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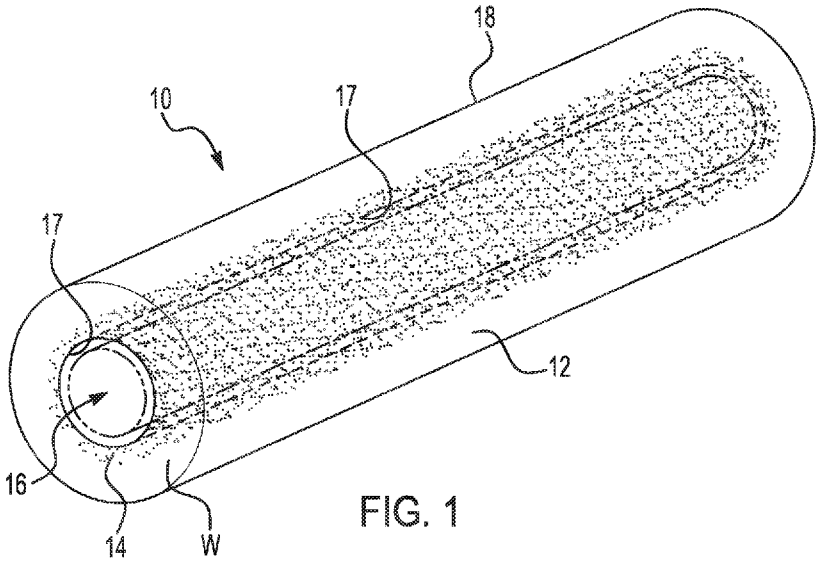

FIG. 1

(57) Abstract: In an example method, a solution is introduced to an interior opening of a nitric oxide permeable polymeric object. The solution includes a solvent and *S*-nitroso-1-adamantanethiol dissolved in the solvent. The solution is allowed to soak in the interior opening of the nitric oxide permeable polymeric object for a time up to 12 hours. The solution is then removed from the interior opening of the nitric oxide permeable polymeric object.

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NITRIC OXIDE GENERATING DEVICES AND METHODS OF MAKING THE SAME

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application Serial Number 63/521,207, filed June 15, 2023, the content of which is incorporated by reference herein in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] This invention was made with government support under EB024038 and HL155100 awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND

[0003] Nitric oxide (NO) is an endogenous gas molecule that has been shown to have several important physiological functions, including its unique vasodilating properties, wound healing properties, angiogenesis promoting properties, cancer-fighting potency, anti-platelet activity, and anti-microbial/anti-viral activity. In some instances, NO can be used to control infection, prevent biofilm formation, and minimize inflammation and fibrosis.

BRIEF DESCRIPTION OF THE DRAWINGS

[0004] Features of examples of the present disclosure will become apparent by reference to the following detailed description and drawings, in which like reference numerals correspond to similar, though perhaps not identical, components. For the sake of brevity, reference numerals or features having a previously described function may or may not be described in connection with other drawings in which they appear.

[0005] Fig. 1 is a schematic and perspective illustration of one example of a nitric oxide generating device, namely an extracorporeal circuit tubing, including S-

nitroso-1-adamantanethiol (SNAT) impregnated into a portion of the wall adjacent to an interior opening of the tubing;

[0006] Fig. 2A is a schematic illustration of a nitric oxide generating device, namely a Y connector for an extracorporeal blood circuit;

[0007] Fig. 2B is a cross-sectional view taken along line 2B-2B of Fig. 2A, schematically depicting S-nitroso-1-adamantanethiol (SNAT) impregnated into a portion of the wall adjacent to the interior opening of the Y connector and an optional coating of a direct thrombin inhibitor attached to a surface of the interior opening;

[0008] Fig. 2C schematically depicts an example of the direct thrombin inhibitor coating on a surface of the interior opening;

[0009] Fig. 3A is a black and white reproduction of a photograph of a portion of a control tubing;

[0010] Fig. 3B is a black and white reproduction of a photograph of a portion of a SNAT semi-impregnated tubing;

[0011] Fig. 4 is a graph depicting the nitric oxide (NO) flux (Y axis, $x10^{-10}$ $mol*min^{-1}*cm^{-2}$) versus the time (X axis, days) for the SNAT semi-impregnated tubing;

[0012] Fig. 5 depicts microscopic images (reproduced in black and white) of the performance of the control tubing and the SNAT semi-impregnated tubing on biofilm formation using *S.aureus and P.aeruginosa* strains;

[0013] Fig. 6 is a schematic illustration of an experimental setup for *in vivo* tests described herein in the Example section;

[0014] Fig. 7A is a photograph, reproduced in black and white, of the interior of the SNAT semi-impregnated tubing after 4 hours *in vivo* testing;

[0015] Fig. 7B is a photograph, reproduced in black and white, of the interior of the control tubing after 4 hours of *in vivo* testing;

[0016] Fig. 8 is a bar graph depicting the platelet count (Y axis, % of baseline) for SNAT semi-impregnated tubings and control tubings after being exposed on the extracorporeal circuit for different time periods (X axis, minutes);

[0017] Fig. 9 depicts several field-emission scanning electron microscope images (reproduced in black and white) of control (unmodified) polymers (row A),

example SNAT doped polymers (row B), and comparative *S*-nitroso-*N*-acetylpenicillamine (SNAP) doped polymers (row C);

[0018] Fig. 10 is a bar graph depicting the nitric oxide (NO) flux (Y axis, $x10^{-10}$ $mol*min^{-1}*cm^{-2}$) versus the time (X axis, days) for two different SNAT fully-impregnated tubings;

[0019] Fig. 11 is a bar graph depicting the 1-year stability of SNAT fully-impregnated tubings exposed to different storage temperatures, where the stability is shown in terms of nitric oxide (NO) flux (Y axis, $x10^{-10}$ $mol*min^{-1}*cm^{-2}$) versus the time (X axis, 1year + number of days 0, 1, or 2);

[0020] Fig. 12 is a bar graph depicting the stability of control tubings and SNAT fully-impregnated tubings exposed to different sterilization conditions, where the stability is shown in terms of nitric oxide (NO) flux (Y axis, $x10^{-10}$ $mol*min^{-1}*cm^{-2}$) versus the time (X axis, days);

[0021] Fig. 13A is a bar graph depicting the platelet aggregation (Y axis, %) for SNAT coated tubings and control tubings after being exposed on the extracorporeal circuit for different time periods (X axis, minutes);

[0022] Fig. 13B is a bar graph depicting the extracorporeal circuit thrombus area (Y axis, $pixels/cm^2$) for SNAT coated tubings and control tubings exposed to 4 hours of extracorporeal circuit flow;

[0023] Fig. 14A is a bar graph depicting the platelet aggregation (Y axis, %) for SNAT fully impregnated tubings and control tubings after being exposed on the extracorporeal circuit for different time periods (X axis, minutes);

[0024] Fig. 14B is a bar graph depicting the extracorporeal circuit thrombus area (Y axis, $pixels/cm^2$) for SNAT fully-impregnated tubings and control tubings exposed to 4 hours of extracorporeal circuit flow;

[0025] Fig. 15 depicts confocal images (reproduced in black and white) of the performance of the control tubing and the SNAT fully-impregnated tubing on biofilm formation using *S.aureus and E.coli* strains; and

[0026] Fig. 16 is a graph depicting the nitric oxide (NO) flux (Y axis, $x10^{-10}$ $mol*min^{-1}*cm^{-2}$) versus the time (X axis, days) for another example SNAT semi-impregnated tubing (n = 3).

DETAILED DESCRIPTION

[0027] Nitric oxide (NO) donor molecules have been incorporated into coatings on polymer objects, or have been impregnated into polymer objects. Example polymer objects include extracorporeal circuit components, such as tubing, catheters (e.g., hemodialysis catheters), and/or connectors, other catheters (e.g., urinary catheters, angiocatheters), pump chambers, or the like. The NO emitting polymer objects have been used in several biomedical applications, including extracorporeal circuits, to decrease thrombus formation, platelet aggregation, and to prevent bacterial infections, and biofilm formation.

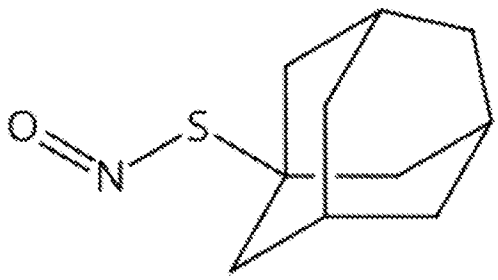
[0028] The present inventors have found that some NO donor molecules, such as diazeniumdiolate NO donors and *S*-nitrosothiol type NO donors, may be limited in terms of use and/or lead to undesirable results. For one example, diazeniumdiolate NO donors (e.g., DBHD-N_2O_2) involve use limitations because they cannot be impregnated, or are difficult to impregnate into a host material. For another example, diazeniumdiolate NO donors coated on extracorporeal circuit tubing can be contaminated with carcinogenic nitrosamines. For another example, *S*-nitrosothiol type NO donors (e.g., *S*-nitroso-*N*-acetylpenicillamine (SNAP)) coated on or impregnated within polymer objects can unfavorably modify the surface roughness and/or the mechanical properties of the polymer objects. The solubility of *S*-nitrosothiol type NO donors is also limited. As such, with impregnated *S*-nitrosothiol type NO donors, mechanical property alteration may be due to the material re-crystallizing after impregnation.

[0029] The present inventors have also found that some NO donor introduction techniques, such as coating techniques (e.g., dip coating, spin coating, or the like) or full impregnation, may also lead to undesirable results. Coating techniques may lead to surface roughness, undesirable increases in tubing (or other component) thickness, and/or peeling. Full impregnation can compromise the mechanical integrity of the tubing (or other component) and may require a significant amount of the NO donor molecule.

[0030] The examples disclosed herein utilize a tertiary nitrosothiol bearing an adamantane molecule (S-nitroso-1-adamantanethiol or SNAT) as the NO donor molecule and also utilize a method which semi-impregnates the polymer object with SNAT. By "semi-impregnation," it is meant that a portion of the polymer object that is exposed to the SNAT containing solution becomes impregnated, while other portions of the polymer object remain free of the SNAT. The resulting NO generating object provides a desirable level of NO (e.g., a minimum of 0.5 flux units to 4 flux units) for a desirable time (e.g., ranging from weeks to months) without compromising the mechanical integrity of the polymer object.

[0031] In some examples, the semi-impregnated polymer object also includes a direct thrombin inhibitor linked polymer coating on the surface(s) of the polymer object that will be exposed to blood or other fluid. The direct thrombin inhibitor linked polymer coating can aid in anticoagulation. As two examples, the direct thrombin inhibitor may be bivalirudin or argatroban.

[0032] Adamantane moieties increase the lipophilicity of drug molecules. The simplest tertiary *S*-nitrosothiol with adamantane is *S*-nitroso-1-adamantanethiol (SNAT, thionitrous acid (HNOS), S-tricyclo[3.3.1.13,7]dec-1-yl ester):



In an example, the *S*-nitroso-1-adamantanethiol is a tertiary thiol having a molecular weight ranging from about 195 Daltons to about 350 Daltons and a predicted n-octanol-water partition ratio over 5 (e.g., 5.9). This molecule is capable of spontaneous release of NO when an interior opening of the polymer object (in which SNAT is semi-impregnated) is exposed to solutions and/or blood under physiological conditions. In one specific example, the NO generating device exhibits antimicrobial and bacterial killing effects (with a minimum of a 2 log reduction in bacteria CFU/mL)

for at least 7 days. In another specific example, the NO generating device is able to continuously release a sufficient amount of NO within or above the normal endothelial range (0.5-4.0 x 10^{-10} mol min^{-1} cm^{-2}) for over a month (e.g., about 35 days).

[0033] In the examples disclosed herein, this molecule is used as a hydrophobic NO donor for creating biocompatible surfaces with antithrombotic and antimicrobial properties. In particular, the SNAT provides desirable surface anticoagulation within the interior opening of the polymer object in which it is semi-impregnated. This may obviate the need for systemic anticoagulation. It is believed that SNAT may be useful for modifying all of the extracorporeal circuit components and may be utilized in applications such as hemodialysis, hemofiltration, cardiopulmonary bypass (CPB), cardiac pacing lead, urinary catheters, and artificial lung housing. Additionally, the SNAT polymer's NO releasing capacity provides an anti-thrombogenic effect by inhibiting platelet activation and platelet adhesion (aggregation) to the surface of the interior opening (as exhibited by the *in vivo* models in the Example section).

[0034] SNAT is very lipophilic (logD=5.9), and thus it is less prone (e.g., compared to other NO donors such as SNAP or diazeniumdiolates) to leach from a lipophilic polymer into the aqueous media and/or blood. SNAT is also pH independent, which is unlike other NO donors (thiol: *S*-Nitroso-N-acetylpenicillamine (SNAP), *S*-Nitrosoglutathione (GSNO), or diamine: DBHD-N_2O_2). Further, SNAT is highly soluble in the solvent or solvent system disclosed herein, thus simplifying the semi-impregnation process. After being semi-impregnated, SNAT remains dissolved within the polymer phase, thus avoiding crystal formation and enabling the polymer object to preserve its mechanical properties. The solubilized SNAT also enables higher payloads within the polymer object compared to an NO donor that crystalizes. Additionally, SNAT provides relatively continuous release of NO (i.e., no lag time upon exposure to solutions and/or blood under physiological conditions). SNAT also does not contain amine functional groups, thus eliminating the formation of carcinogenic nitrosamines. Thus, SNAT semi-impregnated polymer objects may exhibit reduced cytotoxicity compared to other polymer objects modified for NO generation.

[0035] Example NO generating devices 10, 10' are shown in Fig. 1 and Fig. 2A. The devices 10, 10' generally include a polymer object 12 that has an interior opening

16 and SNAT 14 semi-impregnated in a portion of the polymer object 12 adjacent to a surface 17 of the interior opening 16.

[0036] In each example, the nitric oxide permeable polymeric object 12 is formed of a polymer that is capable of releasing NO generated from SNAT, i.e., the NO donor in the examples disclosed herein. In one example, the nitric oxide permeable polymeric object 12 is made of poly(vinyl chloride) (PVC, medical or non-medical grade), silicone, polyurethane, combinations of silicone and polyurethane, polycarbonate, polypropylene, or polytetrafluoroethylene. The polymeric object 12 may also include a plasticizer, such as Tris (2-Ethylhexyl) Trimellitate (i.e., trioctyl trimellitate (TOTM), a phthalate-free plasticizer) or a bio-based plasticizer. Bio-based plasticizers may be any plasticizer that is derived from biomass resources, such as vegetable oil, cardanol, vegetable fatty acid, glycerol, and/or citric acid. These plasticizers have been widely studied to replace petroleum-based o-phthalate plasticizers. As one example, a PVC tubing which includes the plasticizer is commercially available under the tradename TYGON S3™ E-3603 (from Saint-Gobain Performance Plastics Corp.). As other examples, silicone, polyurethane, polycarbonate, and polypropylene may not include the plasticizer.

[0037] The polymer object 12 may be any medical device that has an interior opening 16 that will, in use, come in contact with blood or another bodily fluid, such as urine. As examples, the polymeric object 12 may be any extracorporeal circuit component, such as tubing (schematically shown in Fig. 1), catheters, and/or connectors (schematically shown in Fig. 2A), other catheters, pump chambers, or any other medical device that would benefit from nitric oxide generation at its interior opening 16 during its use. The interior opening 16 may be the inner lumen of the tubing or catheter or connector, the interior of the pump chamber, or the like.

[0038] One example of the NO generating device 10 is shown in Fig. 1. This example is an extracorporeal circuit tubing. As shown in Fig. 1, the NO generating device 10 includes the nitric oxide permeable polymeric object 12; and S-nitroso-1-adamantanethiol (SNAT) 14 impregnated in a portion of a wall W of the nitric oxide permeable polymeric object 12 that is adjacent to an interior opening 16 of the nitric oxide permeable polymeric object 12, wherein an exterior surface 18 of the wall W of

the nitric oxide permeable polymeric object 12 is substantially free of the S-nitroso-1-adamantanethiol 14. By "substantially free," it is meant that less than 1% of the SNAT 14 (that is semi-impregnated) is present at the exterior surface 18 of the polymeric object 12. In some examples, no SNAT 14 is present within the portion of wall W of the polymeric object 12 that is adjacent the exterior surface 18 of the wall W. The phrases "at the exterior surface 18" and "within the portion of wall W of the polymeric object 12 that is adjacent the exterior surface 18" include both the exterior surface 18 and a distance measured from the exterior surface 18 that extends into the wall thickness. In one example, from about 25% to about 80% of the wall W thickness (as measured from the exterior surface 18) is substantially free of the SNAT 14. As such, the portion of the wall W that is substantially free of the SNAT 14 will depend upon the wall thickness. In one specific example, when the wall thickness is 1/16 of an inch (1,587.5 μm), there is no SNAT 14 in the wall W within 500 μm of the exterior surface 18. In this particular example, about 31% of the wall W (measured inward from the exterior surface 18) is free of SNAT 14.

[0039] The SNAT 14 is semi-impregnated using the method disclosed herein, and thus the SNAT 14 is located within a portion of the wall W of the nitric oxide permeable polymeric object 12 that is adjacent to its interior opening 16, and is not at the exterior surface 18 of the wall W (as described above). The SNAT 14 is positioned near the surface 17 that is adjacent to the interior opening 16. The phrase "near the surface 17" means the SNAT 14 is present at the interior surface 17 and a distance measured from the surface 17 that extends into the wall thickness. As described above, this distance is less than the total thickness of the wall W, as a portion at the exterior surface 18 is substantially free of SNAT 14. In one example, from about 20% to about 75% of the wall W thickness (as measured from the interior surface 17) includes the SNAT 14. In one example, the wall W has a thickness of about 3/32 of an inch (about 2,381 μm) and the SNAT 14 extends from about 500 μm to about 1000 μm into the wall W from the interior surface 17. The extent to which the SNAT 14 semi-impregnates may depend upon the thickness of the wall W. Thinner walls may have a smaller impregnation distance, while thicker walls may have a larger impregnation distance (measure from the interior surface 17).

[0040] As noted, the *S*-nitroso-1-adamantanethiol remains dissolved in the polymer phase, and thus the NO generating device 10 does not include solid *S*-nitroso-1-adamantanethiol.

[0041] As shown in phantom in Fig. 1, the interior surface 17 may also be coated with a direct thrombin inhibitor linked polymer 20. The direct thrombin inhibitor linked polymer 20 will be described further in reference to Fig. 2C.

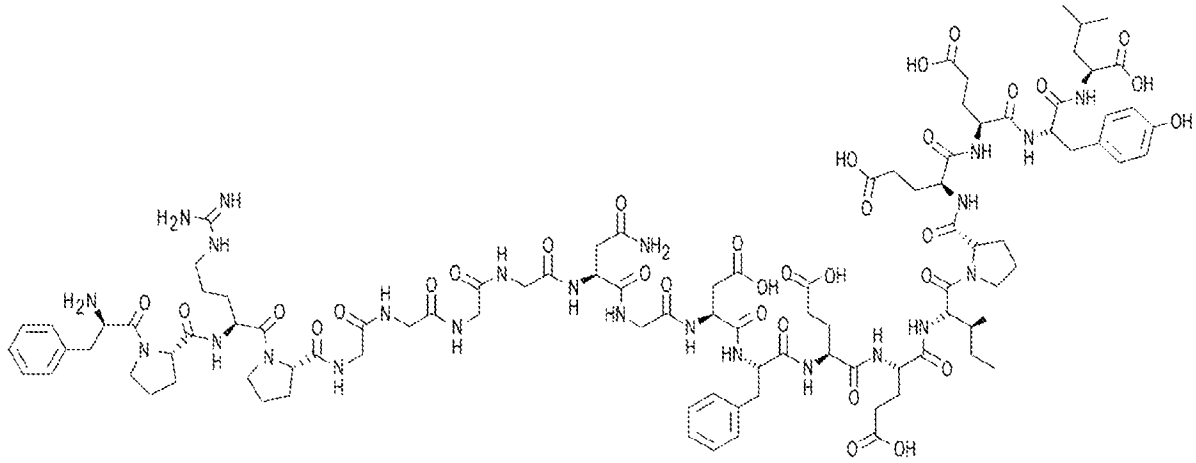
[0042] Another example of the NO generating device 10' is shown in Fig. 2A. This example is an extracorporeal circuit Y connector. As shown in Fig. 2A and the cross-section of Fig. 2B, the NO generating device 10' includes the nitric oxide permeable polymeric object 12; and S-nitroso-1-adamantanethiol (SNAT) 14 impregnated in a portion of the wall W of the nitric oxide permeable polymeric object 12 that is adjacent to the interior opening 16 of the nitric oxide permeable polymeric object 12. Similar to Fig. 1, the exterior surface 18 of the wall W of the nitric oxide permeable polymeric object 12 is substantially free of the S-nitroso-1-adamantanethiol 14.

[0043] As shown in phantom in Fig. 2B, the interior surface 17 may also be coated with the direct thrombin inhibitor linked polymer 20.

[0044] Referring now to Fig. 2C, an example of the direct thrombin inhibitor linked polymer 20 is schematically depicted. The direct thrombin inhibitor linked polymer 20 includes an anchor polymer 22, linking molecules 24 attached to the anchor polymer 22, and direct thrombin inhibitor molecules 26 attached to the linking molecules 24. Any anchor polymer 22 that is capable of forming a coating on the interior surface 17 of a base polymer (i.e., the polymer object 12) and that includes surface functional groups to attach to the linking molecules 24 may be used. Similarly, any linking molecules 24 that include functional groups to attach to the anchor polymer 22 and to the direct thrombin inhibitor molecules 26 may be used.

[0045] In one example, the anchor polymer 22 is a polyurethane or a polyurethane copolymer (e.g., CARBOSIL® - a copolymer of silicone and polycarbonate-urethane – owned by DSM IP Assets), and the linking molecule is 4,4'-Methylene-bis(cyclohexyl isocyanate) (HMDI) or polyethylene glycol (PEG). In one

example, the direct thrombin inhibitor molecule 26 is bivalirudin, which has the structure:



, and the guanidine group on the N-(amino) terminal may be used as an anchorage point to an HMDI linking molecule, via the linker's isocyanate group. The direct thrombin inhibitor molecule 26 may be 100% pure bivalirudin or its trifluoroacetate salt (Bivalirudin TFA). As other examples, the direct thrombin inhibitor may be pirudin, desirudin, and argatroban. Other anchor polymers 22 include poly(vinyl chloride), silicone, or polyurethane, or the combinations of any of the listed polymers. The linking molecules 24 are then selected to attach to the particular anchor polymer 22.

[0046] The semi-impregnation method disclosed herein includes introducing a solution to an interior opening 16 of a nitric oxide permeable polymeric object 12, the solution including a solvent, and *S*-nitroso-1-adamantanethiol 14 dissolved in the solvent; allowing the solution to soak in the interior opening 16 for a time up to 12 hours; and removing the solution from the interior opening 16 of the nitric oxide permeable polymeric object 12.

[0047] SNAT 14 is apolar, and thus is hydrophobic. The solvent or solvent system that is selected should dissolve the SNAT 14. In some instances, the solvent that is selected may also be apolar. In some examples, the solvent is used alone (i.e., without any other solvents or a plasticizer). In other examples, the solvent is part of a solvent system that includes one or more other solvents. When a combination of solvents is used, one of the solvents that is selected is more apolar than the other of the solvents. For example, acetone may be selected with any of methanol, ethyl

acetate, methyl ethyl ketone, tetrahydrofuran, chloroform, or cyclohexane. In still other examples, the solvent is part of a solvent system that includes a plasticizer. In still further examples, the solvent is used in combination with another solvent and a plasticizer.

[0048] The solvent(s) used in the semi-impregnation method can dissolve the SNAT but cannot dissolve the polymer object 12. The solvent(s) can, however, permeate into the polymer object 12. The solvent is selected from the group consisting of acetone, methanol, ethyl acetate, methyl ethyl ketone, tetrahydrofuran, chloroform, cyclohexane, and ethanol.

[0049] In one example of a single solvent system, the nitric oxide permeable polymeric object 12 is made of silicone, and the solvent is tetrahydrofuran.

[0050] When multiple solvents are used, the hydrophobicity and lipophilicity of the selected solvents will contribute to the volume ratio that is used. In one example, first and second solvents, which are different from each other, are used. The solvent and the second solvent are different and are independently selected from the group consisting of acetone, methanol, ethyl acetate, methyl ethyl ketone, tetrahydrofuran, chloroform, cyclohexane, and ethanol. In this example, the solvent system includes a predetermined volume ratio of the solvent and the second solvent, and the predetermined volume ratio of ranges from 0.1:10 to 10:0.1. In one specific example when two solvents are used, the volume ratio of the two solvents may range from 2:1 to 1:2. It is to be understood that any volume ratio between the broadest range may be used, such as 1:1, 1.5:1, 1:1.5, etc.

[0051] In one example of the two solvent system, the nitric oxide permeable polymeric object 12 is made of PVC, and the two solvents are acetone and methanol.

[0052] In another example of the two solvent system, the nitric oxide permeable polymeric object 12 is made of polyurethane, and the two solvents are methanol and chloroform. With polyurethane, the solvent(s) may be selected from the group consisting of methanol, methyl ethyl ketone, chloroform, cyclohexane, and combinations thereof.

[0053] In some examples, the solvent system further includes a plasticizer. The plasticizer is added when the NO permeable polymeric object 12 includes a plasticizer

as part of its composition (e.g., some PVC polymeric objects). The additional plasticizer in the solvent system can supplement plasticizer that leaches out from the NO permeable polymeric object 12 during the semi-impregnation method. The minimum value for the plasticizer in the predetermined volume ratio is ≥0.1 to ensure that the overall mechanical properties of the NO permeable polymeric object 12 are substantially unchanged after the semi-impregnation process is performed. When the plasticizer is added to the solvent(s), the predetermined volume ratio is a volume ratio of a first solvent : plasticizer : a second solvent. As one example, this predetermined volume ratio is from 0.1 to 10 of the first solvent : from 0.1 to 10 of the plasticizer : from 0.1 to 10 of the second solvent. As specific examples, this volume ratio may be 1:3:1, 1.5:2:1.5, 1:2:2, 2:1:2, or 2:2:1. Ratios between the broadest volume ratios may also be used. Still other volume ratios may be used, as long as they are able to dissolve the selected amount of SNAT. While volume ratios are set forth herein, it is to be understood that the given ratios are suitable for weight ratios as well.

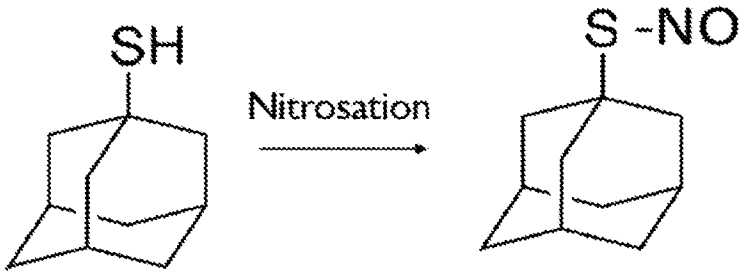
[0054] Due, in part, to SNAT's very high lipophilicity, the amount of SNAT included in the solution is application dependent. In general, the solution includes up to 2000 mg/mL of *S*-nitroso-1-adamantanethiol. For polymeric objects 12 having a wall W that is 1/16" (1,587.5 μm) thick, the concentration of *S*-nitroso-1-adamantanethiol in the solution may be 1000 mg/mL. For a thinner wall W, the *S*-nitroso-1-adamantanethiol concentration may be less than 1000 mg/mL (e.g., from 50 mg/mL to 900 mg/mL). For a thicker wall W, the *S*-nitroso-1-adamantanethiol concentration may be greater than 1000 mg/mL (e.g., from greater than 1000 mg/mL up to 2000 mg/mL).

[0055] After introducing the solution into the interior opening 16 of the nitric oxide permeable polymeric object 12, the interior opening 16 may be sealed for the incubation period. The incubation period is up to 12 hours. In some instances, the incubation period is less than 12 hours. In one example, the incubation period ranges from 1 hour to about 8 hours. As a specific example, when the wall W thickness is about 0.6 mm (600 μm), the incubation period ranges from about 30 minutes to about 2.5 hours. In another example, the incubation period is 4 hours (which is particularly

desirable for maintaining the mechanical properties of the polymer object having a wall W thickness of about 1.5875 mm (1,587.5 μm).

[0056] After the incubation period has expired, the solution is removed from the interior opening 16. When the polymeric object 12 is transparent or translucent, the SNAT impregnated portion of the polymeric object 12 will appear green.

[0057] Prior to introducing the solution into the interior opening 16, the method may further include generating the solution by dissolving the *S*-nitroso-1-adamantanethiol in the solvent system. Prior to generating the solution, the method further comprises generating the *S*-nitroso-1-adamantanethiol by exposing 1-adamantanethiol to nitrosation:



In the examples disclosed herein, the nitrosation process is performed as a solvent-free nitrosation with t-butyl nitrite (i.e., *tert*-butylnitrite ($tBuNO_2$)). The t-butyl nitrite acts as both the solvent and the reagent in the nitrosation process, and thus is considered to be solvent-free. The solvent-free process avoids solvents that can otherwise deleteriously affect the stability of the SNAT. During the method, 1-adamantanethiol is mixed with a desired amount (e.g., 1.1 molar equivalent) of the tert-butylnitrite, and the two are allowed to react. Initially stirring may be performed, and then the reaction vessel is kept on ice for a predetermined time period (e.g., 30 minutes) because the reaction is exothermic. The reaction may then be allowed to perform at room temperature (e.g., 18°C to 21°C) for the desired time period up to 12 hours. Following the reaction, excess tert-butylnitrite and the side product tert-butanol (t-BuOH) are removed, for example, using a rotary at room temperature. This may be followed by the vacuum drying of the product overnight.

[0058] It is to be understood that the solvent-free nitrosation used to form the SNAT may be performed along with the semi-impregnation method, or as a

standalone method to generate the SNAT. In the latter instance, the solvent-free nitrosation is not performed in conjunction with the semi-impregnation method disclosed herein.

[0059] As a result of the semi-impregnation method, the *S*-nitroso-1-adamantanethiol 14 extends partially into the wall W from the interior surface 17 as described herein. In one example, the SNAT 14 is present at the interior surface 17 and then extends from about 500 μm to about 1000 μm into the wall W. In other words, the *S*-nitroso-1-adamantanethiol is present at the interior surface 17 of the interior opening 16 and extends a certain distance into the wall thickness, where this distance is less than the total thickness as described herein. The remainder of the wall thickness extending out to the exterior surface 18 is substantially free of the *S*-nitroso-1-adamantanethiol.

[0060] Some examples of the method further include the incorporation of the direct thrombin inhibitor linked polymer 20 as a coting on the interior surface 17. The direct thrombin inhibitor linked polymer 20 may be formed before it is applied to the interior surface 17.

[0061] In one example method, the direct thrombin inhibitor linked polymer 20 may be formed by dissolving the anchor polymer 22 in a solvent; introducing the linking molecule 24 to the solvent, whereby the linking molecule 24 covalently attaches to the anchor polymer 22; introducing a direct thrombin inhibitor 26 to the solvent, whereby the direct thrombin inhibitor 26 covalently attaches to the linking molecule 24 attached to the anchor polymer 22 to form the direct thrombin inhibitor linked polymer 20. Any examples of the anchor polymer 22 and linking molecule 24 set forth herein may be used, and the solvent that is selected is capable of dissolving each of the components 22, 24, 26. In the example method, precipitation, drying, and redissolution may be performed between the reactions. For example, after the linking molecule 24 is attached to the anchor polymer 22, precipitation may be performed with hexane, and the solid product (e.g., pellets) may be dried and redissolved in fresh solvent before the direct thrombin inhibitor 26 is introduced. Then, after the direct thrombin inhibitor 26 is introduced, the reaction may be allowed to proceed for a predetermined amount of time (e.g., 20 minutes to 40 minutes), and then precipitation

may be performed with water. This solid product (e.g., pellets of the direct thrombin inhibitor linked polymer 20) may be dried and redissolved in fresh solvent. In one example, the final solution includes about 10 μM of the direct thrombin inhibitor linked polymer 20.

[0062] In one example of the method, the anchor polymer 22 is in the form of small polymer beads. In another example of the method, the solvent is tetrahydrofuran (THF).

[0063] Once the direct thrombin inhibitor linked polymer solution is formed, it is introduced into the interior opening 16. After a couple of seconds, the solution is drained from the interior opening 16. The solvent is allowed to evaporate, which occurs relatively rapidly and leaves a coating of the direct thrombin inhibitor linked polymer 20 on the surface 17 of the interior opening 16 (of the base polymer 12). It is to be understood that the direct thrombin inhibitor linked polymer 20 is permeable to NO, and thus does not inhibit the effects of the semi-impregnated SNAT 14.

[0064] To further illustrate the present disclosure, various examples are given herein. It is to be understood that these examples are provided for illustrative purposes and are not to be construed as limiting the scope of the present disclosure.

EXAMPLES

[0065] *Example 1*

[0066] At the outset, several examples of the NO generating polymer tubes disclosed herein were generated using Medical grade PVC TYGON™ ND-100-65 tubing (no topcoat, and 3/8" inner diameter) and SNAT. Two different solutions were tested, along with two different semi-impregnation times (4 hours or 12 hours). The solvent systems of the solutions included acetone:plasticizer:methanol at ratios of 1:3:1 and 2:1:2. Each solution included 1000 mg/mL of SNAT, and the plasticizer was Tris (2-Ethylhexyl) Trimellitate (TOTM).

[0067] The example tubes were filled with the respective solutions, sealed, and allowed to incubate for 4 hours or 12 hours.

[0068] For comparison of the mechanical properties, several control polymer tubes were prepared according to Table 1. These controls were filled with the different

solvent systems (without SNAT) for the semi-impregnation times. The number of each type test is represented by "n" in Table 1). Young's Modulus was measured, and the average results for the control polymer tubes are also shown in Table 1 (where SEM is the standard error mean, *p<0.05). The first naïve control value was supplied by the manufacturer. The second naïve control value represents the measured value of the as-received tubing without any solvent exposure.

TABLE 1

Sample Type	Average Young's Modulus (MPa ± SEM)
Naïve Control (industrial, min)	5.6
Naïve Control (lab)	6.0±0.2 (n = 6)
1:3:1 (4h) solvent control	4.7±0.2 (n = 8)
1:3:1 (12h) solvent control	2.8±0.1 (n = 7)
2:1:2 (4h) solvent control	3.9±0.3 (n = 6)
2:1:2 (12h) solvent control	2.1±0.1 (n = 3)

[0069] These results demonstrate the effect of swelling time and solvent volume ratios on the mechanical properties of the tubing without the added SNAT. The longer incubation period had more of an effect on the Young's modulus, and may be more desirable when thicker polymer objects are utilized.

[0070] Young's Modulus of one example polymer tube (solution containing 1:3:1 solvent system and SNAT, incubated for 4 hours) was also measured, and the average results are also shown in Table 2 (where SEM is the standard error mean, *p<0.05). Table 2 also reiterates some of the controls from Table 1 for ease of comparison.

TABLE 2

Sample Type	Average Young's Modulus (MPa ± SEM)
Naïve Control (industrial, min)	5.6
Naïve Control (lab)	6.0±0.2 (n = 6)
1:3:1 (4h) solvent control	4.7±0.2 (n = 8)
1:3:1 (4h) SNAT	5.4±0.2 (n=3)

[0071] These results indicate that SNAT semi-impregnation did not deleteriously affect the polymer tube's mechanical properties.

[0072] Additional example NO generating polymer tubes were generated using the Medical grade PVC TYGON™ ND-100-65 tubing (no topcoat, and 3/8" inner diameter) and SNAT. The two different solutions (acetone:TOTM:methanol at volume ratios of 1:3:1 and 2:1:2) were tested at a semi-impregnation time of 4 hours. As above, each solution included 1000 mg/mL of SNAT. The tubes were filled with the respective solutions, sealed, and allowed to incubate for 4 hours.

[0073] After the incubation period, the solutions were emptied. A portion of one naïve control sample and one of the additional example samples were photographed, and the black and white reproductions are respectively shown in Fig. 3A and Fig. 3B. The naïve control sample remained transparent, while the example samples included a green portion (depicted as the darker black portion in Fig. 2B). The green in the example tubing was evidence of the semi-impregnation of the SNAT.

[0074] The tubes were tested, *in vitro*, compared to the naïve control (ECC) (Medical grade PVC TYGON™ ND-100-65 tubing (no solution exposure, no topcoat, and 3/8" inner diameter)) for NO donor loading, NO release profile (measured using ozone chemiluminescence, see Fig. 4)), and tensile strength test (measured using a texture analyzer). The antibacterial properties of the example NO generating polymer tubes were also assessed for 7 days using Gram+ (*S. aureus*) and Gram- (*P. aeruginosa*) bacteria strains (see Fig. 5). The tubing was filled with the respective bacteria and was allowed to incubate for 7 days, after which, the bacteria was removed, and the samples were tested using a CDC closed container. All of these results are summarized in Table 3.

TABLE 3

Groups	Loading (%)	NO flux (day32) ($x10^{-10}$ mol/min/cm^2)	Young's (Tensile) modulus (MPa)	Biofilm test (CFU/mL)	
				P.aeruginosa	***S.aureus***
2:1:2 SNAT	11.7%± 0.9* (n=5)	3.3±0.7* (n=3)	3.8±0.2* (n=3)	2E+04±1E+03 * (n=3)	1E+05±1E+05* (n=3)
1:3:1 SNAT	5.1%± 0.4 (n=6)	0.4±0.1 (n=3)	5.4±0.2 (n=3)	8E+05±2E+05 * (n=3)	2E+07±6E+06 (n=3)
Naïve control	-	-	6.0±0.2 (n=6)	1E+07±3E+06 (n=3)	1E+08±6E+07 (n=3)
*p<=0.05 (CI=95%); Data±Standard Error Mean (SEM), n=replicates, CFU=colony forming unit, MPa=N/mm^2,					

[0075] With the semi-impregnation method, an estimated 50% less NO donor was consumed compared to full impregnation, making the method disclosed herein more economical. The 2:1:2 solvent combination yielded significantly higher loading when compared to the 1:3:1 solvent combination ($p \leq 0.05$, Table 3, day 35).

[0076] The tensile strength of the example 1:3:1 group was the closest to the naïve control ($p \geq 0.05$, Table1) and maintained the original properties better than the example 2:1:2 group (Table 3). The example 2:1:2 group had overall higher NO-releasing capacity when comparing values on day 35 of NO release, but the example 1:3:1 group still showed sufficient NO flux (Table 3, Fig. 4).

[0077] The biofilm study also showed adequate antibacterial properties in both groups with about a 2-log reduction in bacterial colonies (Table 3, Fig. 5). The confocal images in Fig. 5 clearly illustrate more biofilm formation on the control examples than the example 1:3:1 group.

[0078] The anticoagulation properties of the example 1:3:1 group (n=3) were also tested *in vivo*, in an acute rabbit model and were compared to the naïve control (n=9). The *in vivo* study was a 4-hour long test. The experimental set up is shown in Fig. 6. An arterio-venous shunt was used in a rabbit. The extracorporeal circuit (ECC) loop with angiocath (16G) was introduced into the lower common carotid artery and

the extracorporeal circuit (ECC) loop with angiocath (14G) was introduced into the right jugular vein and the flow was from the lower common carotid artery through the thrombogenicity chamber and to the right jugular vein. In this experiment, less thrombus area: SNAT 0.5±0.4cm^2 versus the control 9.8±0.8 cm^2; and higher preservation of platelets 108% versus 74% of baseline, respectively, were observed at the end of the test (Fig. 8). Images (reproduced in black and white) of the example and control tubes after 4-hour long blood exposure are shown in Fig. 7A and Fig. 7B, respectively. The SNAT semi-impregnated tube was green, with a minor amount of thrombus. In contrast, the control tube appeared red due to all of the thrombus. Clearly, the SNAT semi-impregnated tube exhibits better anticoagulation properties.

[0079] The data in this example clearly illustrates that the SNAT semi-impregnation provides a more cost-efficient way of surface modification with suitable NO flux and with preserving the original mechanical characteristics of the polymer used. It also has excellent anti-thrombogenic properties without the need of systemic anticoagulation *in vivo*.

[0080] *Example 2*

[0081] At the outset, examples of the NO generating polymer tubes disclosed herein were generated using Medical grade PVC TYGON™ ND-100-65 tubing (no topcoat, and 3/8" inner diameter) and SNAT. One solution was tested. The solvent system of the solution included acetone:plasticizer:methanol at a volume ratio of 1:2:2. The solution included 1000 mg/mL of SNAT, and the plasticizer was Tris (2-Ethylhexyl) Trimellitate (TOTM).

[0082] The example tubes were filled with the solution, sealed, and allowed to incubate for 4 hours.

[0083] Young's Modulus of the control and example tubes was measured, and the average results for four control polymer tubes and four example tubes are shown in Table 4 (where SEM is the standard error mean, *$p<0.05$).

TABLE 4

Sample Type	Average Young's Modulus (MPa ± SEM)
Naïve Control	4.69±1.1 (n = 4)
1:2:2 (4h) SNAT	4.75±0.3 (n = 4)

[0084] Similar to the results in Example 1, these results demonstrate that SNAT semi-impregnation did not deleteriously affect the polymer tube's mechanical properties.

[0085] The example tubes were tested, *in vitro*, compared to the naïve control (ECC) (Medical grade PVC TYGON™ ND-100-65 tubing (no solution exposure, no topcoat, and 3/8" inner diameter)) for NO release profile (measured using ozone chemiluminescence, see Fig. 16)). The 1:2:2 solvent combination yielded desired NO flux (>0.5 x 10^{-10} mol/min/cm^2 flux) for over 14 days, and was at the threshold on day 21.

[0086] One of the example tubes was exposed to the bivalirudin linked polymer to create a coating on the interior surfaces. The bivalirudin linked polymer was prepared as described herein by dissolving CARBOSIL® beads into tetrahydrofuran, and then adding HMDI. The reaction product was precipitated using hexane, and the precipitate was dried. The precipitate was dissolved in fresh THF, and then the trifluoroacetate salt of bivalirudin or bivalirudin (100% pure) was added. The reaction was allowed to occur for about 20 minutes, and then the reaction product was precipitated using water. The precipitate was dried, and redissolved in fresh THF. The solution containing the bivalirudin linked polymer was added to the interior of the SNAT semi-impregnated example tubes, and the solvent was evaporated. The bivalirudin linked polymer coated the interior surface of the example tubes.

[0087] This example tube and one control tube were tested *in vivo* in rabbits as described in Example 1 and Fig. 6. After removal from the rabbits, no clot was observed in the SNAT semi-impregnated tube coated with the bivalirudin linked polymer. In contrast, 10.97±0.95 cm^2 (Data±Standard Deviation, n=7) in the naïve, untreated tubes.

[0088] These results illustrate the additional effect of the bivalirudin linked polymer with the semi-impregnated SNAT.

[0089] *Example 3*

[0090] Examples of the NO generating polymer angiocatheters were generated using polyurethane angiocatheters and SNAT. Two solutions were tested. The solvent system of each solution included methanol:chloroform at a volume ratio of 1:1. One solution included 1000 mg/mL of SNAT, and the other solution included 600 mg/mL of SNAT.

[0091] The example angiocatheters were filled with the solution, sealed, and allowed to incubate for 4 hours.

[0092] These example angiocatheters were tested, *in vitro*, for their NO release profile (measured using ozone chemiluminescence). The average results for three of each example are shown in Table 5.

TABLE 5

	SNAT 1000 in PU (n=3)	**SNAT 600 in PU (n=3)**
Day 0	59.99±35.97	33.4±24.24
Day 1	44.56±32.72	24.20±7.58
Day 4	25.94±10.89	10.17±1.59
Day 7	20.06±9.81	8.27±2.03
Day 14	3.71±1.31	2.24±0.98
Day 21	2.53±1.20	0.27±0.06
Day 31	0.79±0.42	---
Day 35	0.48±0.23	---

[0093] The examples prepared with 1000 mg/mL of SNAT exhibited desired NO flux (>0.5 x 10^{-10} mol/min/cm^2 flux) for over 31 days, and was at the threshold on day 35. The examples prepared with 600 mg/mL of SNAT exhibited desired NO flux (>0.5 x 10^{-10} mol/min/cm^2 flux) for over 14 days, and was under the threshold on day 21. These results illustrate the effectiveness of the semi-impregnation method in polyurethane objects.

[0094] *Example 4*

[0095] One NO generating polymer tubing was generated using a silicone tubing and SNAT. One solution including 70 mg/mL SNAT in tetrahydrofuran was tested.

[0096] The example silicone tubing was filled with the solution, sealed, and allowed to incubate for 4 hours.

[0097] This example tubing was tested, *in vitro*, for its NO release profile (measured using ozone chemiluminescence). The results are shown in Table 6.

TABLE 6

	SNAT in silicone
Day 0	4.8
Day 1	4.7
Day 2	5.1
Day 4	6.8
Day 7	2.3
Day 10	0.9
Day 14	0.3
Day 23	0.1

[0098] The example prepared with 70 mg/mL of SNAT in silicone exhibited near desirable NO flux (>0.5 x 10^{-10} mol/min/cm^2 flux) for 4 days. It is believed that these results would be improved with a higher SNAT loading. These results illustrate the effectiveness of the semi-impregnation method in a silicone object.

[0099] *Comparative Example*

[00100] The comparative examples used SNAT with a full impregnation technique or a coating technique. SNAT was synthesized as described herein, and extracorporeal circuits (PVC tubing (ND 100-65 TYGONT™, 3/8" ID) and connectors, and cannulas) were modified with the NO donor via full impregnation or a coating technique. The full impregnation technique involved fully immersing the soaking the extracorporeal circuits in a solution of the SNAT (1000 mg/mL) in a combination of

organic solvents and plasticizer (acetone : plasticizer : methanol, 1:3:1) until the circuits were swollen with the solution. The circuits were then dried. The coating technique involved dissolving the SNAT in a polymer solution and applying the solution to the inner surface of the ECC component and drying. SNAP was used as the NO donor in other full impregnation techniques. The control examples were the same as the naïve control of the Example.

[00101] To evaluate the surface roughness of the NO donor-doped polymers (SNAP, SNAT) and unmodified control polymers, a field-emission scanning electron microscope (JEOL JSM-7800F) was used. These images are shown in Fig. 9, where row A depicts the naïve control examples, row B depicts the SNAT fully impregnated PVC tubes (1000 mg/ml), and row C depicts the SNAP fully impregnated PVC tubes (250 mg/ml, maximum loading limit). The SNAT loaded PVC surface looked similar to, but more exaggerated than, the naïve control. The SNAP loaded PVC surface clearly showed differences.

[00102] The *in vitro* NO release profile of the SNAT (600 mg/ml and 1000 mg/ml) fully impregnated PVC circuit tubing was measured by a Nitric Oxide Analyzer (NOA). As shown in Fig. 10, the 1000 mg/ml SNAT impregnated PVC tubing demonstrated up to 49 days of endothelial levels of NO release capacity (Fig. 10).

[00103] The storage stability of the 1000 mg/ml SNAT impregnated PVC tubing was tested. The storage conditions were as follows: dry state; under air; different temperatures 21°C, 4°C, and -20°C; and a period of 1 year – where d0 marks the 1-year date of storage and d1 and d2 are subsequent days after the 1 year). The 1-year results (Fig. 11) illustrate that fairly stable storage stability is possible for the 1000 mg/ml SNAT impregnated PVC tubing at 4°C and -20°C.

[00104] The NO release capacity of the 1000 mg/ml impregnated SNAT polymer examples and the naïve controls were also tested after exposure to different sterilization techniques. Sterilization was performed with ethylene oxide (EtO), hydrogen peroxide (H_2O_2), and OPS liquid chemical (shown as PVC liquid). The results are shown in Fig. 12. These results illustrate that the OPS liquid chemical is the best sterilization liquid, as it did not change the NO release up to day 14. The

results also showed that the widely used H_2O_2 can be a further option to sterilize the example tubing while maintaining the original NO releasing capacity.

[00105] To evaluate the antithrombogenic activity and platelet preservation capability of the new NO donor (SNAT) coated or impregnated extracorporeal circuit tubing, and to determine if the modified polymer tubing is less thrombogenic compared to the unmodified polymer material, an arterio-venous (A-V) rabbit thrombogenicity model (similar set up as shown in Fig. 6) without systemic heparinization was used. Endpoint parameters that were determined included thrombus area in tubing, clotting, plasma fibrinogen levels, platelet count and aggregation via aggregometry. In total, 13 white male New Zealand rabbits (n=7 controls, n=3 NO-600 mg/ml coat, n=3 NO-1000 mg/ml impregnation) were evaluated. A 1000 mg/ml SNAT coated tubing (data not shown), but the NO concentration was so high in the thin coated layer, that it created technical difficulties: 1) the surface became uneven, 'bumpy' due to the formation of gas phase NO; and 2) flow readings via a transonic system were not possible due to the uneven surface and the very dark color of the coating.

[00106] Blood pressures and blood flow rates remained steady compared to baseline over the 4-hour test period in the coated NO group. Platelet function as measured by aggregometry (77.9+/1.4% SNATi-1000 vs. 56.0+/4.2% Control compared to baseline 78.1+/5.9%) and platelet count (Fig. 13A) were also maintained over the course of study, and no significant clot formation (Fig. 13B) was observed in this group.

[00107] However, the control group showed higher variances in the above-mentioned parameters. The platelet count dropped below 50% and all the circuits were heavily clotted (Fig. 13A and Fig. 13B, 1.2+/-1.2 cm2 SNAT-600, n=3 vs 11.0+/-0.4 cm2 Control, n=7). Two of the control circuits clotted within the first hour of the experiment.

[00108] As noted, the impregnation technique (1000 mg/ml) was also used to prepare the NO releasing surface. This technique treated the whole polymer on both the inner and the outer surfaces.

[00109] The impregnated NO circuit showed even better antithrombotic properties (0.001+/-0.0 cm2 (SNATi 1000, n=3) vs. 10.28 +/-0.20 cm2 Control, n=7)

than the coated group and had no clot formation observed throughout the entire circuit (Fig. 14A and Fig. 14B). The aggregometry of the impregnated NO group (SNATi) did not differ significantly from the baseline values (79.8+/0.3% SNATi-1000 vs 56.0+/4.2% Control compared to baseline 74.1+/1.9%). The SNAT impregnated PVC also showed great antibacterial (5 day, 3 log reduction). The confocal images are shown in Fig. 15. Tables 7A and 7B illustrate the high antibacterial effect (reduced bacteria colonies) of the SNAT impregnated polymer on Gram+ and Gram- strains after a 5-day long biofilm study (Table 7A), and the bacterial killing (7 day, 7 log reduction (Table 7B)) effect compared to controls.

TABLE 7A - average bacteria colony numbers (CFU/ml) of the biofilm tests

Biofilm	***S. aureus***	**St.Dev.**	***E.coli***	**St.Dev.**
Control	8.50E+07	1.91E+07	9.00E+07	2.58E+07
SNAT Fully impregnated NO circuit	5.60E+04	3.95E+04	3.20E+04	1.39E+04
	3 log reduction		3 log reduction	

TABLE 7B - average bacteria colony numbers (CFU/ml) of the bacteria killing tests

	Average CFU/mL					
Bacterial Killing Effects	***S. aureus***	**St.Dev.**	***E.coli***	**St.Dev.**	***P.aeruginosa***	**St.Dev.**
Control	5.94E+07	8.76E+07	9.33E+07	3.06E+07	1.13E+09	3.06E+08
SNAT Fully impregnated NO circuit	6.67E+00	1.15E+01	4.67E+01	5.03E+01	2.53E+02	3.01E+02
	7 log reduction		6 log reduction		7 log reduction	

[00110] All of these results suggest that the SNAT treated (both coated or impregnated) circuits exhibit excellent antithrombotic and antibacterial activity compared to the untreated control ECCs and does help preserve platelet counts over the course of the study. The antithrombotic and antibacterial activity is likely to be similar for the SNAT semi-impregnated examples. However, these results also

demonstrate the deleterious effects that these techniques can have on the surface roughness, and potentially the mechanical properties of the coated or fully impregnated polymers, which is unlike the semi-impregnated examples disclosed herein.

[00111] Reference throughout the specification to "one example", "another example", "an example", and so forth, means that a particular element (e.g., feature, structure, and/or characteristic) described in connection with the example is included in at least one example described herein, and may or may not be present in other examples. In addition, it is to be understood that the described elements for any example may be combined in any suitable manner in the various examples unless the context clearly dictates otherwise.

[00112] It is to be understood that the ranges provided herein include the stated range and any value or sub-range within the stated range. For example, a molecular weight ranging from about 195 Daltons to about 350 Daltons should be interpreted to include not only the explicitly recited limits of from about 195 Daltons to about 350 Daltons, but also to include individual molecular weights (e.g., 197 Daltons, 250 Daltons, 275.5 Daltons, etc.), and sub-ranges of molecular weights (from about 250 Daltons to about 350 Daltons, from about 197 Daltons to about 297 Daltons, etc.). Furthermore, when "about" is utilized to describe a value, this is meant to encompass minor variations (up to +/- 10%) from the stated value.

[00113] In describing and claiming the examples disclosed herein, the singular forms "a", "an", and "the" include plural referents unless the context clearly dictates otherwise.

[00114] While several examples have been described in detail, it is to be understood that the disclosed examples may be modified. Therefore, the foregoing description is to be considered non-limiting.

What is claimed is:

1. A method for generating a nitric oxide generating device, comprising:

introducing a solution to an interior opening of a nitric oxide permeable polymeric object, the solution including:

a solvent; and

S-nitroso-1-adamantanethiol dissolved in the solvent;

allowing the solution to soak in the interior opening for a time up to 12 hours, whereby the *S*-nitroso-1-adamantanethiol impregnates into a portion of a wall of the nitric oxide permeable polymeric object adjacent to the interior opening; and

removing the solution from the interior opening.

2. The method as defined in claim 1, wherein:

the nitric oxide permeable polymeric object is made of poly(vinyl chloride); and

the solvent is part of a solvent system that further includes a plasticizer.

3. The method as defined in claim 2, wherein:

the solvent system further includes a second solvent; and

the solvent, the plasticizer, and the second solvent are present in a predetermined volume ratio.

4. The method as defined in claim 3, wherein the predetermined volume ratio is from 0.1 to 10 of the solvent : from 0.1 to 10 of the plasticizer : from 0.1 to 10 of the second solvent.

5. The method as defined in claim 3, wherein the solvent and the second solvent are different and are independently selected from the group consisting of acetone, methanol, ethyl acetate, ethyl ketone, tetrahydrofuran, chloroform, and ethanol.

6. The method as defined in any one of claim 3 through claim 5, wherein:

the solvent is acetone;

the plasticizer is trioctyl trimellitate; and

the second solvent is methanol.

7. The method as defined in any one of claim 1 through claim 6, wherein solution includes up to 2000 mg/mL of *S*-nitroso-1-adamantanethiol.

8. The method as defined in any one of claim 1 or claim 7, wherein the nitric oxide permeable polymeric object is made of silicone, polyurethane, combinations of silicone and polyurethane, polycarbonate, polypropylene, or polytetrafluoroethylene.

9. The method as defined in claim 8, wherein:

the nitric oxide permeable polymeric object is made of silicone; and

the solvent is tetrahydrofuran.

10. The method as defined in claim 8, wherein:

the nitric oxide permeable polymeric object is made of polyurethane; and

the solvent is selected from the group consisting of methanol, methyl ethyl ketone, chloroform, cyclohexane, and combinations thereof.

11. The method as defined in any one of claim 1 through claim 10, wherein prior to introducing the solution, the method further comprises generating the solution by dissolving the *S*-nitroso-1-adamantanethiol in the solvent.

12. The method as defined in claim 11, wherein prior to generating the solution, the method further comprises generating the *S*-nitroso-1-adamantanethiol by exposing 1-adamantanethiol to a solvent-free nitrosation with t-butyl nitrite.

13. The method as defined in claim any one of claim 1 through claim 12, further comprising:

introducing a coating solution to the interior opening, the coating solution including a direct thrombin inhibitor linked polymer dissolved in a third solvent; and

allowing the third solvent to evaporate, thereby forming a coating on a surface of the interior opening, the coating including the direct thrombin inhibitor linked polymer.

14. The method as defined in claim 13, wherein prior to introducing the coating solution, the method further comprises forming the coating solution by;

dissolving an anchor polymer in the third solvent;

introducing a linking molecule to the third solvent, whereby the linking molecule covalently attaches to the anchor polymer;

introducing a direct thrombin inhibitor to the third solvent, whereby the direct thrombin inhibitor covalently attaches to the linking molecule attached to the anchor polymer to form the direct thrombin inhibitor linked polymer;

precipitating the direct thrombin inhibitor linked polymer; and

redissolving the direct thrombin inhibitor linked polymer in fresh third solvent.

15. The method as defined in any one of claim 1 through claim 14, wherein the *S*-nitroso-1-adamantanethiol is a tertiary thiol having a molecular weight ranging from about 195 Daltons to about 350 Daltons and a predicted n-octanol-water partition ratio over 5.

16. The method as defined in claim 1, wherein:

the solvent is part of a solvent system including a second solvent;

the solvent and the second solvent are different and are independently selected from the group consisting of acetone, methanol, ethyl acetate, methyl ethyl ketone, tetrahydrofuran, chloroform, cyclohexane, and ethanol;

the solvent system includes a predetermined volume ratio of the solvent and the second solvent; and

the predetermined volume ratio of ranges from0.1:10 to 10:0.1.

17. A nitric oxide generating device, comprising:

a nitric oxide permeable polymeric object; and

S-nitroso-1-adamantanethiol impregnated in a portion of a wall of the nitric oxide permeable polymeric object that is adjacent to an interior opening of the nitric oxide permeable polymeric object, wherein an exterior surface of the wall of the nitric oxide permeable polymeric object is substantially free of the *S*-nitroso-1-adamantanethiol.

18. The nitric oxide generating device as defined in claim 17, wherein the *S*-nitroso-1-adamantanethiol extends from about 500 μm to about 1000 μm into the wall.

19. The nitric oxide generating device as defined in any one of claim 17 or claim 18, wherein the nitric oxide permeable polymeric object is made of poly(vinyl chloride), silicone, polyurethane, combinations of silicone and polyurethane, polycarbonate, polypropylene, or polytetrafluoroethylene.

20. The nitric oxide generating device as defined in any one of claim 17 through claim 19, further comprising a direct thrombin inhibitor linked polymer attached to a surface of the interior opening.

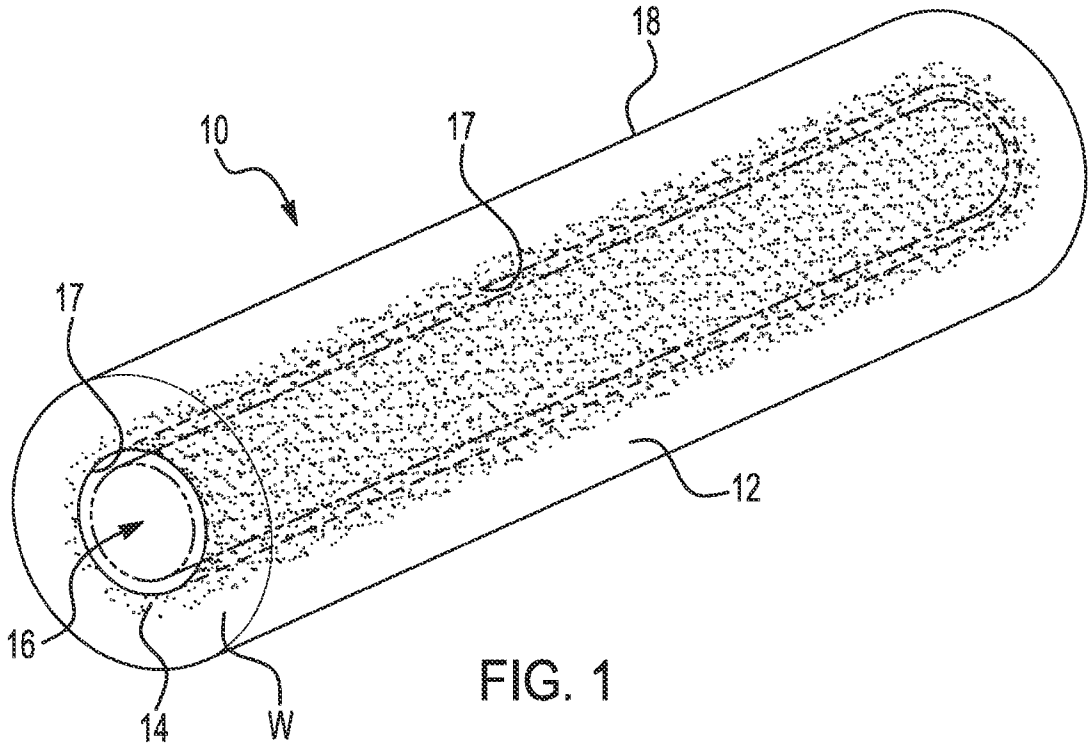


FIG. 1

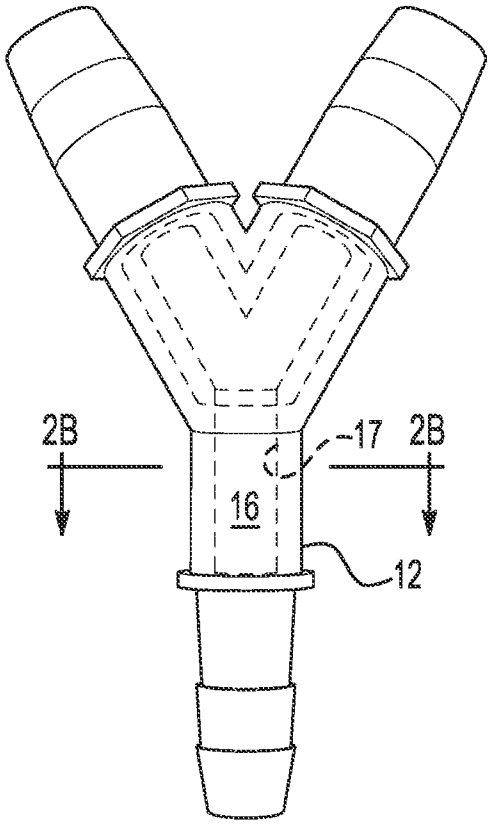


FIG. 2A

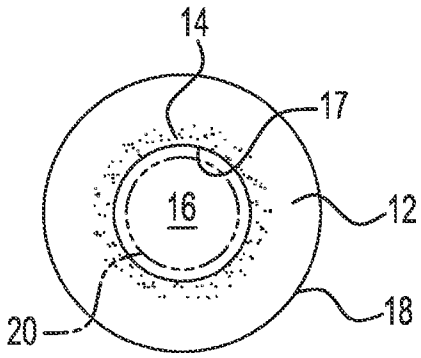


FIG. 2B

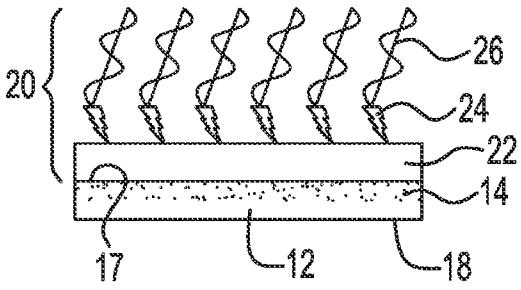


FIG. 2C

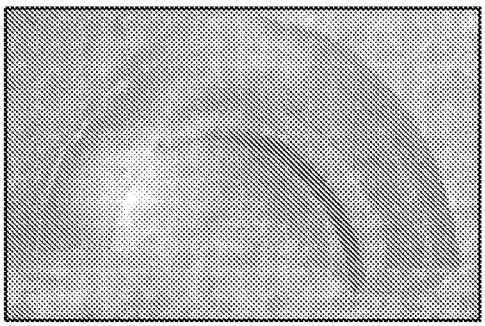


FIG. 3A

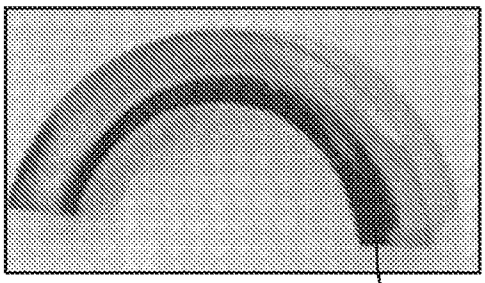


FIG. 3B

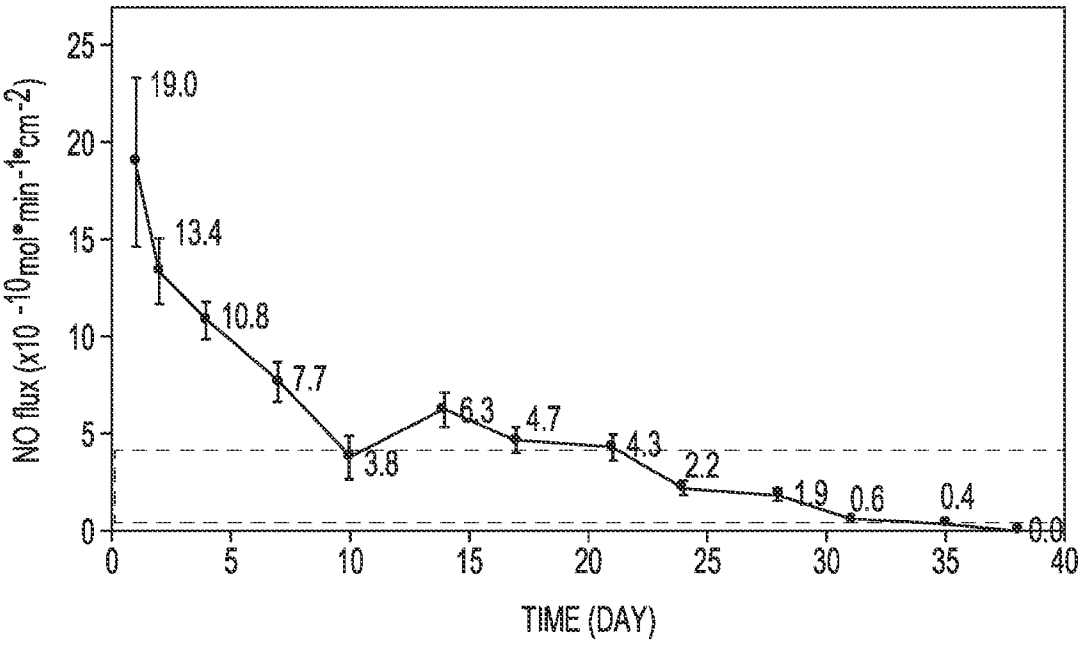


FIG. 4

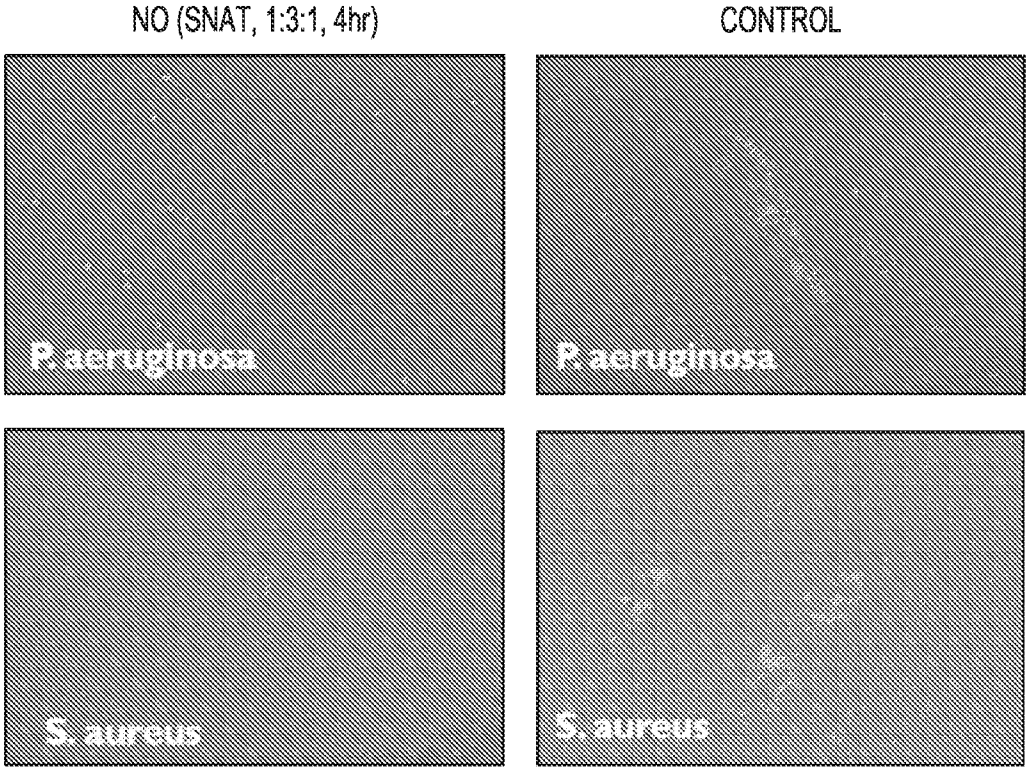


FIG. 5

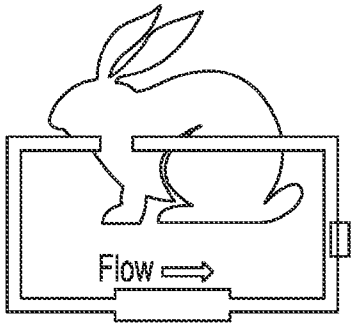


FIG. 6

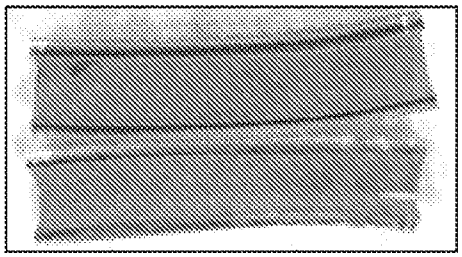


FIG. 7A

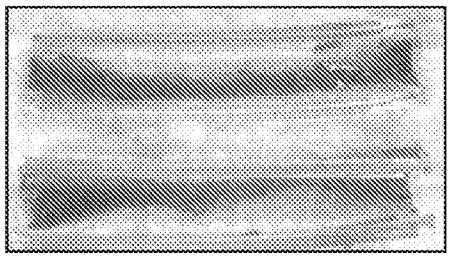


FIG. 7B

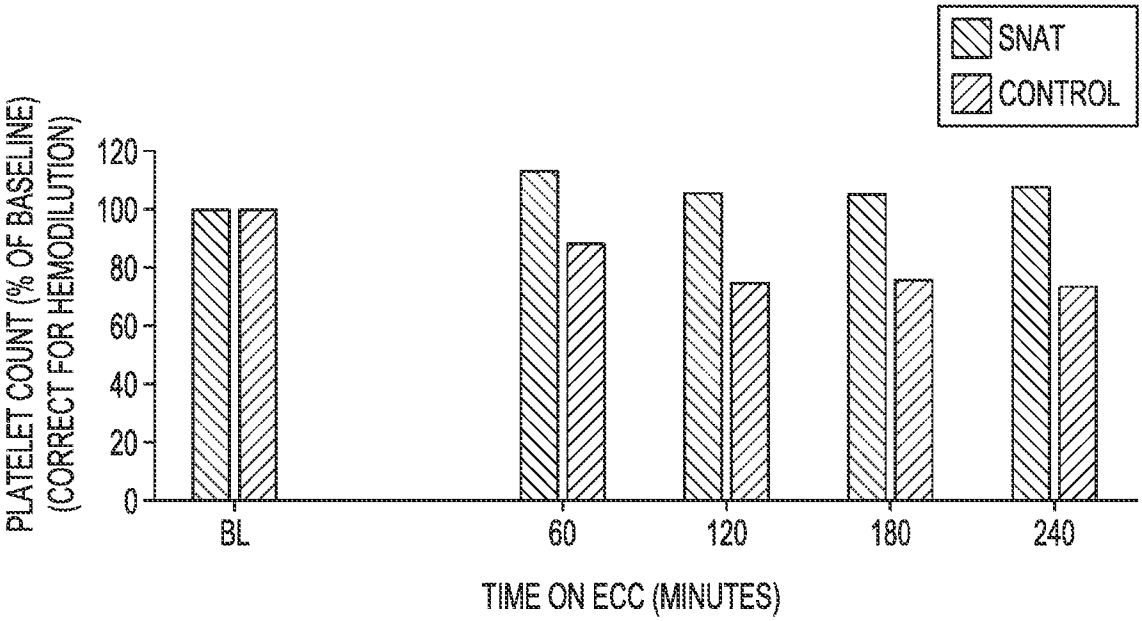

SNAT
CONTROL
PLATELET COUNT (% OF BASELINE)
(CORRECT FOR HEMODILUTION)
120
100
80
60
40
20
0
BL
60
120
180
240
TIME ON ECC (MINUTES)

FIG. 8

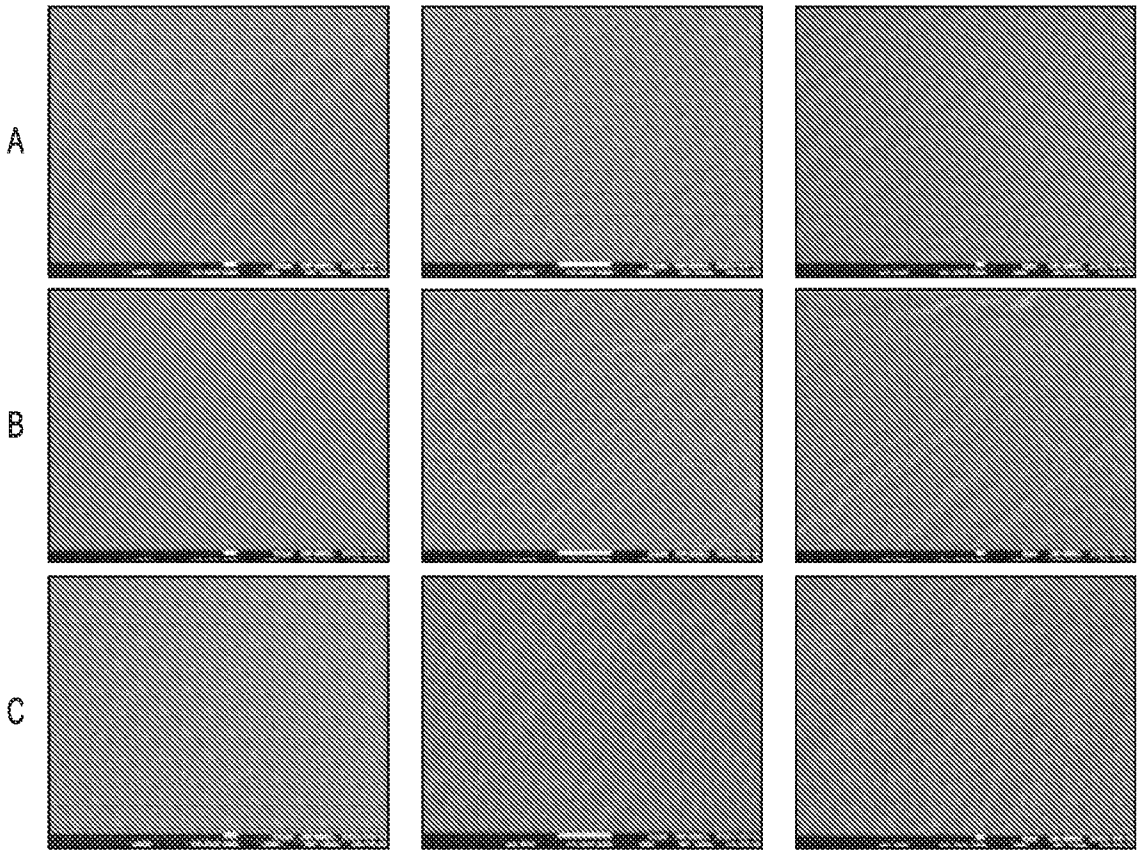

A
B
C

FIG. 9

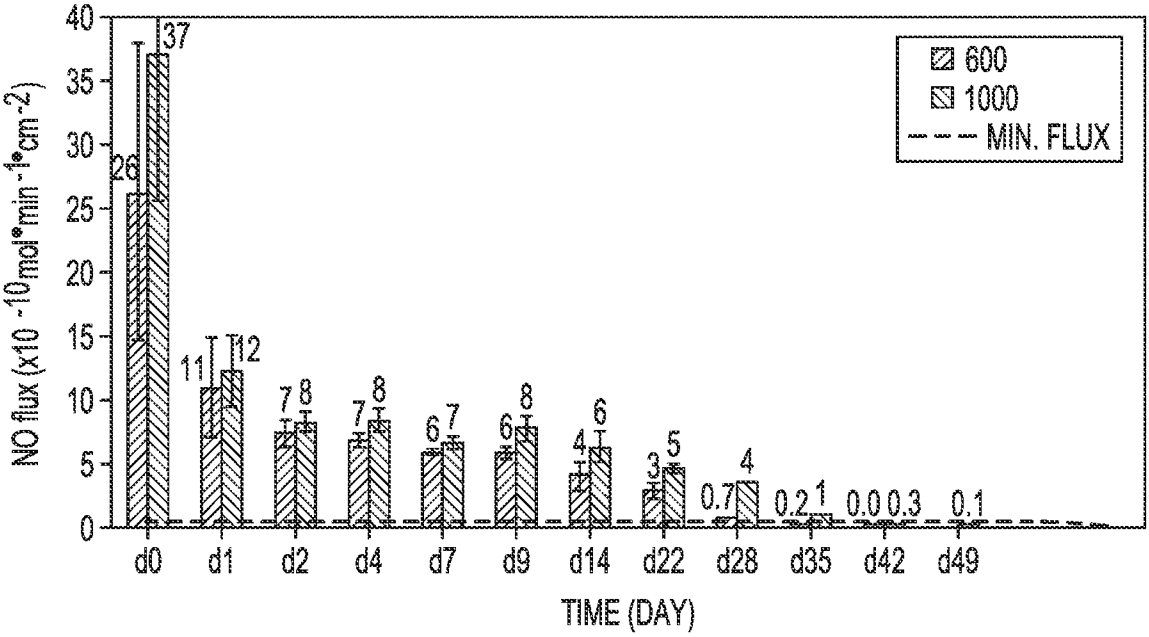

NO flux (x10 -10mol*min-1*cm-2)
40
35
30
25
20
15
10
5
0
600
1000
MIN. FLUX
26
37
11
12
7
8
7
8
6
7
6
8
4
6
3
5
0.7
4
0.2
1
0.0
0.3
0.1
d0
d1
d2
d4
d7
d9
d14
d22
d28
d35
d42
d49
TIME (DAY)

FIG. 10

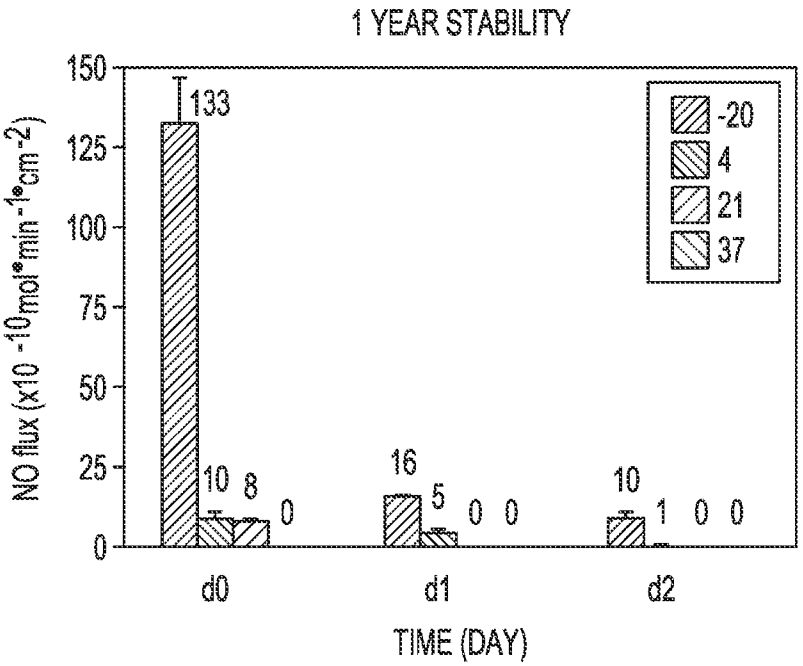

1 YEAR STABILITY
NO flux (x10 -10mol*min-1*cm-2)
150
125
100
75
50
25
0
-20
4
21
37
133
10
8
0
16
5
0
0
10
1
0
0
d0
d1
d2
TIME (DAY)

FIG. 11

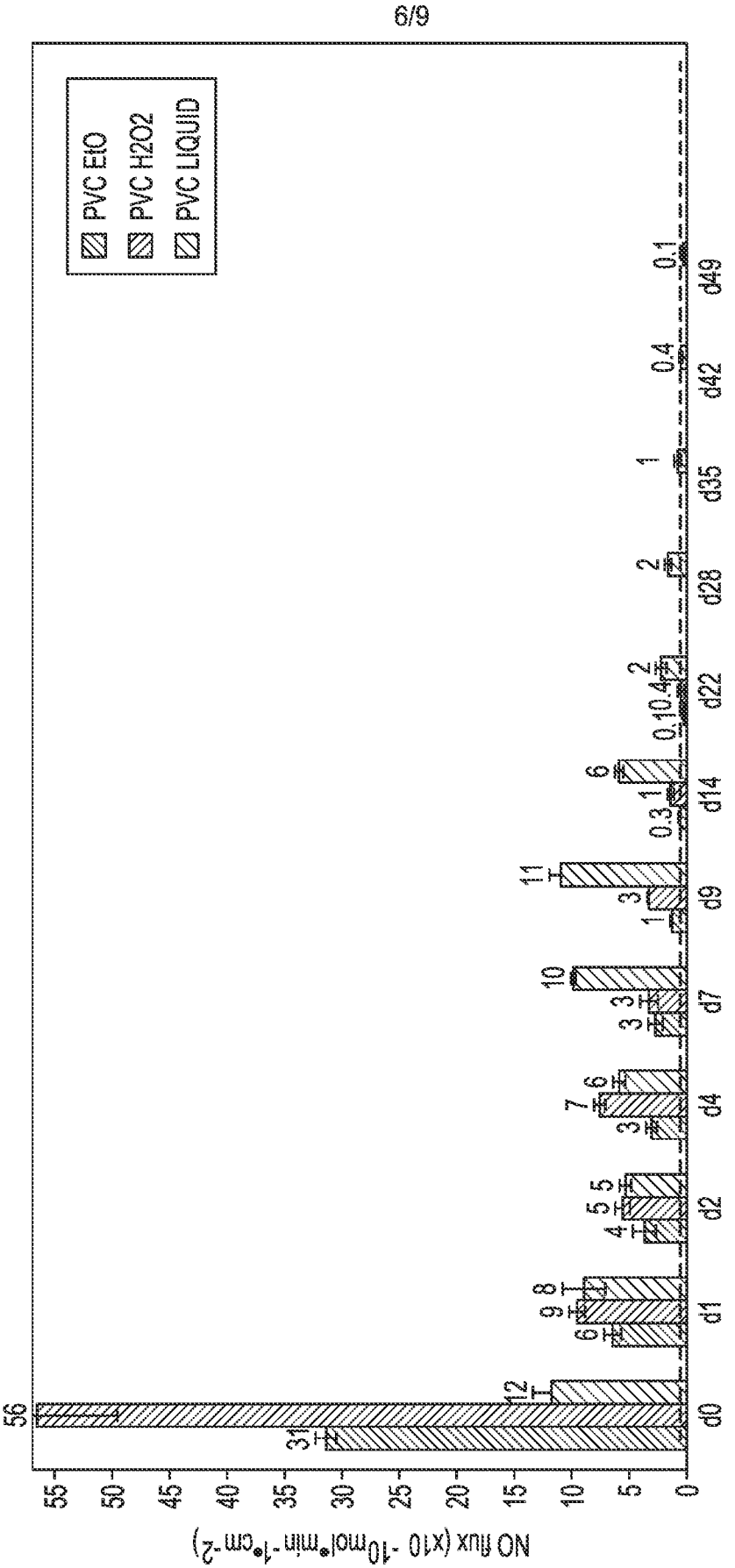

PVC EtO
PVC H2O2
PVC LIQUID
NO flux (x10 -10mol*min-1*cm-2)
TIME (DAY)
d0
d1
d2
d4
d7
d9
d14
d22
d28
d35
d42
d49

FIG. 12

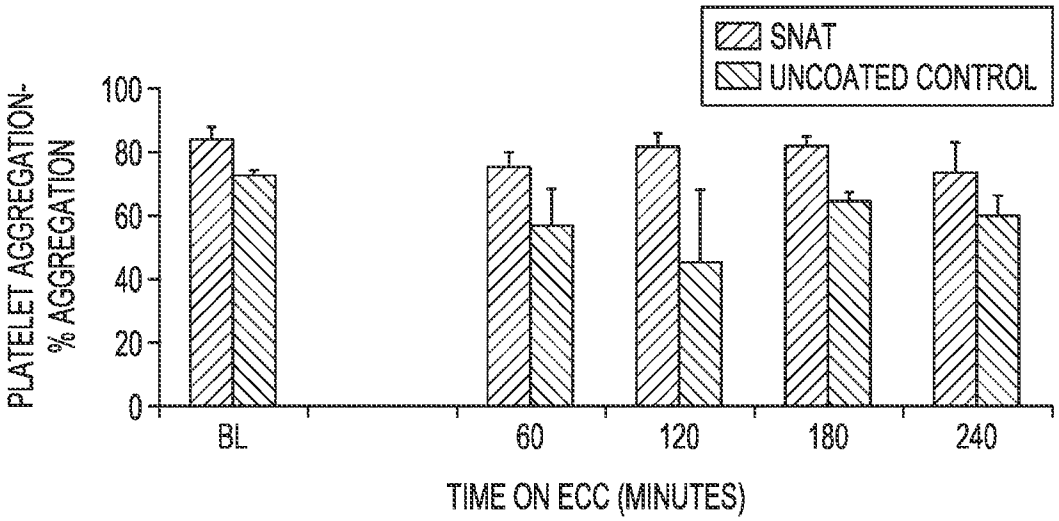


FIG. 13A

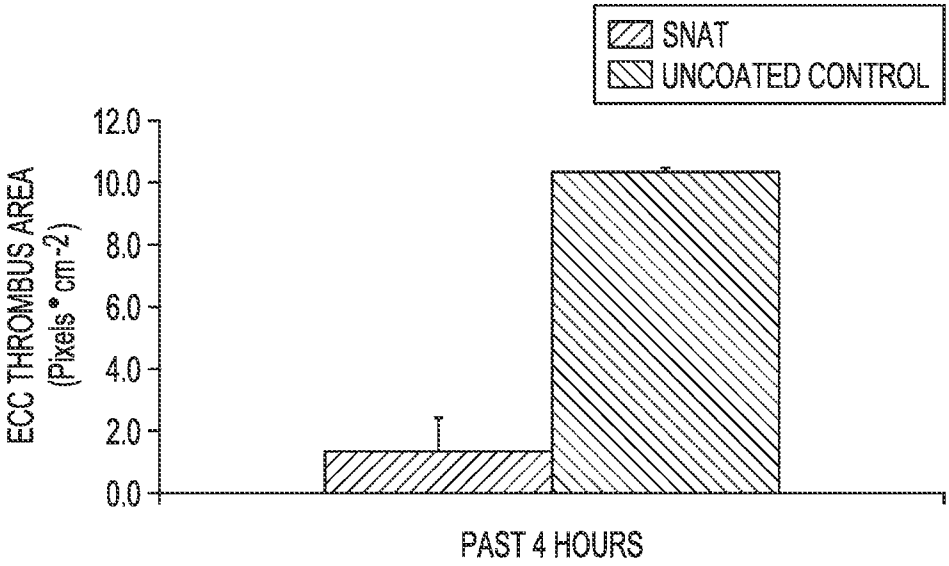


FIG. 13B

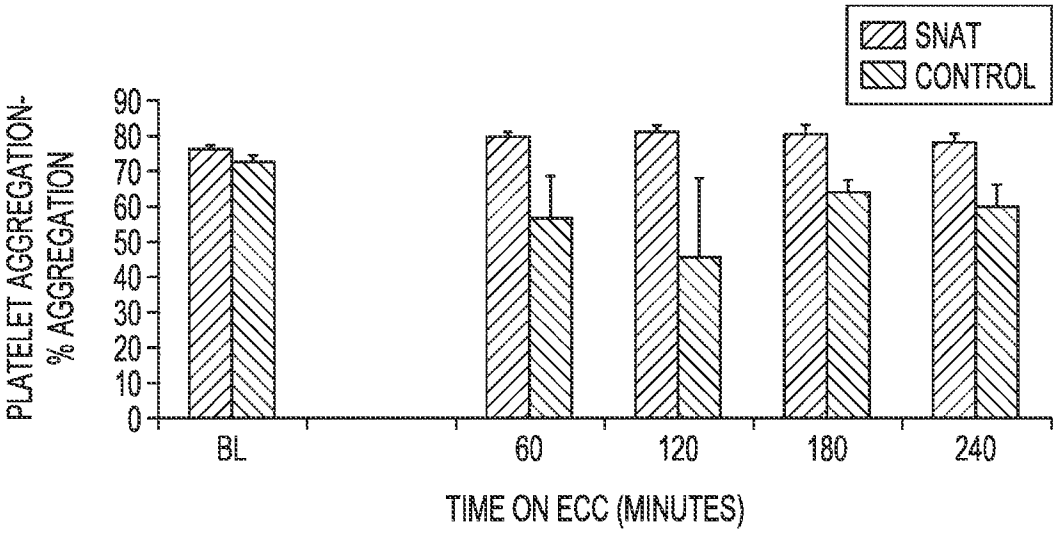


FIG. 14A

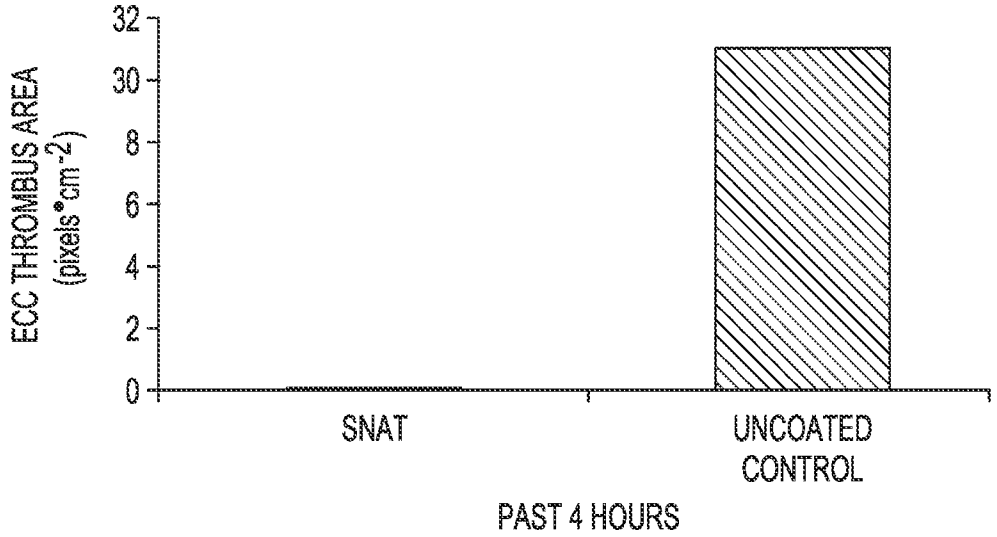


FIG. 14B

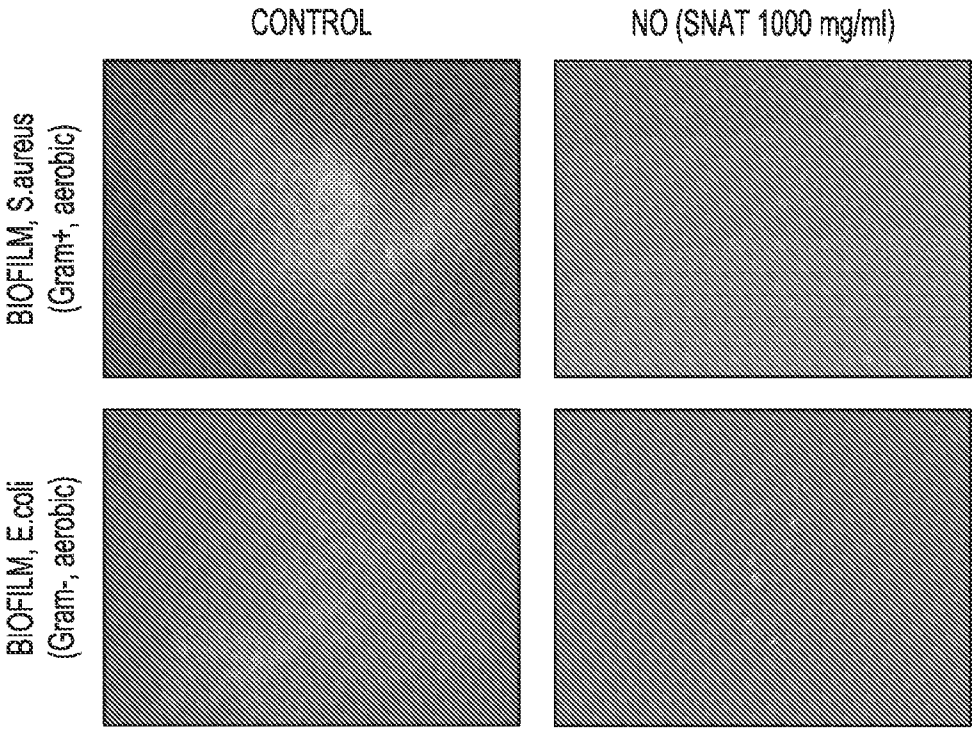

CONTROL
NO (SNAT 1000 mg/ml)
BIOFILM, S.aureus (Gram+, aerobic)
BIOFILM, E.coli (Gram-, aerobic)

FIG. 15

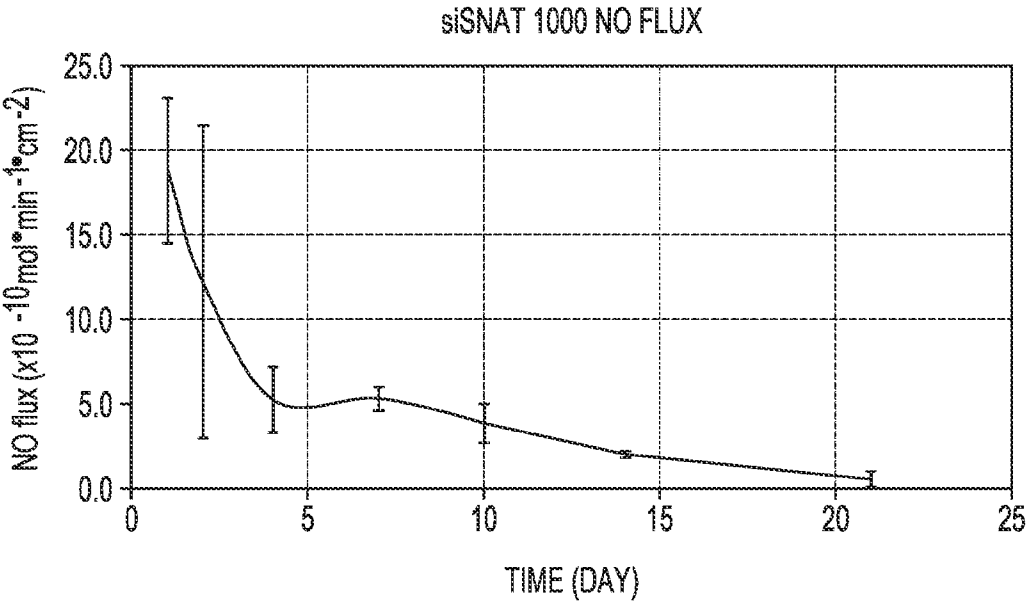

siSNAT 1000 NO FLUX
NO flux (x10 -10mol*min-1*cm-2)
25.0
20.0
15.0
10.0
5.0
0.0
0
5
10
15
20
25
TIME (DAY)

FIG. 16

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2024/031413

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61L29/12 C01B21/24 A61M25/00
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61L C01B A61M

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 03/086282 A2 (NITROMED INC [US]; FANG XINQIN [US] ET AL.) 23 October 2003 (2003-10-23)	17-20
A	page 1, line 6 - page 37, line 16	1-16
A	US 6 087 479 A (STAMLER JONATHAN S [US] ET AL) 11 July 2000 (2000-07-11) the whole document	1-20

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[x] Further documents are listed in the continuation of Box C.

[x] See patent family annex.

* Special categories of cited documents :

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

"E" earlier application or patent but published on or after the international filing date

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

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

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
1 August 2024	02/09/2024

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Zsigmond, Zoltán

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2024/031413

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Orsolya Lautner-Csorba ET AL: "BIOENGINEERING ORAL ABSTRACTS 26 BIO30 A Novel Lipophilic Nitric Oxide (NO) Donor in Extracorporeal Life", American Society for Artificial Internal Organs (ASAIO), 26 June 2022 (2022-06-26), XP093191846, Retrieved from the Internet: URL:https://journals.lww.com/asaiojournal/fulltext/2022/06002/bio30__a_novel_lipophilic_nitric_oxide__no__donor.38.aspx the whole document -----	1-20
A	US 2019/231936 A1 (CHEN HAO [US]) 1 August 2019 (2019-08-01) the whole document -----	1-20

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2024/031413

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		CA 2480832 A1	23-10-2003
		EP 1497268 A2	19-01-2005
		JP 2005537223 A	08-12-2005
		US 2003203915 A1	30-10-2003
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		US 6471978 B1	29-10-2002
		US 2003072783 A1	17-04-2003
		US 2004037836 A1	26-02-2004
		US 2007248676 A1	25-10-2007
		WO 9638136 A1	05-12-1996
US 2019231936 A1	01-08-2019	US 2019231936 A1	01-08-2019
		WO 2019152842 A2	08-08-2019