in water, and of untreated control samples after one week of storage in water.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>untreated</th>
<th>NO-treated</th>
<th>T-test</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>vertical</td>
<td>26.7 ± 0.8</td>
<td>29.6 ± 0.8</td>
<td>+</td>
<td>0.0001</td>
</tr>
<tr>
<td>average L*</td>
<td>1.5 ± 0.3</td>
<td>0.85 ± 0.3</td>
<td>+</td>
<td>0.0001</td>
</tr>
<tr>
<td>horizontal</td>
<td>25.6 ± 0.8</td>
<td>28.5 ± 0.8</td>
<td>+</td>
<td>0.0001</td>
</tr>
<tr>
<td>average a*</td>
<td>1.57 ± 0.2</td>
<td>0.84 ± 0.3</td>
<td>+</td>
<td>0.0006</td>
</tr>
</tbody>
</table>

[0496] The “b*” values exhibited high variability for both sets of samples, showing statistically different results (data not shown).

[0497] As can be seen while comparing Table 3 and Table 6, a slight change in lightness (Value “L*”) was observed in the NO-treated samples after one week of storage in water.

[0498] Table 7 and Table 8 present the results of the tensile strength and hardness-springiness measurements, respectively, as obtained for NO-treated and untreated samples after storage in water for one week.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>untreated</th>
<th>NO-treated</th>
<th>T-test</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Force (g)</td>
<td>3.1 ± 0.1</td>
<td>3.3 ± 0.05</td>
<td>+</td>
<td>0.001</td>
</tr>
<tr>
<td>Area</td>
<td>14.8 ± 0.8</td>
<td>15.0 ± 0.6</td>
<td>−</td>
<td>0.05</td>
</tr>
</tbody>
</table>

[0499] As can be seen in Tables 6-8, following 1-week storage in water, no significant differences were observed in tensile strength, hardness and springiness, relative to controls.

[0500] Measurements after Dosing and Dry Storage:

[0501] Table 9 presents the results of the discoloration assessment of NO-treated samples after 24-hour nitric oxide dosing and one week of storage in air, and of untreated control samples after one week of storage in air.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>untreated</th>
<th>NO-treated</th>
<th>T-test</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>vertical</td>
<td>25.7 ± 1.5</td>
<td>25.7 ± 2.2</td>
<td>−</td>
<td>0.97</td>
</tr>
<tr>
<td>average L*</td>
<td>1.3 ± 0.3</td>
<td>1.1 ± 0.2</td>
<td>−</td>
<td>0.09</td>
</tr>
<tr>
<td>horizontal</td>
<td>25.2 ± 1.5</td>
<td>25.7 ± 2.2</td>
<td>−</td>
<td>0.67</td>
</tr>
<tr>
<td>average a*</td>
<td>1.25 ± 0.2</td>
<td>0.91 ± 0.1</td>
<td>+</td>
<td>0.006</td>
</tr>
</tbody>
</table>

[0502] As can be seen while comparing data in Tables 3 and 9, a slight change in “a*” value was observed in the NO-treated samples after one week storage in air.

[0503] Table 10 and Table 11 present the results of the tensile strength and hardness-springiness measurements, respectively, as obtained for NO-treated and untreated samples after storage in air for one week.
As can be seen in Tables 10 and 11, following 1-week storage in air, no significant differences were observed in tensile strength and springiness, relative to controls. A change in the hardness (force values) was observed.

Example 10

Preparation and characterization of Packaged NO-Impregnated Catheters

Individually packaged (in a gas-permeable package) FoleySil® catheters (Coloplast® AA6118) were treated with nitric oxide (20,000 ppm) for 24 hours, at a flow rate of 1 liter/minute using general procedure 1 described hereinabove. After NO-treating, the packaged catheters were stored for one month at ambient temperature and humidity. Following storage, catheters were analyzed for color, tensile strength and hardness-springiness as described hereinabove.

The results are presented in Tables 12-14, whereas Table 12 presents color measurements, Table 13 presents tensile strength, and Table 14 presents results of the hardness-springiness measurements.

Example 11

Preparation and Characterization of Additional Packaged NO-Impregnated Catheters

Individually packaged (with gas-permeable package) catheters, denoted A, B, C as described below, were treated with nitric oxide (20,000 ppm) for 24 hours, at a flow rate of 1 liter/minute as described in general procedure 1 hereinabove. After NO-treating, the packaged catheters were stored for one month at ambient temperature and humidity. Following storage, catheters were analyzed for color, tensile strength and hardness-springiness as described hereinabove.


Exemplary catheter B is a silicone coated, latex based device: bardin, Foley catheter, 2-way silicone elastomer coated, 20 Ch/Fr (6.7 mm) by Bard®.

Exemplary catheter C is an all-silicone device: Catheter urethral drainage Foley 2 way 30 CC balloon all silicone, 18 Fr. Bard®.

The results are presented in Tables 15-17, whereas Table 15 presents color measurements, Table 16 presents tensile strength, and Table 17 presents results of the hardness-springiness measurements.
As can be seen in Table 15, lightness ("L" value) did not change after impregnation with nitric oxide in all three catheter sample types, while "a" and "b" values did demonstrate a significant color change.

### TABLE 15-continued

<table>
<thead>
<tr>
<th>Sample</th>
<th>Parameter</th>
<th>untreated</th>
<th>NO-treated</th>
<th>T-test (P &lt; 0.05)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>horizontal average L</td>
<td>38 ± 0.6</td>
<td>36 ± 1.5</td>
<td>-</td>
<td>0.43</td>
<td></td>
</tr>
<tr>
<td>horizontal average a</td>
<td>12.6 ± 0.4</td>
<td>15.8 ± 0.7</td>
<td>+</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>horizontal average b</td>
<td>22 ± 0.6</td>
<td>29 ± 2.4</td>
<td>+</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>C vertical average L</td>
<td>38 ± 0.9</td>
<td>38 ± 1.4</td>
<td>-</td>
<td>0.98</td>
<td></td>
</tr>
<tr>
<td>C vertical average a</td>
<td>-0.9 ± 0.16</td>
<td>-1.4 ± 0.2</td>
<td>+</td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td>C vertical average b</td>
<td>-5.8 ± 0.6</td>
<td>-4.5 ± 0.7</td>
<td>+</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>horizontal average L</td>
<td>38 ± 1.2</td>
<td>37 ± 1.3</td>
<td>-</td>
<td>0.83</td>
<td></td>
</tr>
<tr>
<td>horizontal average a</td>
<td>-0.8 ± 0.2</td>
<td>-1.3 ± 0.3</td>
<td>+</td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td>horizontal average b</td>
<td>-6 ± 0.5</td>
<td>-4.9 ± 0.9</td>
<td>+</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
</tbody>
</table>

As can be seen in Table 16, tensile strength of the all-silicone catheter C demonstrated a significantly greater maximum force compared to the other catheters.

### TABLE 16

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sample</th>
<th>untreated</th>
<th>NO-treated</th>
<th>T-test (P &lt; 0.05)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Max force (g) A</td>
<td>1.1 ± 0.06</td>
<td>1 ± 0.04</td>
<td>-</td>
<td>0.164</td>
<td></td>
</tr>
<tr>
<td>Max force (g) B</td>
<td>1.56 ± 0.07</td>
<td>1.52 ± 0.06</td>
<td>-</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>Max force (g) C</td>
<td>2.8 ± 0.2</td>
<td>3.5 ± 0.07</td>
<td>+</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Area A</td>
<td>5.4 ± 0.3</td>
<td>5 ± 0.3</td>
<td>-</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>Area B</td>
<td>7.9 ± 0.4</td>
<td>7.2 ± 0.3</td>
<td>+</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Area C</td>
<td>14.2 ± 1.6</td>
<td>18.3 ± 0.4</td>
<td>+</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
</tbody>
</table>

As can be seen in Table 17, springiness did not vary significantly for the catheters tested; however, following nitric oxide treatment, catheters A and C demonstrated a change in hardness.

### TABLE 17

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sample</th>
<th>untreated</th>
<th>NO-treated</th>
<th>T-test (P &lt; 0.05)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Max force (g) A</td>
<td>7.3 ± 0.3</td>
<td>6.1 ± 0.1</td>
<td>+</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Max force (g) B</td>
<td>11.1 ± 0.8</td>
<td>10.6 ± 0.5</td>
<td>-</td>
<td>0.32</td>
<td></td>
</tr>
<tr>
<td>Max force (g) C</td>
<td>13 ± 0.8</td>
<td>14.8 ± 0.7</td>
<td>+</td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td>Force-1 (g) A</td>
<td>7 ± 0.3</td>
<td>5.8 ± 0.1</td>
<td>+</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Force-1 (g) B</td>
<td>10.4 ± 0.8</td>
<td>10.1 ± 0.8</td>
<td>-</td>
<td>0.44</td>
<td></td>
</tr>
<tr>
<td>Force-1 (g) C</td>
<td>11 ± 0.8</td>
<td>12.2 ± 0.8</td>
<td>+</td>
<td>0.024</td>
<td></td>
</tr>
<tr>
<td>L2/L1 A</td>
<td>0.84 ± 0.001</td>
<td>0.79 ± 0.0</td>
<td>+</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>L2/L1 B</td>
<td>0.93 ± 0.008</td>
<td>0.94 ± 0.01</td>
<td>-</td>
<td>0.58</td>
<td></td>
</tr>
<tr>
<td>L2/L1 C</td>
<td>0.94 ± 0.005</td>
<td>0.94 ± 0.002</td>
<td>-</td>
<td>0.91</td>
<td></td>
</tr>
</tbody>
</table>

As can be seen in Table 17, springiness did not vary significantly for the catheters tested; however, following nitric oxide treatment, catheters A and C demonstrated a change in hardness.

For the parameters measured, while some changes are indicated to be statistically significant (e.g., there is a difference in the color, hardness, springiness or tensile strength between the treated and control samples), this may be small, and not indicative of an alteration in physical properties of the medical device that would otherwise render it unsuitable for the intended application after dosing with nitric oxide.

It is noted herein that a visible color change due to nitric oxide dosing may be useful as an indicator of treatment, and/or sterility of the medical device, as further explained in detail hereinabove.

### Example 12

Preparation and Characterization of Packaged and Unpackaged NO-Impregnated Catheters

Commercially available catheters such as six-mm diameter Folysil® silicone Foley catheters and the catheters described in Example 11 hereinafore, are cut into 3 cm sections, and are dosed with nitric oxide either using general procedure I described hereinafore or using general procedure II as described hereinafore. The physical properties of the samples are tested immediately upon NO dosing and after one-week storage either in water or in air (in a sealed plastic vial at room temperature (about 20° C.)).

Color measurements and measurements of tensile strength, and hardness-springiness are performed after dosing and upon one week storage in air or water.

In a different set of experiments, individually packaged commercially available catheters such as Folysil® catheters (Coloplast® AA6118) and the catheters described in Example 11 hereinafore are treated with nitric oxide (20,000 ppm) for 24 hours, at a flow rate of 1 liter/minute using general procedure I described hereinafore, or according to general procedure II described hereinafore.

After NO-treating, the packaged catheters are stored for one month at ambient temperature and humidity. Following storage, catheters are analyzed for color, tensile strength and hardness-springiness as described hereinafore.

### Example 13

Biofilm Sterilization by Gaseous NO

A biofilm was established in each well of a 6-well plate and the time of treatment with nitric oxide gas for eradication of the biofilm was tested using the biofilm formation assay described hereinafore.

Briefly, 3 ml of LB media were placed in each well of 12-well plates and then each well was inoculated with 10 μl of stock bacteria (E. coli or A. baumannii) (6 plates for each bacterial type). Plates were incubated with agitation for 5 days at 37°C to establish a biofilm in each well. The media was removed and each well washed twice with sterile saline.

Plates with established biofilms (one for each bacterial type) were treated with nitric oxide (20,000 ppm, 1 liter/minute flow rate) for 5, 10, 30, 60 or 120 minutes. Control plates were kept at room temperature, without treatment.

Three of the six wells for each plate were stained with crystal violet to confirm biofilm formation. The biofilm in the remaining 3 wells of each plate was re-suspended (with...
30 seconds sonication) in 5 ml sterile saline. Aliquots of 1, 10 and 100 μl of each sample were plated in compartmentalized (3-section) petri plates.

[0526] FIG. 5A shows the results of the 1, 10 and 100 μl aliquots for E. Coli, while

[0527] FIG. 5B shows the results of the 1, 10 and 100 μl aliquots for A. baumannii.

[0528] As can be seen in FIGS. 5A-B, a biofilm was established for all wells (E. coli and A. baumannii). Absorbance (595 nm) of the crystal violet-stained wells was found to be 0.7 to 1 with no significant difference observed between control and nitric oxide treated wells.

[0529] Control plates demonstrated bacterial growth proportional to the inoculum, while for all nitric-oxide treated plates (for either bacterial species), no colonies were observed.

Example 14

Antimicrobial Activity of NO-Impregnated Catheters

[0530] NO-dosed catheters were prepared as described in general procedure I hereinafore. Untreated catheters were used as control.

[0531] One and a half (1.5) ml of 10^7 CFU/ml inoculum of each of the tested strains of bacteria or fungi (ATCC strains or clinical isolates) were incubated with 2 cm of catheter section at 37°C for 24 hours. At time points of 0 and 24 hours, tubes were vortexed and aliquots were plated on LB agar then incubated at 37°C for 24 hours. Colony-forming units were counted and final bacterial load calculated as CFU/ml.

[0532] The following bacterial strains obtained from the ATCC were tested:

[0533] Enterococcus faecalis #29212 (E.f. #29212), Staphylococcus saprophyticus #15305 (S.s. #15305), Staphylococcus epidermidis #35984 (S.e. #35984), Escherichia coli #25922 (E.c. #25922), Pseudomonas aeruginosa #14210 (P.a. #14210), Acinetobacter baumannii #BAA-747 (A.b. #BAA-747), and Candida albicans (C.a. #14053).

[0534] The following bacterial clinical isolates were tested: Enterococcus faecalis (E.f.), Staphylococcus aureus (S.a.), E. coli (E.c.), P. aeruginosa (P.a.), Stenotrophomonas maltophilia (S.m.)

[0535] FIGS. 6 and 7 present the data obtained for selected bacterial strains obtained from the ATCC (FIG. 6) and for selected bacterial clinical isolates (FIG. 7) in nitric oxide-dosed vs. control catheter sections.

[0536] As can be seen in FIGS. 6-7, all tested strains demonstrated a decrease in CFU when incubated with nitric oxide-treated catheter segments. Some species of bacteria or fungi (S. epidermidis #35984, E. coli #25922, P. aeruginosa #14210, C. albicans #14053) were eradicated following incubation with the nitric oxide-treated catheter segments, whereas others (clinical isolates of E. faecalis, S. aureus, E. coli, P. aeruginosa, S. maltophilia; and E. faecalis #29212, S. saprophyticus #15305, A. baumannii #BAA-747) demonstrated a significant reduction in CFU remaining.

Example 15

Antimicrobial Activity of NO-Impregnated Catheters

[0537] NO-dosed catheters are prepared as described in general procedure I or II hereinafore. Untreated catheters are used as control.

[0538] One and a half (1.5) ml of 10^7 CFU/ml inoculum of a bacterial or fungal strain are incubated with 2 cm of catheter section at 37°C for 24 hours. At time points of 0 and 24 hours, tubes were vortexed and aliquots were plated on LB agar then incubated at 37°C for 24 hours. Colony-forming units are counted and final bacterial load calculated as CFU/ml.

[0539] All tested strains demonstrate a decrease in CFU when incubated with nitric oxide-treated catheter segments, with catheters impregnated with NO using general procedure II demonstrating an enhanced decrease in CFU compared to catheters impregnated with NO using general procedure I.

Example 16

Bacterial Colonization on Surfaces of NO-Impregnated Catheters

[0540] Catheter sections were immersed for 24 hours in 10^3 CFU/ml of each of the ATCC and clinical strains of bacteria or fungi described in Example 15 hereinafore. Catheter sections were washed twice with sterile distilled water and then aseptically transferred to agar plates containing the appropriate growth media for the particular strain. Each section was rolled once on the plate and the plate incubated at 37°C overnight.

[0541] Table 18 presents the data inspected for the presence of bacteria on the surface of nitric oxide-treated catheter compared to untreated control samples, wherein sections denoted “2” exhibited the same growth density as that of the control; “1” exhibited less growth density compared to control; and “0” exhibited no growth.

<table>
<thead>
<tr>
<th>Bacterial Strain</th>
<th>Untreated Catheter</th>
<th>NO-Treated Catheter</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. faecalis #29212</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Staphylococcus saprophyticus #15305</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Staphylococcus epidermidis #35984</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>E. coli #25922</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>P. aeruginosa #14210</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>A. baumannii #BAA-747</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>C. albicans #14053</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>E. faecalis</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>S. aureus</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>E. coli</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>S. maltophilia</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

Example 17

Bacterial Colonization on Surfaces of NO-Impregnated Catheters

[0542] As can be seen in Tables 18, reduction or complete eradication of colonizing bacteria on the surface of the NO-treated catheter was demonstrated for the majority of the tested strains tested.

Example 18

Bacterial Colonization on Surfaces of NO-Impregnated Catheters

[0543] NO-dosed catheters are prepared as described in general procedure I or II hereinafore. Untreated catheters are used as control.

[0544] Catheter sections are immersed for 24 hours in 10^3 CFU/ml of a bacterial or fungal strain, washed twice with sterile distilled water and then aseptically transferred to agar plates containing the appropriate growth media for the par-
ticular strain. Each section is rolled once on the plate and the plate incubated at 37°C overnight.

**Example 18**

Biofilm Formation in Urine on Surfaces of NO-Impregnated Catheters

**[0546]** Measurements of Biofilm Formation and Biofilm-Embedded Bacteria in NO-Treated Catheters:

**[0547]** To simulate a static, clinically-relevant milieu, such as found for example in an implanted urinary catheter, catheters were incubated in urine instead of media. Urine was collected from male volunteer and placed into sterile vial (1.8 ml in each vial), 200 μl of 10^5 of bacteria were inoculated into each vial to reach a final concentration of 10^6 CFU/ml.

**[0548]** The following bacteria were tested: *E. faecalis* #29212, *S. saprophyticus* #15305, *S. epidermidis* #35984, *E. coli* #25922, *P. aeruginosa* #14210, *A. baumannii* #BAA-747, *C. albicans* #14053.

**[0549]** NO-treated and untreated control catheters (2 cm length) were placed into each of the vials. Vials were incubated for 72 hours at 37°C. Thereafter catheters were aseptically removed, washed with sterile water, cut into 1 cm pieces, and the formation of a biofilm was assessed as described hereinabove.

**[0550]** FIG. 8 presents the relative biofilm formation on luminal surfaces of nitric oxide-sequestering catheter sections following 72 hour incubation in urine inoculated with 10^6 CFU/ml of the tested bacteria.

**[0551]** As can be seen in FIG. 8, biofilm formation was reduced for all tested strains where catheters were treated with nitric oxide before incubation in urine.

**[0552]** In order to measure biofilm-embedded bacteria, each lengthwise-cut piece of catheter was washed and put into 4 ml sterile water. Following sonication for 30 seconds, samples were plated on compatible agar plates in triplicates and incubated at 37°C for 24 hours.

**[0553]** FIG. 9 presented the data obtained for the growth of biofilm-embedded bacteria on nitric oxide-sequestering catheter sections following 72 hours incubation in urine inoculated with 10^6 CFU/ml bacteria.

**[0554]** As can be seen in FIG. 9, bacteria embedded within the biofilm were eradicated in 6 of the 7 species tested, with only *P. aeruginosa* exhibiting colony growth.

**[0555]** Scanning Electron Microscopy assessment of Biofilm:

**[0556]** Catheter sections of 1 cm length from the above-described biofilm studies, inoculated with *Staphylococcus epidermidis* or *A. baumannii*, were used. Samples were washed 3 times with sodium cacodylate (0.1 M, pH 7.4) then postfixed in 0.5% tannic acid and 1% buffered OsO4 using a Pelco 3450 laboratory microscope (Redding, Calif., USA). The samples were rinsed 3 times with water, dehydrated in a graded ethanol series, and dried in a Tousimis 815B critical point drier (Tousimis, Rockville, Md., USA) or chemically dried with hexamethydisilazane (Sigma, St. Louis, Mo., USA) mounted on aluminum SEM stubs and coated with 8 nm of gold using a Cressington 208HR sputter coater. Catheter pieces were imaged using a Hitachi 54700 FESEM (Hitachi, Tokyo, Japan).

**[0557]** FIGS. 10A-C present scanning electron micrographs *Staphylococcus epidermidis* biofilms on NO-sequestering and untreated control catheter sections, wherein untreated control sample at magnification 2.5 k (inset at 20.0 k) is shown in FIG. 10A, untreated control sample at magnification 2.5 k (right inset at 15 k, left inset at 20 k) is shown in FIG. 10B and nitric oxide-treated catheter sample at magnification 1.5 k is shown in FIG. 10C.

**[0558]** FIGS. 11A-C present scanning electron micrographs of *A. baumannii* (ATCC #BAA-747) biofilms on nitric oxide-sequestering and untreated control catheter sections, wherein untreated control sample at magnification 2.5 k (inset at 15 k) is shown in FIG. 11A, untreated control sample at magnification 1 k (inset at 5 k) is shown in FIG. 11B and nitric oxide treated catheter sample at magnification 1 k is shown in FIG. 11C.

**[0559]** As can be seen in FIGS. 10 and 11, little to no biofilm was observed on the nitric oxide-treated catheter sections, while biofilm and embedded bacteria was observe on the control untreated catheter sections.

**Example 19**

Comparison of Antiseptic Activity of NO-Impregnated Urinary Catheters and Commercially Available Catheters

**[0560]** Antiseptic urinary catheters have recently become commercially available and others are in the development stage. The efficacy of both commercially available and emerging urinary catheter technologies in relation to their effects on bacteriuria caused by *E. coli* in vitro has been studied.

**[0561]** Materials and Methods:

**[0562]** An untreated control and three different treated catheters were used in this study, as follows:

**[0563]** Control: 6 mm diameter Folsysil silicone Foley catheter Ch/Fr 18 (catalog no. AA6118, Coloplast Corp. Minneapolis, Minn.);

**[0564]** NOX: the same catheter as control after impregnation with NO as described in general procedure Hereinafter;

**[0565]** AG: BARDEX I.C. silver-coated Anti-Infective Foley Catheter Ch/Fr 18 (Catalog No. 0165118; Bard, Inc. Covington, Ga.); and

**[0566]** NFC: Release NF—Nitrofurazonecoated silicone Foley catheter Ch/Fr 16 (Catalog No. 95216; Rochester Medical, Stewartville, Minn.).

**[0567]** Catheter pieces not treated with NO, used as controls, were stored in a sterile sealed vial until use.

**[0568]** *E. coli* bacterial culture was obtained from American Type Culture Collection (ATCC #25922).

**[0569]** Bacteria were grown to 0.5 McFarland standard. 1 ml aliquots of grown bacteria containing approximately 2.5x10^6 CFU/ml were stored at −70°C. On the day of the experiments the fresh stock was removed from the freezer, thawed, and 2 ml of Luria Broth (LB) was added. Cultures were further diluted with LB to 10^4 CFU/ml.

**[0570]** *E. coli* culture (2 ml) at a concentration of 10^5 CFU/ml was added to a vial containing a 2 cm piece of catheter and incubated for 24 hours at 37°C. After 24 hours, samples were vortexed and plated (using a 10^−4 time dilution) on LB agar
plates and were then incubated at 37° C. for 24 hours. The CFU was counted and calculated to represent the CFU per ml.

[0571] E. coli culture (200 µl) at a concentration of 10^5 CFU/ml was added to 1.8 ml of urine (reaching a final concentration of 10^6 CFU/ml) containing a 2 cm section of a tested catheter and incubated at 37° C. for 72 hours. Urine was collected in a sterile vial from a male volunteer on the day of the experiment. After 72 hours, samples were vortexed and plated onto LB agar plates and incubated at 37° C. overnight. The CFU were counted and calculated to represent the CFU/ml.

[0572] To qualitatively evaluate the colonization potential, 1, 10, and 100 µl of each sample were plated on a three compartment LB agar petri plate. Plates were incubated overnight at 37° C.

[0573] Catheter sections (2 cm) were immersed in tubes containing 3 ml of E. coli culture at 10^5 CFU/ml and incubated at 37° C. for 24 hours. After 24 hours, catheter sections were washed twice using 3 ml of sterile saline (0.9% wt/v NaCl) and transferred aseptically to a LB agar plate. Each catheter section was rolled once on the plate then incubated at 37° C. for 24 hours.

[0574] Catheter pieces (2 cm each) were added, aseptically, to 1.8 ml of urine (collected as stated above) plus 200 µl of E. coli culture at 10^5 CFU/ml and incubated at 37° C. for 72 hours. After 72 hours, catheter pieces were washed twice in 3 ml sterile saline then cut into two parts of equal size, aseptically. One half of each catheter section was added to 1.5 ml of Crystal Violet dye in water (1% wt/v) for 15 minutes then washed twice in 4 ml distilled water (dH2O). Washed catheter pieces were then added to 2 ml of 95% ethanol to reliquish crystal violet bound to the surface of the catheter.

[0575] The absorbance at 595 nm of each ethanol sample was measured using a spectrophotometer and used as an indicator of biofilm formation.

[0576] The other half of each catheter section was added to 2 ml of sterile distilled water (dH2O) and sonicated at a level of 5 for 30 seconds. The extent of colonial E. coli released from each catheter was determined by plating each sample on LB agar plates and incubating at 37° C. overnight. The CFU were counted and calculated to represent the CFU per ml.

[0577] Results:

[0578] FIG. 12 presents the comparative data obtained for E. coli growth in media containing pieces of the NOX, AG and NFC catheters versus media from control catheter, after immersion of the catheters for 24 hours in suspension comprising 10^5 CFU/ml and incubated for 24 hours at 37° C.

[0579] As can be seen in FIG. 12, after being immersed in bacterial culture for 24 hours, the antibacterial activity of the NOX and NFC catheters vastly exceeded that of both the untreated (control) and AG catheters. Control and AG catheters reached concentrations of 1.8x10^8 and 2.1x10^8 CFU/ml, respectively. NOX catheter pieces contained an average bacterial concentration of 2.5x10^6 CFU/ml following the 24 hours incubation, revealing an effective reduction in the concentration of planktonic E. coli. No bacteria were observed in the sample containing NFC catheters.

[0580] FIG. 13 presents the comparative data obtained for E. coli growth in urine after 72 hours exposure to pieces of NOX, AG and NFC and control catheters. Within each three compartment LB agar petri plate 1, 10, and 100 µl of each sample were plated and incubated overnight at 37° C.

[0581] As can be seen in FIG. 13, after being immersed in urine plus bacteria for 72 hours, the solution containing the AG catheter had a similar bacterial concentration to the control, 2.0x10^6 and 5.0x10^6 respectively, while the solutions containing the NOX and NFC catheters completely eradicated bacteria in urine.

[0582] The rolling of catheter pieces on LB agar plates provides a qualitative measure of the presence of bacterial colonization on the surface of each catheter.

[0583] FIG. 14 presents the comparative data obtained for E. coli colonization on NOX, AG and NFC catheters versus control after immersion of catheters for 24 hours in suspension containing 10^5 CFU/ml of E. coli. In each LB Petri dish a catheter was rolled over the surface and then incubated at 37° C. overnight.

[0584] As shown in FIG. 14, both the control and AG catheters contained extensive colonization on the surface of the catheter, whereas for catheters NOX and NFC, no bacterial colonization was observed.

[0585] Crystal Violet studies indicate the extent of biofilm formation on each catheter type. Each experiment was repeated three times with three replicates in each one of the experiments. FIGS. 15A and 15B present comparative data of colonized biofilm formation on NOX, AG and NFC catheters versus control after 72 hours of incubation, demonstrated by absorbance at 595 nm of the Crystal Violet that was attached to the catheter pieces after extraction of color with ethanol (FIG. 15A) and the bacterial growth from the biofilms from the different catheters (FIG. 15B).

[0586] As can be seen in FIG. 15A, the AG catheter showed the highest average absorbance indicating extensive biofilm formation. The AG catheter rendered an average absorbance 8-10 times greater than all other catheter types, even the control. The NOX catheter had half the absorption of the control and the NFC catheter had 40% less. This indicates that both the NOX and NFC catheters had less biofilm formation on their surface when compared to the control. Comparative ANOVA test (p<0.005) shows that all three tested catheters were significantly different from the control and NF and NOX were found to be significantly different from AG. FIG. 15B shows the amount of colonized bacteria released from the catheter surface. The NOX and NFC catheters proved effective at eradicating bacteria embedded within the biofilm whereas the control and AG catheters produced bacterial concentration of 7.5x10^6 and 9.8x10^6 CFU/ml, respectively.

[0587] The obtained data demonstrate that NF and NO impregnated urinary catheters possess similar antimicrobial properties, whereby the silver coated catheter was found to be effective.

[0588] NO impregnated catheters are shown to be comparable to antimicrobial coated urinary catheters in their level of antimicrobial activity. NO coated catheters prevented E. coli growth in urine for 72 hours, same as the NF catheters.

[0589] Antibiotics and NO kill bacteria in different ways, thus, their specificity is not the same. Antibiotics are specific to an organism or a group of organisms whereas NO is not. NF was found to be effective against E. coli in this study, but most gram-negative isolates are nonfermenters, a feature which imparts resistant to NF. NO, however, has a broad range of antimicrobial, antifungal and antiviral activity.

Example 20

Nitric Oxide impregnation of Tracheal Tubes using Reduced Pressure

[0590] A whole pack of individually-packaged Tracheal Tubes was placed in a NO chamber as described herein. The
chamber was subjected to a reduced pressure (~10 psi) atmosphere for 5 minutes and was thereafter filled up with 20,000 ppm NO during 16 hours, following general procedure II described hereinafter.

[0591] A NO-impregnated tube was thereafter cut into 1 cm sections which were put into 2.5 cm of ddH₂O in screw-capped vials.

[0592] NO release from the NO-impregnated tube was assayed for nitrates and nitrites, as described hereinafter, using the Griess reagent, during 1 week.

[0593] The following Tracheal tubes were tested: Mallincrodt: Hi-Lo Tracheal tube 6.5 mm ID ref No. 86110 (triangles) and Mallincrodt: Hi-Contour Tracheal tube 4.5 oral/nasal 6.2, 11 mm ID ref No. 107-45.

[0594] The data is presented in FIG. 16 and clearly show continuous release of NO during the entire period.

Example 21

Nitric Oxide Impregnation of Wrapped Tampons

[0595] Four types of commercially available tampons were selected to determine their anti-infective efficacy after being charged with gaseous nitric oxide, according to embodiments of the present invention.

[0596] Table 19 presents the compositions of the tampons selected for testing in this example.

<table>
<thead>
<tr>
<th>TABLE 19</th>
</tr>
</thead>
<tbody>
<tr>
<td>Composition</td>
</tr>
<tr>
<td>Rayon and/or cotton fiber</td>
</tr>
<tr>
<td>Cotten and/or polyester thread</td>
</tr>
<tr>
<td>Weight (grams)</td>
</tr>
<tr>
<td>Water Absorption in 1 min (ml)</td>
</tr>
</tbody>
</table>

[0597] Tampons were put in a petri dish in their individual packaging and impregnated with nitric oxide using a 20,000 ppm cylinder, using general procedure I described hereinafter. Tampons were charged overnight (12-16 hours) at a flow rate of 0.05 liter/minute. Control tampons were used directly out of their packaging.

[0598] Determination of Nitrites Released from Nitric Oxide Impregnated Tampons:

[0599] Nitric oxide impregnated tampons were removed from their individual wrappers and cut in half into equally weighted pieces. Each piece was placed inside a separate 50 ml vial to which 25 ml of distilled water (ddH₂O) was added. At time points of 10, 30, 60, and 120 minutes, nitrites were measured using Griess test, using absorbance at 543 nm, essentially as described hereinafter. The concentration of nitrites was determined using a standard curve. The results are presented in Table 20 below.

[0600] FIG. 17 presents a bar-graph showing the amount of nitric oxide released after 30 minutes from four exemplary NO-impregnated tampons, as measured by the respective total amount of nitrites released from the tampons impregnated with nitric oxide, wherein the letters under each bar denote the tampon type presented in Table 19 hereinafter.

[0601] As can be seen in FIG. 17, all the tampon types sequestered nitric oxide as demonstrated by their ability to release nitric oxide once immersed in solution. During the two hours of immersion, nitric oxide was released from the tampons at different rates. After 30 minutes of immersion in water, tampon B released the highest amount of NO, at 100 μmol nitrites per tampon, while tampon D released the lowest amount at 14 μmol nitrites per tampon.

[0602] FIG. 18 presents comparative plots showing the accumulated of nitric oxide production during the first 5 hours for four types of NO-impregnated tampons, as measured by the respective total accumulation of nitrites in water produced from tampons impregnated with nitric oxide, wherein nitrites released were calculated per 1 tampon.

[0603] As can be seen in FIG. 18, NO release from all tampons in water reached a peak after 2 hours, with tampon B releasing NO at the highest rate, totaling about 196 μmol nitrite, as compared to the other tampons. Tampons C cumulatively released about 88 μmol nitrite each, tampon A released 55 μmol nitrite and tampon D released the lowest amount of about 32 μmol nitrite. All tampons released between 46-60% of their nitric oxide respectively (measured as nitrites) within the first 30 minutes, thus demonstrating a slower release rate.

[0604] Without being bound by any particular theory, the difference in release rates is assumed to be a function of tampon composition, penetration of NO through the packaging, absorption capacity, and other substance related factors. All four tampons tested reached a peak in their respective release profiles within the first three hours of immersion in water. The total amount of nitric oxide released from a single tampon in 25 ml water varied from 200 to 450 μmoles (reaching concentrations of 8 to 18 mM). All four tampon brands tested released between 46-60% of the charged NO within the first 30 minutes (out of 4 hours tested). Thus, some slow release was demonstrated, although it was more rapid than that of other medical devices such as Foley catheters.

Anti-Infective Activity of Nitric Oxide Impregnated Tampons:

[0605] Yeast Preparation:

[0606] Candida albicans yeast culture was obtained from the American Type Culture Collection (ATCC #14053). A starter was prepared by overnight growth of the colony taken from a plate of C. albicans in Difco™ Sabouraud.

[0607] Dextrose Broth (SAB) media at 30° C. Once turbid, the Optical Dispersion at 600 nm (OD₆₀₀) was measured and the culture was diluted with sterile SAB to give an OD₆₀₀ of 0.1, representing a culture containing 10⁶ CFU/ml. From the culture of 10⁶ CFU/ml two separate cultures containing yeast concentrations of 10⁴ and 10² CFU/ml were prepared in sterile SAB inside 1 L sterile containers.

[0608] Presence of C. albicans on the Surfaces of Charged Tampons:

[0609] In order to determine whether fungal growth could be prevented on the surface of impregnated tampons, treated tampons were inoculated with C. albicans culture for 4 hours at 30° C. Growth was assessed by rolling the treated tampons on a petri dish incubated overnight at 30° C. FIG. 19 shows representative plates from each brand of tampon. No growth of C. albicans in plates rolled with NO impregnated tampons was observed. In contrast, controls from all brands showed
growth of C. albicans with total numbers of colonies on each plate being approximately 10 times greater for controls inoculated with 10⁵ CFU/ml than those inoculated with 10⁴ CFU/ml. Controls of all four brands of tampons showed similar total numbers of colonies on corresponding plates.

Antimicrobial Activity of Impregnated Tampons against C. albicans and E. coli:

Tampons were aseptically removed from their individual wrappers and inserted into separate sterile 50 ml vials. The string of each tampon was left hanging on the outside of each vial. Twenty-five (25) ml of C. albicans culture at either 10³ or 10⁴ CFU/ml was added to each vial and vortexed thoroughly. Vials were then incubated at 30°C for 4 (10² CFU/ml) or 6 (10⁴ CFU/ml) hours and thoroughly vortexed every 2 hours. Following incubation, aliquots (100 µl) were plated onto SAB agar petri plates and incubated overnight at 30°C. CFU were counted and calculated to represent the CFU/ml.

FIG. 20 is a bar-graph showing the anti-infective activity of NO-impregnated tampons, according to some embodiments of the present invention, comparing the growth of C. albicans in media after immersion of the tampons for 6 hours in suspension comprising 10⁴ CFU/ml of C. albicans for NO-impregnated tampons and for untreated control tampons, wherein numbers represent viable counts of the triplicate CFUs, white bars represent data from the control experiments and black bars represent data from the NO-impregnated tampons, with error bars representing standard deviation.

As can be seen in FIG. 20, the effect of impregnated tampons on C. albicans growth was measured using various initial inoculums of yeast. Using an initial concentration of 250 CFU in total, tampon B has shown to eradicate all yeast, tampon C was shown to eradicate over 95% of yeast compared to the control, tampon D was shown to have a 50% reduction over 4 hours, and tampon A did not have any effect compared to the control tampons.

FIG. 21 is a bar-graph showing the anti-infective activity of NO-impregnated tampons, according to some embodiments of the present invention, comparing the growth of C. albicans in media after immersion of the tampons for 4 hours in suspension comprising 10⁴ CFU/ml of C. albicans for NO-impregnated tampons and for control untreated tampons, wherein numbers represent viable counts of the triplicate CFUs, white bars represent data from the control experiments and black bars represent data from the NO-impregnated tampons, with error bars representing standard deviation.

As can be seen in FIG. 21, an effect similar to that described above has been demonstrated when the media was inoculated with 2500 CFU in total. The only exception in results between the 10³ and 10⁴ inoculum seen was in the higher inoculi, tampon D did not appear to have any reduction compared to the control, while it did have an anti-infective effect using the lower inoculum of 10³.

Table 20 summarizes the results of the abovementioned comparison of the obtained results.

<table>
<thead>
<tr>
<th>Amount of nitrites reached after 2 hours</th>
<th>Slow release effect during 5 hours</th>
<th>Efficiency in controlling yeast infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>lowest (55 µmole)</td>
<td>Yes (46%)</td>
<td>100%</td>
</tr>
<tr>
<td>highest (88 µmole)</td>
<td>Yes (50%)</td>
<td>94% reduction at 10³</td>
</tr>
<tr>
<td>(32)</td>
<td>Yes (60%)</td>
<td>5% reduction at 10³, none</td>
</tr>
</tbody>
</table>

As can be seen in Table 20, the capacity of various tampons to absorb nitric oxide, according to a process embodiment of the present invention, and to release nitric oxide in an aqueous environment, according to other embodiments of the invention, has been demonstrated. Once immersed in an aqueous solution, tampons released nitric oxide, which was converted to its more stable metabolites, namely nitrates. Nitric oxide was released from the various brands of tampons at different rates as seen in FIG. 18.

Further experiments were performed for determining the antibacterial effect of NO-charged tampons, using 10⁻¹⁰⁵ CFU/ml C. albicans and E. coli. The results are presented in Tables 21 and 22, respectively.

NO charged tampons were shown to eradicate 100% of C. albicans when inoculated with 10⁻¹⁰⁵ CFU/ml and incubated for 4 hours (Table 21). In contrast, untreated tampons showed similar numbers of colonies, regardless of tampon type. Untreated tampons contained average numbers of colonies of 3×10², 2×10², and 2×10¹ for starting concentrations of 10⁴, 10³, and 10², respectively.

NO charged tampons were shown to either reduce, or eradicate E. coli at concentrations 10⁻⁵⁻⁻⁻⁴ CFU/ml whereas tampon A eradicated bacteria at concentrations of 10⁴ and 10³ CFU/ml, and showed a significant reduction in colonies at 10² CFU/ml. Untreated tampons contained average numbers of colonies of 3×10⁴, 4×10³, and 1×10² for starting concentrations of 10⁴, 10³ and 10² CFU/ml, respectively.

<p>| TABLE 20 |</p>
<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amount of nitrites reached after 2 hours</td>
<td>Slow release effect during 5 hours</td>
<td>Efficiency in controlling yeast infection</td>
<td></td>
</tr>
<tr>
<td>lowest (55 µmole)</td>
<td>Yes (46%)</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>highest (88 µmole)</td>
<td>Yes (50%)</td>
<td>94% reduction at 10³</td>
<td></td>
</tr>
<tr>
<td>(32)</td>
<td>Yes (60%)</td>
<td>5% reduction at 10³, none</td>
<td></td>
</tr>
</tbody>
</table>

| TABLE 21 |
| Control * 10³ | Treated | Control * 10⁴ | Treated | Control * 10⁵ | Treated |
| A | 1.3 ± 0.04 | 0.5 ± 0.09 | 0 | 1.0 ± 0.30 | 0 |
| B | 1.3 ± 0.20 | 0.5 ± 0.50 | 0 | 1.0 ± 0.20 | 0 |
| C | 1.7 ± 0.40 | 0.9 ± 0.30 | 0 | 1.1 ± 0.06 | 0 |
| D | 1.3 ± 0.01 | 0.6 ± 0.09 | 0 | 0.7 ± 0.20 | 0 |

| TABLE 22 |
| Control * 10³ | Treated * 10³ | Control * 10⁴ | Treated * 10⁴ | Control * 10⁵ | Treated * 10⁵ |
| A | 31.0 ± 15 | 0 | 51 ± 18 | 0 | 8.5 ± 0.40 | 0.01 ± 0.01 |
| B | 20.0 ± 6.4 | 0 | 23 ± 10 | 0 | 8.5 ± 0.60 | 0 |
Inhibition of Bacterial Growth by Tampons Releasably Sequestering NO:

To check whether NO inhibits bacterial growth in the proximity of the device, the clear zone surrounding the NO impregnated tampons was measured. Charged tampons were cut lengthwise into two, then placed onto an LB agar petri dish that had been inoculated with 200 μl of E. coli at 10⁶ CFU/ml and incubated overnight at 37°C. Following incubation, the radius of the zone from the center of the tampon to the first viable colony was measured.

Results, shown in FIG. 22 and Table 23, show that all charged tampons created a zone of bacterial growth inhibition with tampon B showing the highest zone of inhibition with 4.2 cm to the first shown colony. In all untreated tampons, no measureable zone of inhibition was observed.

TABLE 23

<table>
<thead>
<tr>
<th>Zone of inhibition</th>
<th>Control</th>
<th>Treatment (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0</td>
<td>3.9 ± 0.4</td>
</tr>
<tr>
<td>B</td>
<td>0</td>
<td>4.2 ± 0.2</td>
</tr>
<tr>
<td>C</td>
<td>0</td>
<td>3.2 ± 0.7</td>
</tr>
<tr>
<td>D</td>
<td>0</td>
<td>3.8 ± 0.7</td>
</tr>
</tbody>
</table>

Inhibition of Bacterial Growth in a Vaginal Model:

NO-treated and control tampons were suspended inside sterile 250 ml Erlenmeyer flasks containing LB, 2% (w/v) gelatin, and E. coli at 10⁶ CFU/ml. Erlenmeyer flasks were incubated overnight at 37°C. The opening of an Erlenmeyer flask was utilized to model the shape of a vagina, as shown in the set-up illustration presented in FIG. 23.

FIG. 25 shows a picture comparing the effect of a NO-treated tampon B against an untreated tampon when placed inside an Erlenmeyer flask containing LB, 2% (w/v) gelatin, and E. coli at 10⁶ CFU/ml. It can be readily seen that the NO treated tampon rendered the solid matrix inside the flask much clearer than the untreated tampon, indicating either a significant reduction or eradiication of E. coli. In contrast, the matrix of the untreated tampon was evenly turbid throughout, indicating considerable bacterial growth. Additionally, the clear matrix of the NO-treated tampon in FIG. 25 suggests that the NO released from the tampon (as NO⁻) diffused over a great enough distance to create a large zone of inhibition in liquid.

The data presented herein corroborate the effectiveness of NO-treated tampons as an antimicrobial delivery system inside the vagina, and hence as an effective treatment of, for example, vulvovaginal candidiasis (VVC), BC and/or RVVC.

Example 22

Nitric Oxide Impregnation of Unwrapped Tampons

Unwrapped tampons were treated with nitric oxide using general procedure described hereinabove, except that the tampons were charged with nitric oxide outside their packaging material and additional concentrations of C. albicans were used during the experiment. The results are summarized in FIG. 25 and Tables 24 and 25.

FIG. 25 presents comparative plots of the total accumulation of nitrates produced in water during 4 hours of immersion as a function of time, as measured by the Griess test from NO-treated and untreated tampons, all outside their wrappers, wherein letters denote the type of tampon and the nitrite levels are calculated per single tampon.

Table 24 presents a comparative summary of tampons' ability to release NO and efficacy in controlling yeast growth. Table 25 presents a comparison of growth of C. albicans in media from tampons impregnated with nitric oxide without their wrappers versus media from control tampons, after immersion of the tampons for 4 hours in suspension comprising 10⁴-10⁵ CFU/ml of C. albicans. The numbers represent the averages and standard deviations of viable counts of the triplicate CFUs. “C” denotes negative control samples.

TABLE 25

<table>
<thead>
<tr>
<th>Tampon Type</th>
<th>Inocula (CFU/ml)</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10³</td>
<td>30</td>
<td>35</td>
<td>20</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>10⁴</td>
<td>60</td>
<td>165</td>
<td>125</td>
<td>170</td>
</tr>
<tr>
<td></td>
<td>10⁵</td>
<td>1780</td>
<td>1710</td>
<td>1345</td>
<td>2330</td>
</tr>
</tbody>
</table>

TABLE 25-continued

<table>
<thead>
<tr>
<th>Control * 10³</th>
<th>Treated * 10³</th>
<th>Control * 10⁴</th>
<th>Treated * 10⁴</th>
<th>Control * 10⁵</th>
<th>Treated * 10⁵</th>
</tr>
</thead>
<tbody>
<tr>
<td>C 0.9 ± 3.0</td>
<td>0.6 ± 0.07</td>
<td>15 ± 13</td>
<td>0.1 ± 0.05</td>
<td>8.5 ± 0.04</td>
<td>0.3 ± 0.20</td>
</tr>
<tr>
<td>D 0.9 ± 1.8</td>
<td>0.0 ± 0</td>
<td>43 ± 17</td>
<td>0.0 ± 0</td>
<td>7.9 ± 3.10</td>
<td>0</td>
</tr>
</tbody>
</table>
TABLE 25-continued

<table>
<thead>
<tr>
<th>Tampon Type</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inocula (CFU/ml)</td>
<td>C-avg</td>
<td>C-stdev</td>
<td>Test</td>
<td>C-avg</td>
</tr>
<tr>
<td>$10^4$</td>
<td>19050</td>
<td>4879</td>
<td>0</td>
<td>8600</td>
</tr>
<tr>
<td>$10^5$</td>
<td>121000</td>
<td>21213</td>
<td>0</td>
<td>107500</td>
</tr>
</tbody>
</table>

[0631] As can be seen in FIG. 25 and Tables 24 and 25, the charged tampons were very effective in eradicating and/or controlling yeast growth. In fact, as demonstrated in Tables 24 and 25, all of the tampons charged with nitric oxide had zero yeast count after 4 hours of immersion in $10^4$-$10^5$ CFU/ml yeast, indicating complete eradication of the yeast.

Example 23

Nitric Oxide Impregnation of Tampons

[0632] Wrapped and unwrapped tampons are treated with nitric oxide using general procedure II described herein-above. As opposed to the prolonged time (e.g., overnight) of exposure to nitric oxide required to impregnate tampons with NO using general procedure I (see, Example 22 herein-above), only a few (e.g., 2-4) hours of exposure to nitric oxide are required using general procedure II.

[0633] Total accumulation of nitrates produced in water during 4 hours of immersion as a function of time is measured by the Griess test for NO-treated and untreated tampons. Nitrite levels of tampons impregnated with NO using general procedure II are higher than those impregnated with NO using general procedure I.

[0634] The tampons’ efficacy in controlling yeast growth is determined by measuring growth of C. albicans in media from NO-treated and untreated, after immersion of the tampons for 4 hours in suspension comprising $10^4$-$10^5$ CFU/ml of C. albicans.

[0635] Complete eradication of the yeast is observed in all NO-treated tampons.

[0636] Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims.

[0637] All publications, patents and patent applications mentioned in this specification are herein incorporated in their entirety by reference into the specification, to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference. In addition, citation or identification of any reference in this application shall not be construed as an admission that such reference is available as prior art to the present invention. To the extent that section headings are used, they should not be construed as necessarily limiting.

1.17. (canceled)

118. A process of preparing an article having gaseous nitric oxide sequestered therewithin, the process comprising: placing an article within a chamber; generating a reduced pressure in said chamber; and filling said chamber with a gaseous nitric oxide-containing environment, thereby preparing the article having gaseous nitric oxide sequestered therewithin.

119. The process of claim 118, wherein said generating said reduced pressure comprises reducing said pressure by from −1 psi to −50 psi.

120. The process of claim 118, wherein at least a portion of the article comprises a plurality of voids for sequestering nitric oxide.

121. The process of claim 118, wherein the article is a medical device.

122. The process of claim 121, wherein said medical device is an implantable device.

123. The process of claim 121, wherein said medical device is a tampon.

124. The process of claim 121, wherein said medical device comprises a polymeric material.

125. The process of claim 118, wherein said article is selected from the group consisting of a packaged article and a bare (unpackaged) article.

126. The process of claim 125, wherein said packaged article comprises a gas-permeable package.

127. An article having a gaseous nitric oxide sequestered therewithin, prepared by the process of claim 118.

128. The article of claim 127, further comprising an enclosure.

129. The article of claim 127, comprising at least 1 ppm per cm$^3$ nitric oxide sequestered there within.

130. The article of claim 127, wherein said sequestered nitric oxide is releasable in an aqueous solution during a time period that ranges from 1 hour to 1 month.

131. An article having sequestered therewithin at least 1 ppm nitric oxide per cm$^3$ and comprising less than 1 ppm per cm$^3$ nitrogen-containing and/or oxygen containing reactive species.

132. The article of claim 131, having sequestered therein from 1 ppm to 200 ppm per cm$^3$ nitric oxide.

133. The article of claim 131, wherein said sequestered nitric oxide is releasable in an aqueous solution during a time period that ranges from 1 hour to 1 month.

134. The article of claim 131, further comprising an enclosure.

135. The article of claim 127, being a medical device.

136. The article of claim 135, wherein said medical device is selected from the group consisting of an indwelling catheter and a tracheal tube.

137. The article of claim 135, wherein said medical device is a tampon.

138. A tampon having sequestered therein gaseous nitric oxide.
139. A process of preparing a tampon having sequestered therein nitric oxide, the process comprising:
exposing a tampon to gaseous nitric oxide-containing environment, thereby preparing the tampon having sequestered therein nitric oxide.

140. The process of claim 139, wherein said exposing comprises:
placing the tampon in a chamber; and
filling the chamber with said nitric oxide-containing environment.

141. The process of claim 140, further comprising, prior to said filling, generating a reduced pressure in said chamber.

142. A method of treating a vaginal medical condition in a subject in need thereof, the method comprising placing a tampon having gaseous nitric oxide sequestered therein in a vagina of the subject.

143. A method of treating a vaginal medical condition, the method comprising intravaginally administering to a subject in need thereof gaseous nitric oxide.

144. The method of claim 143, wherein said intravaginally administering gaseous nitric oxide comprises placing a tampon having gaseous nitric oxide sequestered therein.

145. A process of preparing a packaged article, wherein the packaged article comprises a gas-permeable package, the process comprising:
exposing a packaged article to a gaseous nitric oxide-containing environment, thereby preparing the packaged article.

146. The process of claim 145, wherein said exposing comprises:
placing said packaged article in a chamber; and
filling the chamber with said nitric oxide-containing environment.

147. The process of claim 146, further comprising, prior to said filling, sealing said chamber.

148. The process of claim 146, further comprising, prior to said filling, generating a reduced pressure in said chamber.

149. The process of claim 145, wherein the packaged article has gaseous nitric oxide sequestered within the article.

150. A process of preparing a packaged article, wherein the packaged article comprises a non-gas-permeable enclosure, the process comprising:
positioning an intact article within said non-gas-permeable enclosure, to thereby obtain a non-gas-permeable enclosure having said article disposed therewithin;
exposing said enclosure with a gaseous nitric oxide-containing environment, so as to introduce into said enclosure said nitric oxide-containing environment; and
sealing said enclosure, thereby preparing the packaged article.

151. The process of claim 150, wherein said exposing comprises:
placing said enclosure in a chamber; and
filling said chamber with said gaseous nitric oxide-containing environment.

152. The process of claim 151, further comprising, prior to said filling, sealing said chamber.

153. The process of claim 152, further comprising, prior to said filling, reducing a pressure in said chamber.

154. The process of claim 150, wherein the packaged article has gaseous nitric oxide sequestered within the article.

155. A packaged article prepared by the process of claim 145.

156. A packaged article prepared by the process of claim 150.

157. A package comprising:
a material configured to form an enclosure;
a article disposed within the enclosure; and
a gaseous nitric oxide-containing environment within said enclosure.

158. The package of claim 157, wherein said package is a non-gas-permeable package.

159. The package of claim 157, wherein said enclosure is a sealed enclosure.

160. The package of claim 157, wherein said environment is an ambient environment.

161. The package of claim 157, wherein said environment comprises gaseous nitric oxide in an amount sufficient to sterilize said article and an interior of said enclosure.

162. The package of claim 157, wherein said article has gaseous nitric oxide sequestered therewithin.

163. The package of claim 162, wherein said gaseous nitric oxide sequestered in said article is releasable in an aqueous solution during at least 1 minute.

164. A charging device comprising:
a chamber comprising an inlet for receiving a gaseous nitric-oxide-containing environment and an outlet for releasing said gaseous nitric-oxide-containing environment; and
an article disposed within the chamber.

165. The device of claim 164, further comprising an outlet for generating a reduced pressure in said chamber.

166. A charging device comprising:
a sealed chamber having a reduced pressure therewithin; and
an article disposed within the chamber.

167. The device of claim 166, wherein the chamber further comprises an outlet for generating said negative pressure within said chamber.

168. The device of claim 167, further comprising an inlet configured for receiving a gaseous nitric oxide-containing environment and an outlet for releasing said gaseous nitric oxide-containing environment.