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(71) Applicant(s)
UAB Research Foundation

(72) Inventor(s)
Jun, Ho-Wook;Kushawaha, Meenakshi;Brott, Brigitta C.;Anderson, Peter

(74) Agent / Attorney
Griffith Hack, GPO Box 1285, Melbourne, VIC, 3001

(56) Related Art
JUN, et al.: "Enzyme-Mediated Degradation of Peptide-Amphiphile Nanofiber Networks", ADVANCED MATERIALS, (2005) Vol. 17, pages 2612-2617
US 2008/0175883 A1 (HSU et al.) 24 July 2008
JUN, et al. :"Drug Eluting Stent Thrombosis II: Novel Stent Designs: Abstract 4928: Natural Endothelium MimickingSelf-Assembled Nanomatrix for Drug Eluting Stent Applications", Circulation, (2008) Vol. 118 S-962

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(71) **Applicant** *(for all designated States except US)*: **UAB RESEARCH FOUNDATION** [US/US]; 1530 Third Avenue South, Ab 112g, Birmingham, AL 35294-0111 (US).

(72) **Inventors; and**
(75) **Inventors/Applicants** *(for US only)*: **JUN, Ho-Wook** [KR/US]; 5709 Park Side Pass, Hoover, AL 35244 (US). **KUSHAWAHA, Meenakshi** [IN/US]; 117 A 14th Avenue South, Birmingham, AL 35205 (US). **BROTT, Brigitta, C.** [US/US]; 13 Brush Creek Farm, Columbiana, AL 35051 (US). **ANDERSON, Peter** [US/US]; 406 Deleris Drive, Birmingham, AL 35226 (US).

(74) **Agents**: **LANIER, Gibson, J.** et al.; Ballard Spahr LLP, 999 Peachtree Street, N.e., Suite 1000, Atlanta, GA 30309 (US).

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(54) **Title**: ENDOTHELIUM MIMICKING NANOMATRIX

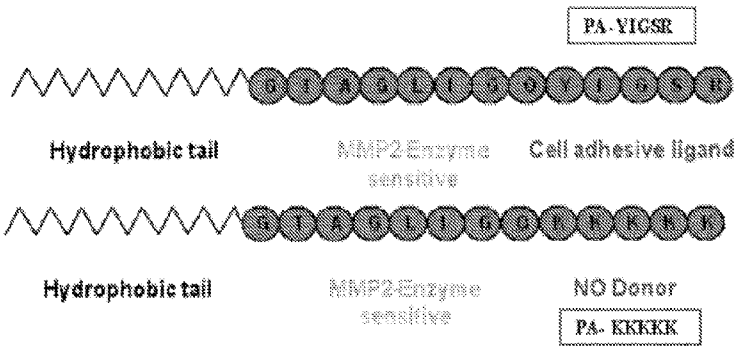

Figure 3

(57) **Abstract**: Disclosed herein are peptide amphiphiles for use in producing a natural endothelium mimicking nanomatrix. The disclosed natural endothelium mimicking nanomatrix can be used to coat medical devices such as vascular stents to promote endothelializaiton and inhibit restenosis and thrombosis. This abstract is intended as a scanning tool for purposes of searching in the particular art and is not intended to be limiting of the present invention.

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ENDOTHELIUM MIMICKING NANOMATRIX

CROSS-REFERENCE TO RELATED APPLICATIONS

[001] This application claims benefit of U.S. Provisional Application No. 61/112,578, filed November 7, 2008, and U.S. Nonprovisional Application No. 12/497,305, filed July 2, 2009, and each is hereby incorporated herein by reference in its entirety.

BACKGROUND

[002] Cardiovascular diseases (CVDs) are the number one cause of death in U.S. They claim approximately one million lives and more than 400 billion US Dollars every year. The main cause of CVDs is arterial obstruction by the deposition of cholesterol in the inner lining of the artery. This is termed atherosclerosis (Silverthorn DU. 2004).

[003] For the treatment of atherosclerosis, a non-invasive technique called balloon angioplasty was introduced in 1970s, which provided an attractive alternative to coronary artery bypass grafting. It used a slender collapsed balloon catheter that is inserted and inflated at the site of plaque. Upon inflation, the balloon compresses and ruptures the plaque, removing the blockage. This technique provided immediate relief to the patient. However, it was limited by the problem of abrupt closure of the artery after withdrawal of the catheter (Windecker S, et al. 2000). These challenges lead to the design of new biomedical solutions, such as bare metal stents.

[004] To prevent the abrupt closure of artery, lattice-shaped expandable metal tubes known as bare metal stents (BMS) were introduced in 1990s. A balloon tipped catheter with a collapsed stent is inserted with a guiding catheter and guide wire. When the balloon is inflated at the plaque site, the stent expands, locks in place, and forms a scaffold holding the artery open. The use of BMS reduced the rate of restenosis compared to balloon angioplasty. While they were successful in preventing elastic recoil of the artery, BMS suffered from the problem of restenosis (i.e. re-closure of the artery)(Sheiban I, et al. 2002).

[005] Figure 1 shows a schematic representation of the principle mechanisms of restenosis, which include elastic recoil, negative vessel remodeling, and neointimal

proliferation (Dobesh PP, et al. 2004). BMS virtually eliminates the problem of elastic recoil (Sheiban I, et al. 2002, Ozaki Y, et al. 1996) and negative vessel remodeling (Sheiban I, et al. 2002). However, the main mechanism of restenosis in BMS is neointimal hyperplasia (Violaris AG, et al. 1997). Neointimal hyperplasia is caused by endothelial denudation due to the penetration of stent struts into the vessel wall. It can be imagined that the fractured plaque exposes the thrombogenic contents of the vessel wall to the lumen, leading to a cascade of platelet adhesion, activation, and thrombosis. In addition, endothelial denudation results in the loss of antithrombotic factors. Activated platelets release factors that favor smooth muscle cell proliferation and migration. Meanwhile, smooth muscle cells also change their morphology from contractile to synthetic. This can result in smooth muscle cell migration and increased extracellular matrix (ECM) synthesis, which leads to neointimal hyperplasia and in-stent restenosis (Bauters C, et al. 1997). Hence, BMS remained limited by the high rates of in-stent restenosis. Thus, further advancements to the biomedical design of BMS were needed, such as the incorporation of localized drug delivery.

[006] The major challenge of catheter-based drug delivery is to achieve the localization of drugs at the site of vascular injury in order to reduce the formation of neointimal hyperplasia. Thus, controlled drug delivery systems have been applied to stents, resulting in the development of drug eluting stents (DES). DES became commercially available in U.S. in 2003 (Ong AT, et al. 2005). They are coated with single or multiple bioactive agents, which are delivered in blood stream and surrounding tissues after implantation. These stents are designed to release drugs that interfere with the process of neointimal hyperplasia by targeting its biochemical pathways. Several drug delivery strategies such as diffusion controlled, dissolution/degradation controlled, and ion exchange-based methods have been investigated for DES (Acharya G, et al. 2006). DES have been shown to reduce restenosis compared with BMS (de Man FH, et al. 2007). They have been implanted in more than 6 million patients from 2004 to 2006 (Colombo A, et al. 2007).

[007] The stent market is shared by only two drug-eluting stents: (1) Cordis CYPHER™, sirolimus-eluting stent and (2) Boston Scientific TAXUS™, paclitaxel-eluting stent. (FDA approved the Cypher stent in April 2003 and Taxus stent in March 2004). Both sirolimus and paclitaxel work by inhibiting the cell cycle. Sirolimus is an immunosuppressive drug

that promotes kinase activation, leading to the inhibition of the cellular growth phase. Paclitaxel binds to microtubules in dividing cells and causes them to assemble, thereby preventing mitosis (Wessely R, et al. 2006). Use of these DES has shown to reduce the risk of restenosis by at least 80%, as shown by numerous randomized controlled trials (Morice MC, et al. 2002; Moses JW, et al. 2003) and meta-analyses (Roiron C, et al. 2006). However, no difference in mortality rate has been observed between DES and BMS (Roiron C, et al. 2006; Babapulle MN, et al. 2004). This can be attributed to the occurrence of late stent thrombosis or blood clots, which is an emerging cause of concern in the first generation of DES (Camenzind E, et al. 2007; Webster MW, et al. 2007; Van Belle E, et al. 2007; Jaffe R, et al. 2007; Leon MB. 2007). This late thrombosis seems to be related to discontinued antiplatelet therapy, (Zimarino M, et al. 2005) rare cases of local hypersensitivity as a reaction to the drug, and "off-label use" of DES (Win HK, et al. 2007). According to FDA standards, DES have only been approved for patients with previously untreated coronary stenosis of less than 30 mm in length and a reference vessel diameter within the range from 2.50 mm to 3.70 mm (Win HK, et al. 2007; Melikian N, et al. 2006). A study from American College of Cardiology reported "off-label use" is common and has increased in frequency over time (Rao SV, et al. 2006). In addition, studies have shown that DES cause significant delay in arterial healing due to persistent fibrin deposition and poor endothelialization when compared with the sites of BMS implantation (Finn A, et al. 2007). Angioscopic findings show incomplete neointimal covering of sirolimus-eluting stents (Kotani J, et al. 2006). Also, patient risk factors like diabetes, renal failure, and previous complications have contributed to the incidences of late thrombosis in the patients who received DES (Jaffe R, et al. 2007).

[008] These concerns have given way to the idea of an ideal stent which should be designed to control and direct vessel repair after surgery without eliciting undesirable inflammatory response and eventually leading to a re-endothelialized vessel wall.

[008a] In the claims which follow and in the preceding description of the invention, except where the context requires otherwise due to express language or necessary implication, the word "comprise" or variations such as "comprises" or "comprising" is used in an inclusive sense, i.e. to specify the presence of the stated features but not to preclude the presence or addition of further features in various embodiments of the invention.

[008b] It is to be understood that, if any prior art publication is referred to herein, such reference does not constitute an admission that the publication forms a part of the common general knowledge in the art, in Australia or any other country.

BRIEF SUMMARY

[009] In accordance with the purpose of this invention, as embodied and broadly described herein, this invention relates to peptide amphiphiles for use in producing a natural endothelium mimicking nanomatrix. The disclosed natural endothelium mimicking nanomatrix can be used to coat medical devices such as vascular stents. Additional

advantages of the disclosed method and compositions will be set forth in part in the description which follows, and in part will be understood from the description, or may be learned by practice of the disclosed method and compositions. The advantages of the disclosed method and compositions will be realized and attained by means of the elements and combinations particularly pointed out in the appended claims. It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention as claimed.

[009a] The invention further provides a peptide amphiphile, comprising a hydrophilic peptide sequence and a hydrophobic tail, wherein the hydrophilic peptide sequence comprises a degradation sequence and one or more of a first cell adhesive sequence and a nitric oxide producing donor sequence, wherein the first adhesive sequence is an endothelial cell adhesive sequence; wherein the cell adhesive sequence comprises YIGSR (SEQ ID NO:2), RGD (SEQ ID NO:8), RGDS (SEQ ID NO:9), DGEA (SEQ ID NO:10), VAPG (SEQ ID NO:11), REDV (SEQ ID NO:12), DGEA (SEQ ID NO:13), or LRSR (SEQ ID NO:14); and wherein the nitric oxide producing donor sequence comprises polylysine, polycysteine sequence, or modified polylysine.

[009b] The invention further provides a composition comprising a first and second peptide amphiphile, each independently comprising a hydrophilic peptide sequence and a hydrophobic tail, wherein the hydrophilic peptide sequence of the first peptide amphiphile comprises a degradation sequence and an endothelial cell adhesive sequence, wherein the cell adhesive sequence comprises YIGSR (SEQ ID NO:2), RGD (SEQ ID NO:8), RGDS (SEQ ID NO:9), DGEA (SEQ ID NO:10), VAPG (SEQ ID NO:11), REDV (SEQ ID NO:12), DGEA (SEQ ID NO:13), or LRSR (SEQ ID NO:14), and wherein the hydrophilic peptide sequence of the second peptide amphiphile comprises a degradation sequence and a nitric oxide producing donor sequence, wherein the nitric oxide producing donor sequence comprises polylysine, polycysteine sequence, or modified polylysine.

[009c] The invention further provides an endothelium mimicking nanomatrix, comprising one or more peptide amphiphiles assembled into nanofibers, wherein the peptide amphiphiles each comprise a hydrophilic peptide sequence and a hydrophobic tail, wherein the hydrophilic peptide sequence comprises a degradation sequence and one or more of a first cell adhesive sequence and a nitric oxide producing donor sequence, wherein the first adhesive sequence is

an endothelial cell adhesive sequence, wherein the cell adhesive sequence comprises YIGSR (SEQ ID NO:2), RGD (SEQ ID NO:8), RGDS (SEQ ID NO:9), DGEA (SEQ ID NO:10), VAPG (SEQ ID NO:11), REDV (SEQ ID NO:12), DGEA (SEQ ID NO:13), or LRSR (SEQ ID NO:14); and wherein the nitric oxide producing donor sequence comprises polylysine, polycysteine sequence, or modified polylysine.

[009d] The invention further provides a composition comprising a medical device coated with an endothelium mimicking nanomatrix, comprising one or more peptide amphiphiles assembled into nanofibers, wherein the peptide amphiphiles each comprise a hydrophilic peptide sequence and a hydrophobic tail, wherein the hydrophilic peptide sequence comprises a degradation sequence and one or more of a first cell adhesive sequence and a nitric oxide producing donor sequence, wherein the first adhesive sequence is an endothelial cell adhesive sequence, wherein the cell adhesive sequence comprises YIGSR (SEQ ID NO:2), RGD (SEQ ID NO:8), RGDS (SEQ ID NO:9), DGEA (SEQ ID NO:10), VAPG (SEQ ID NO:11), REDV (SEQ ID NO:12), DGEA (SEQ ID NO:13), or LRSR (SEQ ID NO:14); and wherein the nitric oxide producing donor sequence comprises polylysine, polycysteine sequence, or modified polylysine.

[009e] The invention further provides a method of making a peptide amphiphile, comprising the steps a) providing a hydrophilic peptide comprising a degradation sequence and one or more of an endothelial cell adhesive sequence and a nitric oxide producing donor sequence, wherein the cell adhesive sequence comprises YIGSR (SEQ ID NO:2), RGD (SEQ ID NO:8), RGDS (SEQ ID NO:9), DGEA (SEQ ID NO:10), VAPG (SEQ ID NO:11), REDV (SEQ ID NO:12), DGEA (SEQ ID NO:13), or LRSR (SEQ ID NO:14); and wherein the nitric oxide producing donor sequence comprises polylysine, polycysteine sequence, or modified polylysine; b) alkylating the N-terminus of the hydrophilic peptide with a hydrophobic moiety.

[009f] The invention further provides a method of treating a subject with cardiovascular disease comprising administering to the subject one or more peptide amphiphiles, wherein at least one peptide amphiphile is the peptide amphiphile as described herein.

[009g] The invention further provides a method of improving graft patency comprising administering to a recipient of a graft one or more peptide amphiphiles, wherein at least one peptide amphiphile is the peptide amphiphile as described herein.

[009h] The invention further provides use of the peptide amphiphile as described herein or for treating a subject with cardiovascular disease or improving graft patency in a recipient of a graft.

[009i] The invention further provides use of the peptide amphiphile as described herein for the preparation of a medicament for treating a subject with cardiovascular disease, and/or for improving graft patency in a recipient of a graft.

[009j] The invention further provides the peptide amphiphile as described herein for use in treating a subject with cardiovascular disease or in improving graft patency in a recipient of a graft.

[009k] The invention further provides the method or the use or the peptide amphiphile as described herein, wherein the peptide amphiphile are coated on a polyester nanofiber.

[009l] The invention further provides the method or peptide amphiphile use as described herein, wherein the polyester nanofiber is a polycarbolactone nanofiber.

BRIEF DESCRIPTION OF THE DRAWINGS

[010] The accompanying drawings, which are incorporated in and constitute a part of this specification, illustrate several embodiments of the disclosed method and compositions and together with the description, serve to explain the principles of the disclosed method and compositions.

[011] Figure 1 shows mechanisms and timelines of restenosis. Neointimal proliferation is the main cause of restenosis in bare metal stents (BMS). See Dobesh, P. P., et al. (2004). Pharmacotherapy. 24(11): 1554-77.

[012] Figure 2A shows chemical structure of diazeniumdiolates. Figure 2B shows formation of diazeniumdiolates by reaction of a nucleophilic amine (X-) with NO. Figure 2C shows

dissociation of diazoniumdiolates on protonation to release free NO. Figure 2D shows structure of Lysine. It has two pendant amine groups for NO binding.

[013] Figure 3 shows molecular structure of PAs. YIGSR (SEQ ID NO:2) is a cell adhesive ligand and KKKKK (SEQ ID NO:3) is a NO donor.

[014] Figure 4 shows TEM images of evaporation induced self-assembled nanofibers. Shown are PA-YIGSR (A), PA-KKKKK (B), PA-YK (C), and PA-YK-NO (D).

[015] Figure 5 shows initial attachment of HUVECs and AoSMCs on PA-YK (9:1 molar ratio of PA-YIGSR and PA-KKKKK) nanomatrix. Cell attachment is normalized to that of attachment on the glass. *HUVECs show significantly higher attachment than AoSMCs after 2 hrs ($p<0.05$). Error bar represents means ± standard deviation for n=12.

[016] Figure 6 shows initial spreading of HUVECs and AoSMCs on PA-YK coatings. *HUVECs show significantly greater spreading than AoSMCs after 2 hrs ($p<0.01$). Error bar represents means ± standard deviation for n=12.

[017] Figure 7 shows fluorescent images of HUVECs and AoSMCs on PA-YK after 2 hours using Calcein AM. Figure 7A shows HUVECs attain their regular spread morphology within 2 hours. Figure 7B shows AoSMCs remain round in shape.

[018] Figure 8 shows platelet attachment on Collagen, PA-YK, and PA-YK-NO nanomatrices after incubation with human blood for 15 minutes. Platelet adhesion is significantly less on PA-YK-NO films compared to PA-YK or collagen I-coated films. Data represent the mean of three samples. Error bar represents mean ± standard deviation. (*: $p < 0.05$ compared to collagen I; #: $p < 0.05$ compared to PA-YK).

[019] Figure 9 shows proliferation of HUVECs and AoSMCs seeded on PA-YK and PA-YK-NO nanomatrices after 48 hours, quantitatively assessed by PCNA staining. PA-YK-NO enhances HUVECs proliferation but reduces AoSMCs proliferation. Results are expressed as the percentage of PCNA positive cells. Data represent the mean of four samples. Error bar represents mean ± standard deviation. (*, #: $p < 0.05$)..

[020] Figure 10 shows NO release from PA-YK-NO films in HBS at pH 7.4, 37 °C. 53 % of total NO released displaying multiphasic profile over one month. Data represent the mean of four samples. Error bar represents mean ± standard deviation.

[021] Figure 11 shows Schematic representation of stent coating technique with nanomatrix.

[022] Figure 12 shows SEM Image of 0.1 wt% PAYK coated stent after handling by the clinician. Notice the smooth uniform coating surface which stays undisturbed after handling (mounting on angioplasty balloon and expansion).

[023] Figure 13A shows balloon inflation to deploy stent in rabbit iliac artery. Figure 13B shows histology section of 1 wt% nanomatrix coated stent after 4 weeks of implant. Little neointimal hyperplasia and no thrombus found on the surface of the nanomatrix coated stents.

[024] Figure 14 shows neointimal thickness at 2 weeks. Apparent trend towards less NI thickness in the high dose stents compared to the control was observed. N=2 stents per group.

[025] Figure 15 shows neointimal thickness at 4 weeks. NI was greater than at 2 weeks, but the trend towards less NI in both the low dose and high dose groups appeared to persist, compared with controls. N=2 stents per group.

[026] Figure 16 shows inflammation scores at 2 weeks. Average inflammation scores for all study groups were less than 0.5. N=2 stents per group.

[027] Figure 17 shows inflammation scores at 4 weeks. Average inflammation scores for all study groups were less than 0.5. N=2 stents per group.

[028] Figure 18 shows thrombus scores at 4 weeks. The average thrombus score was less than 0.1 for all stent groups with no significant difference between the groups. N=2 stents per group.

[029] Figure 19 shows the fabrication of an NO releasing hybrid biomimetic nanomatrix. Figure 19Ashows NO releasing peptide amphiphiles PA-YK-NO, 8 nm in diameter, are coated onto ePCL nanofibers, 300-500 nm in diameter, to create a hybrid nanomatrix, ePCL-PA-YK-NO. Figure 19B shows the presence of YIGSR ligands and release of NO promote the adhesion, spreading and proliferation of endothelial cells, while simultaneously limiting the adhesion, spreading and proliferation of smooth muscle cells.

[030] Figure 20 shows SEM images of hybrid nanomatrices at 5000x. (A) ePCL. (B) ePCL+PA-YK. (C) ePCL+PA-YK-NO.

[031] Figure 21 shows TEM images of hybrid nanomatrices at 67000x showing successful self assembly. (A) ePCL. (B) ePCL+PA-YK. (C) ePCL+PA-YK, tilted to 21°. (D) ePCL+PA-YK-NO. (E) ePCL+PA-YK-NO, tilted to 21°.

[032] Figure 22 shows that HUVEC adhesion increases with increasing concentration of PA-YIGSR. ePCL-PA-YK75 (*) and ePCL-PA-YK90 (#) showed significantly increased HUVEC adhesion when compared to ePCL-PA-YK50, ePCL-PA-YK25 and ePCL.

[033] Figure 23 shows the NO Release profile over 28 days.

[034] Figure 24 shows HUVEC morphology at 2 hours on (A) ePCL (B) ePCL-PA-YK (C) ePCL-PA-YK-NO. Scale bar = 20μm.

[035] Figure 25 shows AoSMC morphology at 2 hours on (A) ePCL (B) ePCL-PA-YK (C) ePCL-PA-YK-NO. Scale bar = 20μm.

[036] Figure 26 shows cell adhesion on hybrid nanomatrix at 2 hrs. HUVECs showed significantly increased adhesion on (*) ePCL+PA-YK and (#) ePCL-PA-YK-NO when compared to ePCL.

[037] Figure 27 shows the percentage of proliferating cells on the hybrid nanomatrices. HUVECs showed significantly greater proliferation on ePCL+PA-YK-NO when compared to ePCL+PA-YK(*). AoSMCs showed significantly lower proliferation on ePCL+PA-YK-NO when compared to ePCL+PA-YK(#).

DETAILED DESCRIPTION

[038] The disclosed method and compositions may be understood more readily by reference to the following detailed description of particular embodiments and the Example included therein and to the Figures and their previous and following description.

[039] Disclosed are materials, compositions, and components that can be used for, can be used in conjunction with, can be used in preparation for, or are products of the disclosed method and compositions. These and other materials are disclosed herein, and it is understood that when combinations, subsets, interactions, groups, etc. of these materials are disclosed that while specific reference of each various individual and collective combinations and permutation of these compounds may not be explicitly disclosed, each is specifically contemplated and described herein. For example, if a peptide is disclosed and discussed and a number of modifications that can be made to a number of molecules including the peptide are discussed, each and every combination and permutation of peptide and the modifications that are possible are specifically contemplated unless specifically indicated to the contrary. Thus, if a class of molecules A, B, and C are disclosed as well as a class of molecules D, E, and F and an example of a combination molecule, A-D is disclosed, then even if each is not individually recited, each is

individually and collectively contemplated. Thus, in this example, each of the combinations A-E, A-F, B-D, B-E, B-F, C-D, C-E, and C-F are specifically contemplated and should be considered disclosed from disclosure of A, B, and C; D, E, and F; and the example combination A-D. Likewise, any subset or combination of these is also specifically contemplated and disclosed. Thus, for example, the sub-group of A-E, B-F, and C-E are specifically contemplated and should be considered disclosed from disclosure of A, B, and C; D, E, and F; and the example combination A-D. This concept applies to all aspects of this application including, but not limited to, steps in methods of making and using the disclosed compositions. Thus, if there are a variety of additional steps that can be performed it is understood that each of these additional steps can be performed with any specific embodiment or combination of embodiments of the disclosed methods, and that each such combination is specifically contemplated and should be considered disclosed.

[040] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the method and compositions described herein. Such equivalents are intended to be encompassed by the following claims.

[041] It is understood that the disclosed method and compositions are not limited to the particular methodology, protocols, and reagents described as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

[042] All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited. The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided herein can be different from the actual publication dates, which can require independent confirmation. The discussion of references states what their authors assert, and Applicant reserves the right to challenge the accuracy and pertinency of the cited documents.

A. Definitions

[043] The present invention can be understood more readily by reference to the following detailed description of the invention and the Examples included therein.

[044] Before the present compounds, compositions, articles, systems, devices, and/or methods are disclosed and described, it is to be understood that they are not limited to specific synthetic methods unless otherwise specified, or to particular reagents unless otherwise specified, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular aspects only and is not intended to be limiting. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, example methods and materials are now described.

[045] It must be noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, reference to "a peptide" includes a plurality of such peptides, reference to "the peptide" is a reference to one or more peptides and equivalents thereof known to those skilled in the art, and so forth.

[046] "Optional" or "optionally" means that the subsequently described event, circumstance, or material may or may not occur or be present, and that the description includes instances where the event, circumstance, or material occurs or is present and instances where it does not occur or is not present.

[047] Ranges can be expressed herein as from "about" one particular value, and/or to "about" another particular value. When such a range is expressed, another embodiment includes from the one particular value and/or to the other particular value. Similarly, when values are expressed as approximations, by use of the antecedent "about," it will be understood that the particular value forms another embodiment. It will be further understood that the endpoints of each of the ranges are significant both in relation to the other endpoint, and independently of the other endpoint. It is also understood that there are a number of values disclosed herein, and that each value is also herein disclosed as "about" that particular value in addition to the value itself. For example, if the value "10" is disclosed, then "about 10" is also disclosed. It is also understood that when a value is

disclosed that "less than or equal to" the value, "greater than or equal to the value" and possible ranges between values are also disclosed, as appropriately understood by the skilled artisan. For example, if the value "10" is disclosed the "less than or equal to 10"as well as "greater than or equal to 10" is also disclosed. It is also understood that the throughout the application, data is provided in a number of different formats, and that this data, represents endpoints and starting points, and ranges for any combination of the data points. For example, if a particular data point "10" and a particular data point 15 are disclosed, it is understood that greater than, greater than or equal to, less than, less than or equal to, and equal to 10 and 15 are considered disclosed as well as between 10 and 15. It is also understood that each unit between two particular units are also disclosed. For example, if 10 and 15 are disclosed, then 11, 12, 13, and 14 are also disclosed.

[048] Throughout the description and claims of this specification, the word "comprise" and variations of the word, such as "comprising" and "comprises," means "including but not limited to," and is not intended to exclude, for example, other additives, components, integers or steps.

[049] As used herein, the term "treatment" refers to the medical management of a patient with the intent to cure, ameliorate, stabilize, or prevent a disease, pathological condition, or disorder. This term includes active treatment, that is, treatment directed specifically toward the improvement of a disease, pathological condition, or disorder, and also includes causal treatment, that is, treatment directed toward removal of the cause of the associated disease, pathological condition, or disorder. In addition, this term includes palliative treatment, that is, treatment designed for the relief of symptoms rather than the curing of the disease, pathological condition, or disorder; preventative treatment, that is, treatment directed to minimizing or partially or completely inhibiting the development of the associated disease, pathological condition, or disorder; and supportive treatment, that is, treatment employed to supplement another specific therapy directed toward the improvement of the associated disease, pathological condition, or disorder. It is further understood and herein contemplated that a treatment does not have to result in the complete ablation of a disease or condition, but can refer to any reduction in the presence of a disease or condition or symptoms thereof such that an improvement over the untreated state of the subject with the disease or condition is observed. Accordingly, treatment can

refer to a 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100%, improvement in any symptom or underlying cause in a disease or condition. For example, a composition or device that results in a 10% reduction in artio plaques relative to the amount of blockage prior to administration of said composition or device would be a treatment.

[050] As used herein, the term "prevent" or "preventing" refers to precluding, averting, obviating, forestalling, stopping, or hindering something from happening, especially by advance action. It is understood that where reduce, inhibit or prevent are used herein, unless specifically indicated otherwise, the use of the other two words is also expressly disclosed.

[051] As used herein, the terms "administering" and "administration" refer to any method of providing a pharmaceutical preparation to a subject. Such methods are well known to those skilled in the art and include, but are not limited to, oral administration, transdermal administration, administration by inhalation, nasal administration, topical administration, intravaginal administration, ophthalmic administration, intraaural administration, intracerebral administration, rectal administration, and parenteral administration, including injectable such as intravenous administration, intra-arterial administration, intramuscular administration, and subcutaneous administration. Administration can be continuous or intermittent. In various aspects, a preparation can be administered therapeutically; that is, administered to treat an existing disease or condition. In further various aspects, a preparation can be administered prophylactically; that is, administered for prevention of a disease or condition.

[052] As used herein, the term "subject" refers to a target of administration. The subject of the herein disclosed methods can be a vertebrate, such as a mammal, a fish, a bird, a reptile, or an amphibian. Thus, the subject of the herein disclosed methods can be a human, non-human primate, horse, pig, rabbit, dog, sheep, goat, cow, cat, guinea pig or rodent. The term does not denote a particular age or sex. Thus, adult and newborn subjects, as well as fetuses, whether male or female, are intended to be covered. A patient refers to a subject afflicted with a disease or disorder. The term "patient" includes human and veterinary subjects.

[053] As used herein, the term "effective amount" refers to an amount that is sufficient to achieve a desired result or to have an effect on undesired symptoms, but is generally insufficient to cause adverse side affects. The specific effective dose level for any particular patient will depend upon a variety of factors including the disorder being treated and the severity of the disorder; the specific composition employed; the age, body weight, general health, sex and diet of the patient; the time of administration; the route of administration; the rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidental with the specific compound employed and like factors well known in the medical arts. For example, it is well within the skill of the art to start doses of a compound at levels lower than those required to achieve the desired effect and to gradually increase the dosage until the desired effect is achieved. If desired, the effective daily dose can be divided into multiple doses for purposes of administration. Consequently, single dose compositions can contain such amounts or submultiples thereof to make up the daily dose. The dosage can be adjusted by the individual physician in the event of any contraindications. Dosage can vary, and can be administered in one or more dose administrations daily, for one or several days. Guidance can be found in the literature for appropriate dosages for given classes of pharmaceutical products. In a further aspect, a preparation can be administered in a "diagnostically effective amount"; that is, an amount effective for diagnosis of a disease or condition. In a further aspect, a preparation can be administered in a "therapeutically effective amount"; that is, an amount effective for treatment of a disease or condition. In a further aspect, a preparation can be administered in a "prophylactically effective amount"; that is, an amount effective for prevention of a disease or condition.

[054] As used herein, the term "pharmaceutically acceptable carrier" refers to sterile aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, as well as sterile powders for reconstitution into sterile injectable solutions or dispersions just prior to use. Examples of suitable aqueous and nonaqueous carriers, diluents, solvents or vehicles include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol and the like), carboxymethylcellulose and suitable mixtures thereof, vegetable oils (such as olive oil) and injectable organic esters such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials such as lecithin, by the maintenance of the required particle size in the case of dispersions and by the use of

surfactants. These compositions can also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of the action of microorganisms can be ensured by the inclusion of various antibacterial and antifungal agents such as paraben, chlorobutanol, phenol, sorbic acid and the like. It can also be desirable to include isotonic agents such as sugars, sodium chloride and the like. Prolonged absorption of the injectable pharmaceutical form can be brought about by the inclusion of agents, such as aluminum monostearate and gelatin, which delay absorption. Injectable depot forms are made by forming microencapsule matrices of the drug in biodegradable polymers such as polylactide-polyglycolide, poly(orthoesters) and poly(anhydrides). Depending upon the ratio of drug to polymer and the nature of the particular polymer employed, the rate of drug release can be controlled. Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissues. The injectable formulations can be sterilized, for example, by filtration through a bacterial-retaining filter or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable media just prior to use. Suitable inert carriers can include sugars such as lactose. Desirably, at least 95% by weight of the particles of the active ingredient have an effective particle size in the range of 0.01 to 10 micrometers.

[055] As used herein, the term "biologically active agent" or "bioactive agent" means an agent that is capable of providing a local or systemic biological, physiological, or therapeutic effect in the biological system to which it is applied. For example, the bioactive agent can act to control infection or inflammation, enhance cell growth and tissue regeneration, control tumor growth, act as an analgesic, promote anti-cell attachment, and enhance bone growth, among other functions. Other suitable bioactive agents can include anti-viral agents, hormones, antibodies, or therapeutic proteins. Other bioactive agents include prodrugs, which are agents that are not biologically active when administered but, upon administration to a subject are converted to bioactive agents through metabolism or some other mechanism. Additionally, any of the compositions of the invention can contain combinations of two or more bioactive agents. It is understood that a biologically active agent can be used in connection with administration to various subjects, for example, to humans (*i.e.*, medical administration) or to animals (*i.e.*, veterinary administration).

[056] As used herein, the term "pharmaceutically active agent" includes a "drug" or a "vaccine" and means a molecule, group of molecules, complex or substance administered to an organism for diagnostic, therapeutic, preventative medical, or veterinary purposes. This term include externally and internally administered topical, localized and systemic human and animal pharmaceuticals, treatments, remedies, nutraceuticals, cosmeceuticals, biologicals, devices, diagnostics and contraceptives, including preparations useful in clinical and veterinary screening, prevention, prophylaxis, healing, wellness, detection, imaging, diagnosis, therapy, surgery, monitoring, cosmetics, prosthetics, forensics and the like. This term may also be used in reference to agriceutical, workplace, military, industrial and environmental therapeutics or remedies comprising selected molecules or selected nucleic acid sequences capable of recognizing cellular receptors, membrane receptors, hormone receptors, therapeutic receptors, microbes, viruses or selected targets comprising or capable of contacting plants, animals and/or humans. This term can also specifically include nucleic acids and compounds comprising nucleic acids that produce a bioactive effect, for example deoxyribonucleic acid (DNA) or ribonucleic acid (RNA). Pharmaceutically active agents include the herein disclosed categories and specific examples. It is not intended that the category be limited by the specific examples. Those of ordinary skill in the art will recognize also numerous other compounds that fall within the categories and that are useful according to the invention. Examples include a radiosensitizer, the combination of a radiosensitizer and a chemotherapeutic, a steroid, a xanthine, a beta-2-agonist bronchodilator, an anti-inflammatory agent, an analgesic agent, a calcium antagonist, an angiotensin-converting enzyme inhibitors, a beta-blocker, a centrally active alpha-agonist, an alpha-1-antagonist, an anticholinergic/antispasmodic agent, a vasopressin analogue, an antiarrhythmic agent, an antiparkinsonian agent, an antiangina/antihypertensive agent, an anticoagulant agent, an antiplatelet agent, a sedative, an ansiolytic agent, a peptidic agent, a biopolymeric agent, an antineoplastic agent, a laxative, an antidiarrheal agent, an antimicrobial agent, an antifungal agent, a vaccine, a protein, or a nucleic acid. In a further aspect, the pharmaceutically active agent can be coumarin, albumin, bromolidine, steroids such as betamethasone, dexamethasone, methylprednisolone, prednisolone, prednisone, triamcinolone, budesonide, hydrocortisone, and pharmaceutically acceptable hydrocortisone derivatives; xanthines such as theophylline and doxophylline; beta-2-agonist bronchodilators such as salbutamol,

fenterol, clenbuterol, bambuterol, salmeterol, fenoterol; antiinflammatory agents, including antiasthmatic anti-inflammatory agents, antiarthritis antiinflammatory agents, and non-steroidal antiinflammatory agents, examples of which include but are not limited to sulfides, mesalamine, budesonide, salazopyrin, diclofenac, pharmaceutically acceptable diclofenac salts, nimesulide, naproxene, acetominophen, ibuprofen, ketoprofen and piroxicam; analgesic agents such as salicylates; calcium channel blockers such as nifedipine, amlodipine, and nicardipine; angiotensin-converting enzyme inhibitors such as captopril, benazepril hydrochloride, fosinopril sodium, trandolapril, ramipril, lisinopril, enalapril, quinapril hydrochloride, and moexipril hydrochloride; beta-blockers (i.e., beta adrenergic blocking agents) such as sotalol hydrochloride, timolol maleate, esmolol hydrochloride, carteolol, propanolol hydrochloride, betaxolol hydrochloride, penbutolol sulfate, metoprolol tartrate, metoprolol succinate, acebutolol hydrochloride, atenolol, pindolol, and bisoprolol fumarate; centrally active alpha-2-agonists such as clonidine; alpha-1-antagonists such as doxazosin and prazosin; anticholinergic/antispasmodic agents such as dicyclomine hydrochloride, scopolamine hydrobromide, glycopyrrolate, clidinium bromide, flavoxate, and oxybutynin; vasopressin analogues such as vasopressin and desmopressin; antiarrhythmic agents such as quinidine, lidocaine, tocainide hydrochloride, mexiletine hydrochloride, digoxin, verapamil hydrochloride, propafenone hydrochloride, flecainide acetate, procainamide hydrochloride, moricizine hydrochloride, and disopyramide phosphate; antiparkinsonian agents, such as dopamine, L-Dopa/Carbidopa, selegiline, dihydroergocryptine, pergolide, lisuride, apomorphine, and bromocryptine; antiangina agents and antihypertensive agents such as isosorbide mononitrate, isosorbide dinitrate, propranolol, atenolol and verapamil; anticoagulant and antiplatelet agents such as coumadin, warfarin, acetylsalicylic acid, and ticlopidine; sedatives such as benzodiazapines and barbiturates; ansiolytic agents such as lorazepam, bromazepam, and diazepam; peptidic and biopolymeric agents such as calcitonin, leuprolide and other LHRH agonists, hirudin, cyclosporin, insulin, somatostatin, protirelin, interferon, desmopressin, somatotropin, thymopentin, pidotimod, erythropoietin, interleukins, melatonin, granulocyte/macrophage-CSF, and heparin; antineoplastic agents such as etoposide, etoposide phosphate, cyclophosphamide, methotrexate, 5-fluorouracil, vincristine, doxorubicin, cisplatin, hydroxyurea, leucovorin calcium, tamoxifen, flutamide, asparaginase, altretamine, mitotane, and procarbazine hydrochloride; laxatives such as

senna concentrate, casanthranol, bisacodyl, and sodium picosulphate; antidiarrheal agents such as difenoxine hydrochloride, loperamide hydrochloride, furazolidone, diphenoxylate hdyrochloride, and microorganisms; vaccines such as bacterial and viral vaccines; antimicrobial agents such as penicillins, cephalosporins, and macrolides, antifungal agents such as imidazolic and triazolic derivatives; and nucleic acids such as DNA sequences encoding for biological proteins, and antisense oligonucleotides. It is understood that a pharmaceutically active agent can be used in connection with administration to various subjects, for example, to humans (*i.e.*, medical administration) or to animals (*i.e.*, veterinary administration).

[057] A residue of a chemical species, as used in the specification and concluding claims, refers to the moiety that is the resulting product of the chemical species in a particular reaction scheme or subsequent formulation or chemical product, regardless of whether the moiety is actually obtained from the chemical species. Thus, an ethylene glycol residue in a polyester refers to one or more $-OCH_2CH_2O-$ units in the polyester, regardless of whether ethylene glycol was used to prepare the polyester. Similarly, a sebacic acid residue in a polyester refers to one or more $-CO(CH_2)_8CO-$ moieties in the polyester, regardless of whether the residue is obtained by reacting sebacic acid or an ester thereof to obtain the polyester.

[058] As used herein, the term "substituted" is contemplated to include all permissible substituents of organic compounds. In a broad aspect, the permissible substituents include acyclic and cyclic, branched and unbranched, carbocyclic and heterocyclic, and aromatic and nonaromatic substituents of organic compounds. Illustrative substituents include, for example, those described below. The permissible substituents can be one or more and the same or different for appropriate organic compounds. For purposes of this disclosure, the heteroatoms, such as nitrogen, can have hydrogen substituents and/or any permissible substituents of organic compounds described herein which satisfy the valences of the heteroatoms. This disclosure is not intended to be limited in any manner by the permissible substituents of organic compounds. Also, the terms "substitution" or "substituted with" include the implicit proviso that such substitution is in accordance with permitted valence of the substituted atom and the substituent, and that the substitution

results in a stable compound, *e.g.*, a compound that does not spontaneously undergo transformation such as by rearrangement, cyclization, elimination, etc.

[059] In defining various terms, "A^1," "A^2," "A^3," and "A^4" are used herein as generic symbols to represent various specific substituents. These symbols can be any substituent, not limited to those disclosed herein, and when they are defined to be certain substituents in one instance, they can, in another instance, be defined as some other substituents.

[060] The term "alkyl" as used herein is a branched or unbranched saturated hydrocarbon group of 1 to 24 carbon atoms, such as methyl, ethyl, *n*-propyl, isopropyl, *n*-butyl, isobutyl, *s*-butyl, *t*-butyl, *n*-pentyl, isopentyl, *s*-pentyl, neopentyl, hexyl, heptyl, octyl, nonyl, decyl, dode cyl, tetradecyl, hexadecyl, eicosyl, tetracosyl, and the like. The alkyl group can also be substituted or unsubstituted. The alkyl group can be substituted with one or more groups including, but not limited to, optionally substituted alkyl, cycloalkyl, alkoxy, amino, ether, halide, hydroxy, nitro, silyl, sulfo-oxo, or thiol, as described herein. A "lower alkyl" group is an alkyl group containing from one to six carbon atoms.

[061] Throughout the specification "alkyl" is generally used to refer to both unsubstituted alkyl groups and substituted alkyl groups; however, substituted alkyl groups are also specifically referred to herein by identifying the specific substituent(s) on the alkyl group. For example, the term "halogenated alkyl" specifically refers to an alkyl group that is substituted with one or more halide, *e.g.*, fluorine, chlorine, bromine, or iodine. The term "alkoxyalkyl" specifically refers to an alkyl group that is substituted with one or more alkoxy groups, as described below. The term "alkylamino" specifically refers to an alkyl group that is substituted with one or more amino groups, as described below, and the like. When "alkyl" is used in one instance and a specific term such as "alkylalcohol" is used in another, it is not meant to imply that the term "alkyl" does not also refer to specific terms such as "alkylalcohol" and the like.

[062] This practice is also used for other groups described herein. That is, while a term such as "cycloalkyl" refers to both unsubstituted and substituted cycloalkyl moieties, the substituted moieties can, in addition, be specifically identified herein; for example, a particular substituted cycloalkyl can be referred to as, *e.g.*, an "alkylcycloalkyl." Similarly, a substituted alkoxy can be specifically referred to as, *e.g.*, a "halogenated

alkoxy," a particular substituted alkenyl can be, *e.g.*, an "alkenylalcohol," and the like. Again, the practice of using a general term, such as "cycloalkyl," and a specific term, such as "alkylcycloalkyl," is not meant to imply that the general term does not also include the specific term.

[063] The term "polyalkylene group" as used herein is a group having two or more CH_2 groups linked to one another. The polyalkylene group can be represented by the formula $—(CH_2)_a—$, where "a" is an integer of from 2 to 500.

[064] The terms "alkoxy" and "alkoxyl" as used herein to refer to an alkyl or cycloalkyl group bonded through an ether linkage; that is, an "alkoxy" group can be defined as $—OA^1$ where A^1 is alkyl or cycloalkyl as defined above. "Alkoxy" also includes polymers of alkoxy groups as just described; that is, an alkoxy can be a polyether such as $—OA^1—OA^2$ or $—OA^1—(OA^2)_a—OA^3$, where "a" is an integer of from 1 to 200 and A^1, A^2, and A^3 are alkyl and/or cycloalkyl groups.

[065] The term "alkenyl" as used herein is a hydrocarbon group of from 2 to 24 carbon atoms with a structural formula containing at least one carbon-carbon double bond. Asymmetric structures such as $(A^1A^2)C=C(A^3A^4)$ are intended to include both the *E* and *Z* isomers. This can be presumed in structural formulae herein wherein an asymmetric alkene is present, or it can be explicitly indicated by the bond symbol C=C. The alkenyl group can be substituted with one or more groups including, but not limited to, optionally substituted alkyl, cycloalkyl, alkoxy, alkenyl, cycloalkenyl, alkynyl, cycloalkynyl, aryl, heteroaryl, aldehyde, amino, carboxylic acid, ester, ether, halide, hydroxy, ketone, azide, nitro, silyl, sulfo-oxo, or thiol, as described herein.

[066] The term "alkynyl" as used herein is a hydrocarbon group of 2 to 24 carbon atoms with a structural formula containing at least one carbon-carbon triple bond. The alkynyl group can be unsubstituted or substituted with one or more groups including, but not limited to, optionally substituted alkyl, cycloalkyl, alkoxy, alkenyl, cycloalkenyl, alkynyl, cycloalkynyl, aryl, heteroaryl, aldehyde, amino, carboxylic acid, ester, ether, halide, hydroxy, ketone, azide, nitro, silyl, sulfo-oxo, or thiol, as described herein.

[067] As used herein, the term "peptide amphiphile" refers to a peptide compound possessing both a hydrophilic portion (e.g., a hydrophilic peptide sequence moiety) and a

hydrophobic portion (e.g., a hydrocarbon moiety). One property typically associated with a peptide amphiphile can be self-assembly.

[068] As used herein, the term "hydrophilic peptide sequence" refers to a peptide residue sequence having hydrophilicity properties relative to a hydrocarbon moiety. A hydrophilic peptide sequence can comprise one or more functional peptide sequences (e.g., degradable peptide sequences, nitric oxide donors, and/or cell adhesive ligands).

[069] As used herein, the term "degradation sequence" refers to a sequence of peptide residues that can be degraded by enzymes or hydrolysis under biological conditions.

[070] As used herein, the term "cell adhesive sequence" refers to a sequence of peptide residues capable of operation as adhesive ligands for cells. In one aspect, due to amphilic characteristic of peptide amphiphiles, the disclosed cell adhesive sequences are exposed at the exterior surface of a nanofiber assembly; thus, such cell adhesive sequence can be available for interaction with one or more cells. One example is an "endothelial cell adhesive sequence," which refers to a peptide sequence that supports endothelial cell adhesion, spreading, migration, and/or growth.

[071] As used herein, the term "nitric oxide producing donor sequence" refers to a peptide residue (e.g., lysine (K) or cysteine (C)) or sequence of peptide residues (e.g., polylysine (KKKKK) or polycysteine (CCCCC)) capable of reversibly binding nitric oxide gas, or equivalent thereof) as a complex (e.g., diazoniumdiolates). Thus, the peptide or sequence can serve as a reservoir for nitric oxide gas and can selectively release nitric oxide over time. It is understood that the term can include other nitric oxide doners, for example any peptide sequences containing cystine or amine groups.

[072] As used herein, the term "self-assembling" refers to the characteristic of a plurality of molecules of a compound in which a disordered system forms a more organized structure or pattern as a consequence of specific, local interactions among the molecules themselves, without external direction. In one aspect, peptide amphiphiles can be self-assembling. In a further aspect, a plurality of molecules of a compound can self-assemble into nanofibers. In a yet further aspect, peptide amphiphiles can self-assemble into nanofibers. In a still further aspect, peptide amphiphiles can self-assemble into nanofibers without the need for cross-linking.

B. COMPOSITIONS

[073] Biological compatibility of artificial implants can be improved by mimicking natural systems. Consequently, an ideal stent should have properties similar to the natural endothelium. Thus, disclosed herein is a natural endothelium mimic nanomatrix.

[074] Natural endothelium consists of endothelial cells embedded in a viscoelastic extracellular matrix (ECM). In addition to providing structural integrity, ECM also provides a dynamic, functional environment, which is crucial for cell proliferation, differentiation, and migration (Ross JM. 1998). This concept has inspired the use of ECM-derived cell adhesive ligands for biomimetic scaffolds to control cellular behaviors. Controlling the degradation kinetics of scaffolds is another important factor because the degradation rate affects the ECM production *in vitro* and tissue formation *in vivo* (Alsberg E, et al. 2003; Bryant SJ, et al. 2002). Cell migration and proliferation are dependent on cell adhesion for many different cell types (Ross JM. 1998). Natural ECM consists of many self-assembled nanostructured fibrillar proteins. Endothelium also serves to maintain the non-thrombogenic environment of the blood vessel by releasing soluble factors like nitric oxide (NO). Therefore, natural endothelium mimicking nanomatrix can be designed to imitate this chemical and biological complexity of the endothelial ECM: 1) endothelial cell adhesive moieties to promote strong endothelial cell retention and migration, 2) cell-mediated degradable sites for endothelial cell migration into the nanomatrix for strong endothelializaiton, 3) self-assembled nanofibrous structure similar to the natural ECM, and 4) release of NO to promote homing of satellite endothelial progenitor cells, along with inhibiting restenosis and thrombosis.

[075] As disclosed herein, a natural endothelium mimic nanomatrix can be produced from peptide amphiphiles comprising a hydrophilic peptide and a hydrophobic tail, wherein the hydrophilic peptide comprises one or more of endothelial cell adhesive moieties to promote strong endothelial cell retention and migration, cell-mediated degradable sites for endothelial cell migration into the nanomatrix for strong endothelializaiton, and release of NO to promote homing of satellite endothelial progenitor cells, along with inhibiting restenosis and thrombosis.

[076] Thus, provided herein is a peptide amphiphile, comprising a hydrophilic peptide sequence and a hydrophobic tail, wherein the hydrophilic peptide sequence comprises a

degradation sequence and one or more of a first cell adhesive sequence and a nitric oxide producing donor sequence, wherein the first adhesive sequence is an endothelial cell adhesive sequence that does not bind to smooth muscle cells and/or platelets.

[077] Thus, the hydrophilic peptide sequence can comprise the formula:

DS---CA,

wherein --- is a direct or indirect covalent linkage, wherein "DS" is a degradation sequence; and wherein "CA" is an endothelial cell adhesive sequence.

[078] The hydrophilic peptide sequence can comprises the formula:

DS---KK,

wherein --- is a direct or indirect covalent linkage, wherein "DS" is a degradation sequence; and wherein "KK" is a nitric oxide producing donor sequence.

[079] The hydrophilic peptide sequence can comprises the formula:

DS---CA---KK,

wherein --- is a direct or indirect covalent linkage, wherein "DS" is a degradation sequence; wherein "CA" is an endothelial cell adhesive sequence; and wherein "KK" is a nitric oxide producing donor sequence.

[080] The hydrophilic peptide sequence can comprise the formula:

DS---KK---CA,

wherein --- is a direct or indirect covalent linkage, wherein "DS" is a degradation sequence; wherein "CA" is an endothelial cell adhesive sequence; and wherein "KK" is a nitric oxide producing donor sequence.

[081] As disclosed herein, the peptide amphiphiles can be used to form a nanomatrix. It is understood and herein contemplated that the nanomatrices herein can comprise amphiphile coated nanofibers. As disclosed herein, the nanofibers can comprise polyester fibers such as, for example, polycaprolactone fibers. Thus, disclosed herein are, for

example, polycaprolactone nanofibers coated with the peptide amphiphiles disclosed herein.

[082] Also disclosed herein is a composition comprising a first and second peptide amphiphile, each independently comprising a hydrophilic peptide sequence and a hydrophobic tail, wherein the hydrophilic peptide sequence of the first peptide amphiphile comprises a degradation sequence and an endothelial cell adhesive sequence, and wherein the hydrophilic peptide sequence of the second peptide amphiphile comprises a degradation sequence and a nitric oxide producing donor sequence.

[083] Thus, the hydrophilic peptide sequence of the first peptide amphiphile can comprise the formula:

DS---CA,

wherein each --- is independently a direct or indirect covalent linkage, wherein "DS" is a degradation sequence; and wherein "CA" is an endothelial cell adhesive sequence; and the hydrophilic peptide sequence of the second peptide amphiphile can comprise the formula:

DS---KK,

wherein "KK" is a nitric oxide producing donor sequence.

[084] The first and second peptide amphiphiles can be present in the disclosed composition at a ratio of from about 1:20 to about 20:1, including about 20:1, 19:1, 18:1, 17:1, 16:1, 15:1, 14:1, 13:1, 12:1, 11:1, 10:1, 9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, 1:10, 1:11, 1:12, 1:13, 1:14, 1:15, 1:16, 1:17, 1:18, 1:19, or 1:20. Thus, the first and second peptide amphiphiles can be present in the composition at a ratio of from about 1:9 to about 9:1.

[085] The herein disclosed compositions can further comprise one or more additional peptide amphiphiles comprising a degradation sequence but not comprising either a endothelial cell adhesive sequence or nitric oxide producing donor sequence. These one or more additional peptide amphiphiles can comprise additional functional moieties. These one or more additional peptide amphiphiles can comprise non-functional sequences. The skilled artisan can use these additional peptide amphiphiles to control the concentration of

the first and second peptide amphiphiles. For example, wherein the first and second peptide amphiphiles are present in the composition at a ratio of about 9:1, the can also be present in the composition with a third peptide amphiphile at a ratio of, for example, about 9:1:0.1, 9:1:0.2, 9:1:0.3, 9:1:0.4, 9:1:0.5, 9:1:0.6, 9:1:0.7, 9:1:0.8, 9:1:0.9, 9:1:1, 9:1:2, 9:1:3, 9:1:4, 9:1:5, 9:1:6, 9:1:7, 9:1:8, 9:1:9, 9:1:10. Many other such combinations and mixtures can be selected by the skilled artisan and tested for optimal performance using routine skill.

[086] The herein disclosed compositions can further comprise polyester nanofibers such as a polycaprolactone nanofiber. Thus, for example, disclosed herein are polycaprolactone nanofibers coated with a first and second peptide amphiphile, wherein the first peptide amphiphile is PA-YIGSR and the second peptide amphiphile is PA-KKKKK; and wherein the ration of the first peptide amphiphile to the second peptide amphiphile is 9:1.

1. Peptide Amphiphile

[087] A peptide amphiphile (PA) is an amphiphilic structure typically with a short hydrophilic peptide sequence conjugated with a single hydrophobic tail (Paramonov SE, et al. 2006). Depending on their shape, charge, and environment these molecules can self-assemble into sheets, spheres, rods, disks, or channels (Lowik D, et al. 2004). Amphiphiles with a conical shape, such that the hydrophilic head group is bulkier than the hydrophobic tail, are known to form cylindrical micelles (Jun HW, et al. 2006). Amphiphilic nature of the PAs is responsible for their self-assembly. In a neutral solution, the negatively charged amino acids in the backbone of the PAs can help to solubilize it. In order to induce self-assembly, the repelling forces due to presence of negative charges can be eliminated. This can be achieved by lowering the pH of the PA solution or addition of divalent ions (Hartgerink JD, et al. 2002; Hartgerink JD, et al. 2001; Jun HW, et al. 2005). In a self-assembled nanofiber structure, the four amino acids closest to the core can form hydrogen bonds. The presence or absence of these hydrogen bonds can define the cylindrical or the spherical orientation of the self-assembled structure (Paramonov SE, et al. 2006).

[088] Amphiphilicity is widely found in natural biological systems, such as cell membranes. It is the major driving force responsible for the self-assembly of biomolecules into supramolecular structures with complex hierarchical order. This concept indicates the use of PAs for biomimetic scaffolds (Curtis A, et al. 2001; Barnes CP, et al. 2007). PAs

consisting of various bioactive sequences, like cell adhesive ligands or biodegradable sequences, can be self-assembled under physiological conditions to form supramolecular structures similar to the naturally occurring biomolecules. Recently, they have been extensively studied for various biomedical applications including growth of blood vessels (Malkar NB, et al. 2003; Hosseinkhani H, et al. 2006; Rajangam K, et al. 2006) and bone tissue repair (Hosseinkhani H, et al. 2007; Hosseinkhani H, et al. 2006; Sargeant TD, et al. 2008).

[089] PAs are attractive templates for biomimetic scaffolds because of the ease of incorporating different cell adhesion and degradation moieties in their backbone (Jun HW, et al. 2006; Jun HW, et al. 2005).

[090] The herein disclosed peptide amphiphile can in some aspects be any modified peptide capable of self-assembling into a nanomatrix. In some aspects, the disclosed peptide amphiphile comprises a hydrophilic peptide and a hydrophobic tail. The length of the hydrophilic peptide and hydrophobic tail can be selected such that the peptide amphiphile maintains the ability to self-assemble into a nanomatrix. Thus, for example, the length of the hydrophobic tail can be increased to accommodate for an increased length in the hydrophilic peptide. The skilled artisan can use routine skill to screen for the ability of a peptide amphiphile with a selected hydrophilic peptide and a selected hydrophobic tail to self-assemble into a nanomatrix.

2. Nitric Oxide (NO)

[091] NO released from the endothelium is known to block a number of key events in the restenosis cascade. NO plays a pivotal role in regulating vessel wall homeostasis (Marin J, et al. 1997; Kuo PC, et al. 1995; Davies KM, et al. 2001). It is continuously released from amino acid L-arginine in healthy endothelial cells by the enzyme, nitric oxide synthase. NO released from the endothelium stimulates soluble guanylyl cyclase, increasing concentrations of cyclic guanosine monophosphate (GMP)(Kuo PC, et al. 1995). The increase of cyclic GMP levels in vascular smooth muscle cells underlying the endothelium leads to activation of GMP-dependent kinases that decrease intracellular calcium, resulting in relaxation of smooth muscle cells (SMCs). Local relaxation of SMCs in response to sudden constriction of a blood vessel is critical for maintaining laminar blood flow (Beckman JS. 1996). NO released into the blood vessel lumen increases cyclic GMP levels

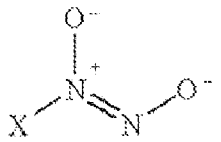
in platelets as well. It decreases platelet activation and adhesion to the surface of the endothelium, rendering the vascular wall non-thrombogenic. NO regulates the cellular environment within the blood vessel by inhibiting the activity of growth factors released from the platelets (Kuo PC, et al. 1995). Given its antithrombogenic role, the incorporation of NO is expected to improve the biological properties of vascular prostheses. NO-releasing polymers have been successfully developed such as poly(vinyl chloride), silicone rubber, polymethacrylate, and polyurethane for uses as non-thrombogenic coatings for cardiovascular devices (Reynolds MM, et al. 2004; Verma S, et al. 2005). For example, NO has been successfully incorporated into vascular grafts in the form of diazeniumdiolates (a special class of compound capable of releasing NO in blood)(Pulfer SK, et al. 1997). Initial studies in rabbits show that controlled release of NO from NO-containing microspheres loaded in channeled stents limited in-stent restenosis (Do Y, et al. 2004). In another 28 day study, stent-based nitric oxide delivery from sodium nitroprusside has been investigated in porcine models and shown to reduce neointimal proliferation (Hou D, et al. 2005). In addition to the aforementioned functions, NO is also known for its ability to promote endothelial cell growth, survival and migration (Ziche M, et al. 1994; Kawasaki K, et al. 2003). NO also plays a critical role in neovascularization partly by recruitment of circulating endothelial progenitor cells (Aicher A, et al. 2003). To this effect, local delivery of NO from hydrogels has been shown to enhance the rate of vessel re-endothelialization (Lipke EA, et al. 2005).

[092] The antithrombogenic, homeostatic and pro-endothelialization properties of NO make it an attractive candidate as a therapeutic drug coating on stents. However, the short half-life of only a few seconds in vivo makes NO unsuitable for direct administration as a systemic drug (Miller M, et al. 2007). Also, NO gas is difficult to handle due to the necessity of complete oxygen exclusion, which is needed to prevent its oxidation. Hence, NO carriers are used to stabilize it until the time of release (Hanson SR, et al. 1995; Miller M, et al. 2007). Currently, two types of NO donor drugs are clinically used: (1) organic nitrates and (2) sodium nitroprusside (Miller M, et al. 2007). Nitroglycerine is a clinically used organic nitrate. It is used in transdermal patches for the treatment of heart failure and chronic angina. Nitroglycerine contains three nitroxy-ester (nitrite) groups, which undergo enzyme-mediated release. The major limiting factor of this drug is the development of tolerance after prolonged continuous use. Sodium nitroprusside is used on-site in hospitals

to reduce blood pressure in hypersensitive crises. It contains an iron molecule coordinated to five cyanide molecules and one molecule of NO. The NO molecule is rapidly released during infusion, whereas the cyanide molecules are liberated gradually. These cyanide molecules can reach toxic levels in some cases, presenting a major limitation to the use of this drug (Gori T, et al. 2002).

[093] Disclosed herein is a class of NO-releasing compound called diazeniumdiolates, also known as NONOates. The chemical structure of these compounds can be inferred from the name: diazen N=N, ium: formal positive charge, diolate: two negative oxygens (Paramonov SE, et al. 2006; Saavedra J, et al. 2000).

[094] The following is the chemical structure of a diazeniumdiolate:



[095] Diazeniumdiolates can be formed by the reaction of a nucleophilic amine (X-) with NO as shown in the following reaction:

$$X^- + 2NO \rightarrow X\text{-}[N(O)NO]^-$$

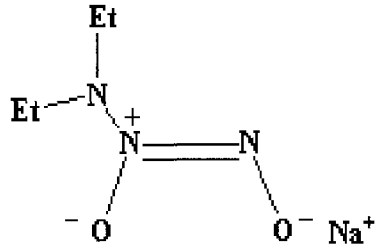
[096] Diazoniumdiolates then dissociate on protonation to release free NO as shown in the following reaction:

$$X\text{-}[N(O)NO]^- \rightarrow X^- + 2NO$$

[097] Owing to its electron acceptor properties, NO can react with nucleophilic amines to form diazoniumdiolates. When dissolved in buffer, blood, or cell culture medium, the diazeniumdiolates undergo protonation and disassociation to release NO (Saavedra J, et al. 2000; Keefer LK, et al. 1996; Hrabie JA, et al. 2002). The release kinetics can be controlled by the structure of the nucleophilic amines. Diazeniumdiolates can be synthesized by direct reaction with amines under high pressure in the absence of air.

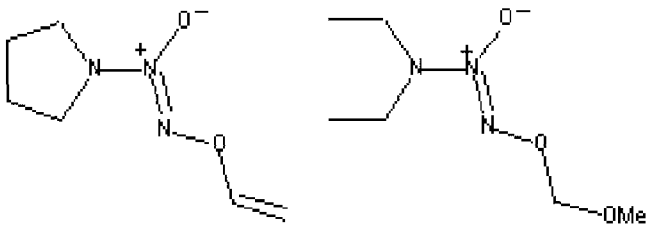
[098] Other nitric oxide-releasing diazeniumdiolate compounds are known in the art. For example, nitrogen- and carbon-based NO donor complexes are available in the art with half-lives at physiological pH ranging from a few seconds to many days.

[099] The most common diazeniumdiolates are formed by the reaction of secondary amines and polyamines with nitric oxide in basic media. These are stable solids, capable of regenerating 2 equivalents of nitric oxide and the starting amine in neutral or acidic buffers. The half-life of NO generation varies from a few seconds to many hours depending on the amine. The decomposition to NO is a spontaneous, first-order reaction at constant pH. An example of an underivatized diazeniumdiolate:



i. O-Derivatized diazeniumdiolates

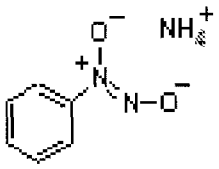
[0100] The diazeniumdiolate anions react with electrophiles to produce stable covalent compounds. These compounds have the ability to act as prodrugs, releasing nitric oxide only when metabolically converted to the diazeniumdiolate anion. Several compounds of this class have been synthesized by reaction of alkyl or aryl halides, sulfate esters, epoxides, etc. with the ionic diazeniumdiolates. Examples of O-derivatized diazeniumdiolates:

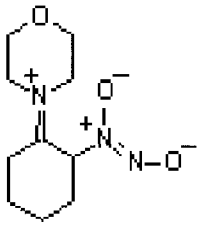


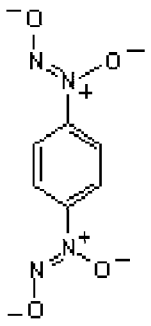
ii. C-Based diazeniumdiolates

[0101] Compounds containing the diazeniumdiolate group bound to carbon have been known for over 100 years under names such as "isonitramines", and "nitrosohydroxylamines" even though these are not accurate descriptions of the bonding as

shown by numerous X-ray structural determinations. While attachment to a carbon obviously presents great advantages of flexibility for the design of new NO donors, it must be recognized that not all of these materials produce NO spontaneously. The range of reactivity of these compounds runs the full scale from materials which are so stable that no NO is ever produced (rare) to those which decompose violently (also rare). Many also produce mixtures of NO and N_2O rather than pure NO. Examples of C-based diazeniumdiolates:

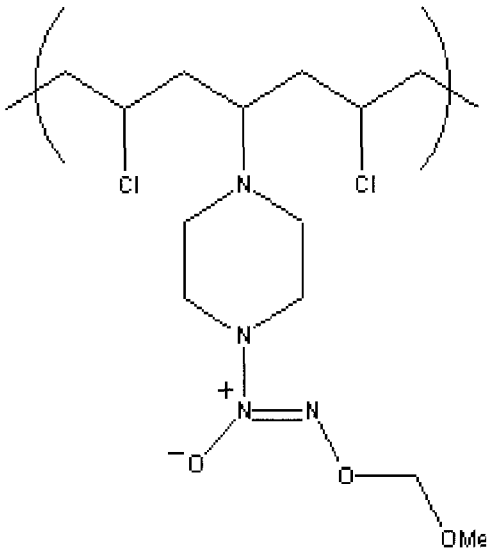






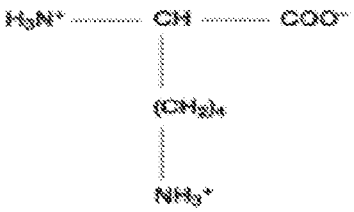
iii. Polymer-based diazeniumdiolates

[0102] To modulate the time course of NO release and also limit NO exposure to selected sites of the body, the diazeniumdiolate functional group can be incorporated into polymeric matrices. The NO-releasing polymers range from films, microspheres and gels to powders and moldable resins. Polymeric diazeniumdiolates have been shown to improve thromboresistivity in intra-arterial devices and can serve as important tools in cardiovascular research. Example of a polymer-based diazeniumdiolate:



iv. Lysine

[0103] Preferrably, the nucleophilic amine is on the side chain (R group) of an amino acid (natural or artificial). For example, the nucleophilic amine can be on the side chain (R group) of lysine. As shown below, lysine has two pendant amine groups for NO binding.



[0104] Controlled release of NO from lysine-based polymers has been shown to reduce platelet attachment and smooth muscle cell proliferation, which are major causes of restenosis (Jun H, et al. 2005; Bohl KS, et al. 2000; Jun HW, et al. 2005). The lysine residues can have pendant amine groups that react with NO to form a diazeniumdiolate-modified peptide $[K[N(O)NO^-]]_n$.

[0105] Thus, the nitric oxide producing donor sequence can comprise one or more diazeniumdiolate-modified lysine residues. Thus, the nitric oxide producing donor sequence can comprise the diazeniumdiolate-modified amino peptide $[K[N(O)NO^-]]_n$, wherein "n" is from 1 to 20. In some aspects, n is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or higher. Thus, the nitric oxide producing donor sequence can comprise the diazeniumdiolate-modified peptide $[K[N(O)NO^-]]_5$.

[0106] The nitric oxide producing donor sequence can comprise one or more lysine residues comprising pendant amine groups that react with NO to form a diazeniumdiolate-modified peptide. Thus, the nitric oxide producing donor sequence can comprise the amino acid sequence Lys-Lys-Lys-Lys-Lys (SEQ ID NO:3), wherein one or more of the lysine residues comprise pendant amine groups that react with NO to form a diazeniumdiolate-modified peptide.

[0107] The nitric oxide producing donor sequence can comprise one or more cysteine residues comprising pendant thiol groups that react with NO to form a modified peptide. Thus, the nitric oxide producing donor sequence can comprise the amino acid sequence

Cys-Cys-Cys-Cys-Cys (SEQ ID NO:17), wherein one or more of the cysteine residues comprise pendant thiol groups that react with NO to form a modified peptide.

[0108] It is understood that each lysine residue can be a donor for two nitric oxide molecules. The number of lysine residues or diazeniumdiolate-modified lysine residues can therefore be selected based on the amount of NO desired. This can further be regulated by the artisan by selecting the amount or concentration of the peptide amphiphiles comprising these lysine residues. For example, disclosed herein is an endothelial mimicking matrix comprising a first PA comprising an endothelial cell adhesion sequence ("PA-YIGSR") and a second PA comprising a NO donor sequence ("PA-KKKKK") at a ratio of 9:1, wherein PA-KKKKK comprises five diazeniumdiolate-modified lysine residues. The skilled artisan could therefore achieve the same result by increasing the number of diazeniumdiolate-modified lysine residues in the second peptide amphiphile and comcomitently increasing the ratio of the first PA to second PA (>9:1).

[0109] Thus, it is understood herein that the rate and duration of NO release can be modulated by increasing or decreasing the number lysines in the second PA or altering the ration of the first and second peptide amphiphile such that an increase in the number of lysines or an increase in the proportion of the second amphiphile increase the rate and/or duration of NO relaease. Thus, for example, the rate and/or duration of NO release can be increased by changing the number of lysines in the second amphiphile from PA-KKKKK to , for example, PA-KKKKKK, PA-KKKKKKK, PA-KKKKKKKK, PA-KKKKKKKKK, or PA-KKKKKKKKKK. Also, by increasing the ratio of the second amphiphile relative to the first amphiphile, the duration and/or rate of NO release can be increased. Thus, the rate and/or duration of NO release will be increased by changing the PA-YIGSR to PA-KKKKK ratio from 9:1 to, for example 9:2, 3:1, 9:4, 2:1, 9:5, 3:2, 9:7, 9:8, 1:1, 9:10, 9:11, 3:4, 3:5, 1:2, 3:7, 3:8, 1:3 or any ration in between.

[0110] Simlarly, the rate and/or duration of NO release can be decreased by reducing the ratio of PA-YIGSR to PA-KKKKK or removing lysines from PA-KKKKK. Thus, a ratio of PA-YIGSR to PA-KKKKK of 10:1, 11:1, 12:1, 13:1 14:1, 15:1, 16:1, 17:1, 18:1, 19:1, 20:1 or any ration in between will decrease the rate and/or duration of NO release relative to a 9:1 ratio. Also, decreasing the number of lysines from five in PA-KKKKK will

decrease the rate and/ or duration of NO release (i.e., PA-KKKK, PA-KKK, PA-KK, or PA-K).

3. EC Adhesion

[0111] Retention of endothelial cells in the lumen of DES is critical to its performance. Endothelialization results in a non-thrombogenic coating to the exposed stent surface, the absence of which leaves the stent struts in direct contact with flowing blood and eventual thrombus formation. Laminin is the major non-collagenous glycoprotein component of basement membranes and is a mediator of cell adhesion, migration, growth, and differentiation (Beck K, et al. 1990). YIGSR (SEQ ID NO: 2) is a laminin-derived cell adhesive sequence, known to enhance attachment, spreading, and migration of endothelial cells (Hubbell JA, et al. 1991). Cell spreading via YIGSR (SEQ ID NO: 2) is mediated by a 67-kDa cell membrane associated receptor (Massia SP, et al. 1993). Use of YIGSR (SEQ ID NO: 2) for modification of surfaces like polyethylene terephthalate and polytetrafluoroethylene was shown to selective enhance EC adhesion, spreading, migration (Massia SP, et al. 1991; Fittkau MH, et al. 2005) and promote EC colony formation.

[0112] The incorporation of YIGSR (SEQ ID NO: 2) sequence in polyurethane has shown to enhance endothelial cell adhesion and spreading (Jun H, et al. 2004). NO releasing polyurethanes were developed by incorporating lysine-based diazeniumdiolates in the polymer (Jun HW, et al. 2005). This polymer has been shown to dramatically decrease platelet adhesion and inhibit smooth muscle cell proliferation, while stimulating endothelial cell adhesion. Furthermore, it has been observed that the incorporation of YIGSR (SEQ ID NO: 2) sequence into the NO releasing polymer backbone enhances endothelial cell proliferation while at the same time inhibiting platelet attachment (Taite LJ, et al. 2008).

[0113] The disclosed hydrophilic peptide can therefore comprise a first adhesive sequence that selectively binds endothelial cells and thus selectively promotes the binding of endothelial cells to a nanomatrix comprising the disclosed peptide amphiphiles. Thus, the disclosed hydrophilic peptide can comprise a first adhesive sequence comprising an endothelial cell adhesive sequence that binds to endothelial cells but does not substantially bind to smooth muscle cells and/or platelets. Thus, the endothelial cell adhesive sequence

of the disclosed hydrophilic peptide can comprise the amino acid sequence Tyr-Ile-Gly-Ser-Arg (YIGSR ; SEQ ID NO:2).

[0114] The hydrophilic peptide of the disclosed peptide amphiphile can further comprise one more additional cell adhesive sequences. In some aspects, the one more additional cell adhesive sequences bind endothelial cells and thus promote the binding of endothelial cells to a nanomatrix comprising the disclosed peptide amphiphiles. Thus, the one more additional cell adhesive sequences can be one or more of Arg-Gly-Asp (SEQ ID NO:8), Arg-Gly-Asp-Ser (SEQ ID NO:9), Asp-Gly-Glu-Ala (SEQ ID NO:10), Val-Ala-Pro-Gly (SEQ ID NO:11), Arg- Glu-Asp-Val (SEQ ID NO:12), Asp-Gly-Glu-Ala (SEQ ID NO:13), and Lys-Arg-Ser-Arg (SEQ ID NO:14). Other such cell adhesive sequences, including endothelial cell adhesive sequences (selective and non-selective) are known and can be used in the disclosed peptide amphiphiles. The skilled artisan can screen peptide amphiphiles comprising candidate cell adhesive sequences using routine *in vitro* methods.

4. Degradation Sequence

[0115] The degradation sequence can comprise an amino acid sequence that undergoes cell-mediated proteolytic degradation.

[0116] In some aspects, the degradation sequence comprises a matrix metalloprotease (MMP) specific cleavage site. (MMPs are zinc-dependent endopeptidases belonging to a larger family of proteases known as the metzincin superfamily.

[0117] The most commonly used groupings of MMPs are based partly on historical assessment of the substrate specificity of the MMP and partly on the cellular localization of the MMP. These groups are the collagenases, the gelatinases, the stromelysins, and the membrane type MMPs (MT-MMPs).

[0118] The collagenases are capable of degrading triple-helical fibrillar collagens into distinctive 3/4 and 1/4 fragments. These collagens are the major components of bone and cartilage, and MMPs are the only known mammalian enzymes capable of degrading them. Traditionally, the collagenases are MMP1, MMP8, MMP13, and MMP18. In addition, MMP14 has also been shown to cleave fibrillar collagen, and more controversially there is evidence that MMP2 is capable of collagenolysis.

[0119] The main substrates of the gelatinases are type IV collagen and gelatin, and these enzymes are distinguished by the presence of an additional domain inserted into the catalytic domain. This gelatin-binding region is positioned immediately before the zinc binding motif, and forms a separate folding unit which does not disrupt the structure of the catalytic domain. The gelatinases are MMP2 and MMP9.

[0120] The stromelysins display a broad ability to cleave extracellular matrix proteins but are unable to cleave the triple-helical fibrillar collagens. The three canonical members of this group are MMP3, MMP10, and MMP11.

[0121] All six membrane type MMPs (MMP14, MMP15, MMP16, MMP17, MMP24, and MMP25) have a furin cleavage site in the pro-peptide, which is a feature also shared by MMP11.

[0122] Examples of additional MMP cleavage sites are known and described, for example, in Handbook of proteolytic enzymes, Edited by Alan J. Barrett, Neil D. Rawlings, J. Fred Woessner, Academic Press.

Table 1: MMPs

MMP	Group	Cleavage Site	SEQ ID NO:
MMP1	collagenases	GPQGIWGQ	SEQ ID NO:16
MMP2	gelatinases	GTAGLIGQ or GPQGLLGA	SEQ ID NO:1 SEQ ID NO:15

[0123] Thus, the degradation sequence comprises a matrix metalloprotease-2 (MMP2) specific cleavage site. For example, the degradation sequence can comprise the amino acid sequence Gly-Thr-Ala-Gly-Leu-Ile-Gly-Gln (SEQ ID NO: 1:)

[0124] Incorporation of an MMP specific sequence, such as an MMP-2 specific sequence, in the PA backbone can ensure that the nanomatrix undergoes cell-mediated proteolytic degradation, enabling cell migration through the nanomatrix and eventual remodeling with natural ECM (Jun HW, et al. 2005; Giannelli G, et al. 1997). For MMP2, this cleavage is expected between glycine and leucine residues (Jun HW, et al. 2005).

5. Hydrophobic Tail

[0125] The hydrophobic tail can comprise a moiety having an optionally substituted C4 or larger alkyl chain. Thus, the hydrophobic tail can comprise a moiety having an optionally substituted C6 to C28 or larger alkyl chain. Thus, the hydrophobic tail can comprise a moiety having an optionally substituted C10 to C25 or larger alkyl chain. Thus, the hydrophobic tail can comprise a moiety having an optionally substituted C5, C5, C6, C7, C8, C9, C10, C11, C12, C13, C14, C15, C16, C17, C18, C19, C20, C21, C22, C23, C24, C25, C26, C27, C28, or larger alkyl chain. Thus, the hydrophobic tail can comprise a moiety having an optionally substituted C16 alkyl chain.

6. Specific Embodiments

[0126] The hydrophilic peptide sequence can comprise the amino acid sequence Gly-Thr-Ala-Gly-Leu-Ile-Gly-Gln (SEQ ID NO:1) and the amino acid sequence Tyr-Ile-Gly-Ser-Arg (SEQ ID NO:2). Thus, the hydrophilic peptide sequence can comprise the amino acid sequence Gly-Thr-Ala-Gly-Leu-Ile-Gly-Gln-Tyr-Ile-Gly-Ser-Arg (SEQ ID NO:4).

[0127] The hydrophilic peptide sequence can comprise the amino acid sequence Gly-Thr-Ala-Gly-Leu-Ile-Gly-Gln (SEQ ID NO:1) and the amino acid sequence Lys-Lys-Lys-Lys-Lys (SEQ ID NO:3), wherein the lysine residues comprises pendant amine groups that react with NO to form a diazeniumdiolate-modified peptide. Thus, the hydrophilic peptide sequence can comprise the amino acid sequence Gly-Thr-Ala-Gly-Leu-Ile-Gly-Gln-Lys-Lys-Lys-Lys-Lys (SEQ ID NO:5), wherein the lysine residues comprises pendant amine groups that react with NO to form a diazeniumdiolate-modified peptide.

[0128] The hydrophilic peptide sequence can comprise the amino acid sequence Gly-Thr-Ala-Gly-Leu-Ile-Gly-Gln (SEQ ID NO:1), the amino acid sequence Tyr-Ile-Gly-Ser-Arg (SEQ ID NO:2), and amino acid sequence Lys-Lys-Lys-Lys-Lys (SEQ ID NO:3), wherein the lysine residues comprises pendant amine groups that react with NO to form a diazeniumdiolate-modified peptide. Thus, the hydrophilic peptide sequence can comprise the hydrophilic peptide sequence comprises the amino acid sequence Gly-Thr-Ala-Gly-Leu-Ile-Gly-Gln-Tyr-Ile-Gly-Ser-Arg-Lys-Lys-Lys-Lys-Lys (SEQ ID NO:6), wherein the lysine residues comprises pendant amine groups that react with NO to form a diazeniumdiolate-modified peptide. Thus, the hydrophilic peptide sequence can comprise the amino acid sequence Gly-Thr-Ala-Gly-Leu-Ile-Gly-Gln-Lys-Lys-Lys-Lys-Lys-Tyr-

Ile-Gly-Ser-Arg (SEQ ID NO:7), wherein the lysine residues comprises pendant amine groups that react with NO to form a diazeniumdiolate-modified peptide.

7. Electrospinning

[0129] The technique of electrospinning, also known within the fiber forming industry as electrostatic spinning, of liquids and/or solutions capable of forming fibers, is well known and has been described in a number of patents as well as in the general literature. Typically, the process of electrospinning generally involves the creation of an electrical field at the surface of a liquid. Fibers produced by this process have been used in a wide variety of applications, and are known, from U.S. Pat. Nos. 4,043,331 and 4,878,908, to be particularly useful in forming non-woven structures. The resulting electrical forces create a jet of liquid which carries electrical charge. Thus, the liquid jets maybe attracted to other electrically charged objects at a suitable electrical potential. As the jet of liquid elongates and travels, it will harden and dry. The hardening and drying of the elongated jet of liquid may be caused by cooling of the liquid, i.e., where the liquid is normally a solid at room temperature; evaporation of a solvent, e.g., by dehydration, (physically induced hardening); or by a curing mechanism (chemically induced hardening). The produced fibers are collected on a suitably located, oppositely charged receiver and subsequently removed from it as needed, or directly applied to an oppositely charged generalized target area.

[0130] In one aspect, electrospinning (ES) is an atomization process of fluid which exploits the interactions between an electrostatic field and the fluid. In one aspect, the fluid can be a conducting fluid. During electrospinning, fibers with micron or sub-micron sized diameters are extruded be means of an electrostatic potential from a polymer solution (see U.S. Pat. No. 1,975,504 to Formhals). When an external electrostatic field is applied to a fluid (e.g., a semi-dilute polymer solution or a polymer melt), a suspended conical droplet is in equilibrium with the electric field. Electrostatic atomization occurs when the electrostatic field is strong enough to overcome the surface tension of the liquid. The liquid droplet then becomes unstable and a tiny jet is ejected from the surface of the droplet. As it reaches a grounded target, the material can be collected as an interconnected web containing relatively fine, i.e. small diameter, fibers. The resulting films (or membranes) from these small diameter fibers have very large surface area to volume ratios

and small pore sizes. This process typically yields non-woven mats or felts composed of round fibers that are extremely pliable. Due to their high-surface area and good mechanical characteristics, electrospun meshes have traditionally found applications in filtration and composite reinforcement. For the very same reasons, felts and meshes derived from biocompatible polymers such as poly(lactic acid) and its copolymer with glycolic acid and other polyesters are being explored as substrates (scaffolds) for association of cells in the engineering of tissue (see Kenawy et al., Biomaterials, 2003, 24 (6), 907 describing making a fiber by electrospinning process from a single-phase system containing ethylene vinyl alcohol, 70% propanol and 30% water). Such pliable porous media is particularly suited for engineering of skin, vascular, and neural prostheses.

[0131] Parameters that can be varied in the ES process are the electric field, the distance between the "Taylor Cone" and the target, and the polymer solution viscosity (Fridrikh et al., G. C. Phys Rev Lett. 2003, 90(14), 144502). Due to the complexity of the fiber forming process, very few attempts have been made to alter geometry of electrospun fibers. Recently, Reneker and coworkers have observed the formation of branched and ribbon-like fibers in some solvent systems and have attributed this to the collapse of a polymer skin due to buckling instability similar to that seen in garden hoses (see Koombhongse et al., Polym. Sci.: Part B: Polym. Phys. 2001, 39, 2598-2606). However, the formation of such fibers is not achievable in a predictable manner under generally known ES operating conditions. U.S. Pat. Nos. 4,323,525 and 4,689,186 to Bornat, incorporated by reference herein, are directed to processes for the production of tubular products by electrostatically spinning a liquid containing a fiber-forming material.

[0132] ES is a process through which fibers with micron or sub-micron sized diameters are extruded from a polymer solution by means of an electrostatic potential (FIG. 5). In a typical ES process, the polymer solution is injected through a nozzle while being subjected to a high voltage DC field (e.g., 5-30 kV). Under such conditions, the polymer solution erupts into a "Taylor Cone" due to the droplet being subjected to a phenomenon called "Raleigh's Instability," which leads to whipping of the polymer jet. As the jet is propelled, the formation of fibers is facilitated by solvent evaporation and thinning of the jet. The parameters that can be varied to affect fiber morphology include the electric field strength, the distance between the "Taylor Cone" and the target and viscosity of polymer solution.

Due to the complexity of the "Taylor Cone" formation, most attempts at controlling fiber morphology have focused on controlling polymer solution properties. This can be achieved by either increasing the polymer concentration or molecular weight or increasing volatility of the organic solvent; all of which accelerate the rate at which the polymer fibers solidifies during spinning. In general, increasing viscosity and solvent volatility results in thicker fibers.

[0133] A limitation of conventional approaches is that they do not enable altering of other fiber properties such as aspect ratio (rounder versus flatter fibers) and fiber porosity both of which can severely impact cellular interactions by increasing surface area, which can be a desired property in cell contacting applications and tissue engineering (TE).

[0134] Good mechanical properties, high surface area to weight ratios, and pliability have made electrospun fibers candidates for a wide range of applications in filtration and composite reinforcement. These characteristics, combined with specific polymer properties, also make electrospun felts ideal for tissue engineering scaffolds as well as drug delivery devices. Electrospun materials typically possess a high aspect ratio, which can be a desired property for various application, for example tissue engineering (TE) applications.

[0135] Fiber diameter is typically controlled by changing electric field strength (either by changing applied voltage or tip-to-target distance), changing evaporation rates (via changing the spinning environment or using solvents of different volatilities), or by changing polymer concentration. The last method enjoys particular popularity among researchers since polymer concentration is an easy variable to control and can have repeatable and drastic effects on fiber diameters. This method works by changing both the amount of solvent that must evaporate before a solid fiber precipitates from the solution and by changing the viscosity of the solution, and hence, "Taylor cone" formation and final jet diameter.

[0136] In conventional methods, surface geometry and morphology of electrospun nanofibers has been more difficult to modify. Typical electrospun fibers adopt a circular cross-section, though porous and flat fiber morphologies have been observed in several polymer/solvent systems, but little research has found success at controlling these

morphologies. Common techniques used to modify fiber cross-sectional shape have been to cospin polymers and selectively remove certain polymer phases. More recent approaches have succeeded in producing hollow fiber morphologies by using an immiscible second phase and coaxial spinnerets. Both techniques involve either complicated processing steps or specialized electrospinning apparatus to achieve the desired final shape.

[0137] One of the most modulated parameters in conventional electrospinning techniques involves changing the concentration of the dissolved polymer. This typically has the effect of being able to control the final fiber diameter by changing how the fiber formation process and timescale during electrospinning. One of the more important parameters coupled with polymer concentration is the viscosity of the solution. Viscosity plays a large role in "Taylor cone" formation and stability.

[0138] However, changing the concentration of the polymer solution has two limits. Low concentration solutions can lack the viscosity to properly form a "Taylor cone." In conventional techniques, instead of drawing a single, electrified jet from the spinneret, the jet is broken down into multiple droplets. This process is called electrospraying and has been utilized in processes like applying surface coatings and inkjet printing.

[0139] The PAs disclosed herein can be coated onto an electrospun nanofiber, such as, for example, a polyester nanofiber (e.g., an electrospun polycaprolactone (ePCL) nanofiber). The PAs disclosed herein can be coated onto an ePCL in one of several ways. A pressure gradient can be used in the form of a syringe. The ePCL nanofibers disc can be placed in the barrel of a syringe, and PA solution can be pushed through it. This can be repeated from the other side. Alternatively, the ePCL nanofibers can be coated with PAs with a rotational technique. The ePCL nanofibers can be mounted on a mandrel and rotated, while they are immersed partially in a PA solution.

[0140] Polymers suitable for nanofiber formation include those suitable for electrospinning. For example, a polymer can be a polyester, polyamide, polyurethane, polyolefin, or polycarbonate. In one aspect, a polymer can be an amorphous biodegradable polyester. It is contemplated that the polymer can be branched or straight-

chain. Copolymers, including block and graft copolymers, are also contemplated. Polymer mixtures can also be employed in the disclosed methods and compositions.

[0141] In one aspect, suitable polymers include polyesters; polyanhydrides; polyorthoesters; polyphosphazenes; polyphosphates; polyphosphoesters; polydioxanones; polyphosphonates; polyhydroxyalkanoates; polycarbonates; polyalkylcarbonates; polyorthocarbonates; polyesteramides; polyamides; polyamines; polypeptides; polyurethanes; polyetheresters; polyalkylene glycols; polyalkylene oxides; polypeptides; polysaccharides; polyvinyl pyrrolidones and combinations thereof.

[0142] In a further aspect, suitable polymers include poly(lactide)-co-(polyalkylene oxide); poly(lactide-co-glycolide)-co-(polyalkylene oxide); poly(lactide-co-caprolactone)-b-(polyalkylene oxide); and poly(lactide-co-glycolide-co-caprolactone)-b-(polyalkylene oxide).

[0143] In a further aspect, suitable polymers include poly(lactide); poly(glycolide); poly(caprolactone); poly(valerolactone); poly(hydroxybutyrate); poly(lactide-co-glycolide); poly(lactide-co-caprolactone); poly(lactide-co-valerolactone); poly(glycolide-co-caprolactone); poly(glycolide-co-valerolactone); poly(lactide-co-glycolide-co-caprolactone); and poly(lactide-co-glycolide-co-valerolactone).

[0144] In a further aspect, suitable polymers include poly(lactide)-co-poly(vinylpyrrolidone); poly(lactide-co-glycolide)-co-poly(vinylpyrrolidone); poly(lactide-co-caprolactone)-b-poly(vinylpyrrolidone); and poly(lactide-co-glycolide-co-caprolactone)-b-poly(vinylpyrrolidone).

[0145] In one aspect, the polymer fibers can comprise any biocompatible polymer known to those of skill in the art. In a further aspect, the polymer fibers comprise poly(lactic acid), poly(glycolic acid), or poly(ε-caprolactone), or a copolymer thereof, or a mixture thereof. In a further aspect, the polymer of the fibers can be polyethylene and/or polyurethane. In a further aspect, a polymer can be poly(lactide-co-glycolide), poly(lactic acid), poly(glycolic acid), poly(glaxanone), poly(orthoesters), poly(pyrolic acid), and poly(phosphazenes). Additional polymers that can be used include, but are not limited to, polyalkylene polymers and copolymers, fluorocarbon polymers and copolymers, polyester polymers and copolymers, polyether polymers and copolymers, silicone polymers and

copolymers, and polyurethane polymers and copolymers. Other polymers that can be used include, but are not limited to, polyethylenes, polypropylenes, polytetrafluoroethylenes, poly(tetrafluoroethylene-co-hexafluoropropenes), modified ethylene-tetrafluoroethylene copolymers, ethylene chlorotrifluoroethylene copolymers, polyvinylidene fluorides, polyethylene oxides, polyethylene terephthalates, silicones, polyurethanes, polyether block amides, and polyether esters. In a further aspect, the polymer can be one or more polymers, for example, polypyrrole, polyaniline, polythiophene, poly(*p*-phenylene vinylene), polyparalene, or a mixture thereof. In a further aspect, the polymer can be poly (ethylene-vinyl acetate).

[0146] In one aspect, the polymer is a biocompatible polymer. In one aspect, the polymer is polycaprolactone. In a further aspect, polycaprolactone is used as the hydrophobic block of amphiphilic synthetic block copolymer.

[0147] In one aspect, the polymer is a degradable polymer. By "degradable," it is meant that the polymer breaks down under certain conditions after a period of time. In a further aspect, the polymer is a "biodegradable" polymer, breaking down under physiological conditions after a period of time. In a further aspect, the polymer is non-degradable and/or non-biodegradable. For example, the polymer can comprises one or more hydrolyzable bonds. The presence of hydrolyzable bonds can facilitate degradation of nanofibers in biological systems. In a further aspect, the polymer does not comprise one or more hydrolyzable bonds.

8. ECM Mimicking Nanomatrix

[0148] Disclosed herein is an endothelium mimicking nanomatrix, comprising one or more of the herein disclosed peptide amphiphiles assembled into nanofibers. The nanofibers can comprise a mixture of peptide amphiphiles having formulas DS---CA and DS---KK.

[0149] The DS---CA and DS---KK peptide amphiphiles can be present in the nanofibers at a ratio of from about 1:20 to about 20:1, including about 20:1, 19:1, 18:1, 17:1, 16:1, 15:1, 14:1, 13:1, 12:1, 11:1, 10:1, 9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, 1:10, 1:11, 1:12, 1:13:, 1:14, 1:15, 1:16, 1:17, 1:18, 1:19, or 1:20. Thus, the nanofibers can comprise a mixture of peptide amphiphiles having formulas DS---CA and

DS---KK. The DS---CA and DS---KK peptide amphiphiles can be present in the nanofibers at a ratio of from about 1:9 to about 9:1. As disclosed herein, this ratio can be selected by the skilled artisan based in part on the number of diazeniumdiolate-modified lysine residues in the one or more peptide amphiphiles and the desired NO release.

[0150] The DS---CA peptide amphiphile of the disclosed endothelium mimicking nanomatrix can comprise the amino acid sequence Gly-Thr-Ala-Gly-Leu-Ile-Gly-Gln (SEQ ID NO:1) and the amino acid sequence Tyr-Ile-Gly-Ser-Arg (SEQ ID NO:2). Thus, the DS---CA peptide amphiphile of the disclosed endothelium mimicking nanomatrix can comprise the amino acid sequence Gly-Thr-Ala-Gly-Leu-Ile-Gly-Gln-Tyr-Ile-Gly-Ser-Arg (SEQ ID NO:4).

[0151] The DS---KK peptide amphiphile of the disclosed endothelium mimicking nanomatrix can comprise the amino acid sequence Gly-Thr-Ala-Gly-Leu-Ile-Gly-Gln (SEQ ID NO:1) and one or more lysine residues comprising pendant amine groups that can react with nitric oxide to form a diazeniumdiolate-modified peptide. Thus, the DS---KK peptide amphiphile of the disclosed endothelium mimicking nanomatrix can comprise the amino acid sequence Gly-Thr-Ala-Gly-Leu-Ile-Gly-Gln (SEQ ID NO:1) and the amino acid sequence Lys-Lys-Lys-Lys-Lys (SEQ ID NO:3), wherein the lysine residues comprises pendant amine groups that react with nitric oxide to form a diazeniumdiolate-modified peptide. Thus, the DS---KK peptide amphiphile of the disclosed endothelium mimicking nanomatrix can comprise the amino acid sequence Gly-Thr-Ala-Gly-Leu-Ile-Gly-Gln-Lys-Lys-Lys-Lys-Lys (SEQ ID NO:5), wherein the lysine residues comprises pendant amine groups that react with NO to form a diazeniumdiolate-modified peptide. In some aspects, the DS---KK peptide amphiphile has reacted with nitric oxide to form a diazeniumdiolate-modified peptide $[K[N(O)NO^-]]_n$. In some aspects, n can be from 1 to 20. In some aspects, n is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or higher. In some aspects, the DS---KK peptide amphiphile has reacted with nitric oxide to form a diazeniumdiolate-modified peptide $[K[N(O)NO^-]]_5$.

[0152] The nanofibers of the disclosed endothelium mimicking nanomatrix can comprise peptide amphiphiles having the formula DS---CA---KK, DS---KK---CA, or a combination thereof. For example, the DS---CA---KK and DS---KK---CA peptide amphiphiles can comprise the amino acid sequence Gly-Thr-Ala-Gly-Leu-Ile-Gly-Gln (SEQ ID NO:1), the

amino acid sequence Tyr-Ile-Gly-Ser-Arg (SEQ ID NO:2), and the amino acid sequence Lys-Lys-Lys-Lys-Lys (SEQ ID NO:3), wherein the lysine residues comprises pendant amine groups that react with NO to form a diazeniumdiolate-modified peptide. For example, the DS---CA---KK peptide amphiphile can comprise the amino acid sequence Gly-Thr-Ala-Gly-Leu-Ile-Gly-Gln-Tyr-Ile-Gly-Ser-Arg-Lys-Lys-Lys-Lys-Lys (SEQ ID NO:6), wherein the lysine residues comprises pendant amine groups that react with NO to form a diazeniumdiolate-modified peptide. The DS---KK---CA peptide amphiphile can comprise the amino acid sequence Gly-Thr-Ala-Gly-Leu-Ile-Gly-Gln-Lys-Lys-Lys-Lys-Lys-Tyr-Ile-Gly-Ser-Arg (SEQ ID NO:7), wherein the lysine residues comprises pendant amine groups that react with NO to form a diazeniumdiolate-modified peptide.

[0153] In some aspects, the peptide amphiphile of the disclosed endothelium mimicking nanomatrix has reacted with nitric oxide to form a diazeniumdiolate-modified peptide $[K[N(O)NO^-]]_n$. In some aspects, n is from 1 to 20. In some aspects, n is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or higher. Thus, in some aspects, the peptide amphiphile has reacted with nitric oxide to form a diazeniumdiolate-modified peptide $[K[N(O)NO^-]]_5$.

[0154] Disclosed herein are hybrid nanomatrices that comprising electrospun polycaprolactone (ePCL) nanofibers coated with NO releasing peptide amphiphiles (PAs). Electrospinning has been garnering a lot of attention recently (Li WJ, *et al.* 2003; Choi JS, *et al.* 2008; Zhang YZ, *et al.* 2005; Matthews JA, et al. 2002; and Heydarkhan-Hagvall S, et al. 2008), due to its ability to fabricate uniform polymer fibers with diameters in the nanoscale ranges. These fibers are structurally similar to nanofibrillar ECM proteins such as collagen. However, these nanofibers are hindered by their lack of bioactivity, which is crucial for interacting with the cells and promoting their adhesion and proliferation. Disclosed herein, this bioactivity can be endowed upon these ePCL nanofibers by coating them with peptide amphiphiles (PAs). These PAs can carry enzyme mediated degradable sites and cell adhesive ligands, and can therefore, mimic the biochemical aspect of ECM. This coating of PAs onto ePCL nanofibers constitutes the hybrid biomimetic nanomatrix, as shown in *Figure 19*. The PAs used in the study consist of matrix metalloprotease-2 (MMP-2) degradable site Gly-Thr-Ala-Gly-Leu-Ile-Gly-Gln (GTAGLIGQ). MMPs are constitutively produced by healthy cells, and therefore, the presence of this site promotes

the remodeling of the nanomatrix by the cells (Jun *et al.* 2005; Massia SP and Hubbell JA: 1991; and Andukuri A, *et al.* 2009). The PAs also contain laminin derived Tyr-Ile-Gly-Ser-Arg (YIGSR) sequence, which is known to promote endothelial cell adhesion and spreading, and a polylysine (KKKKK) sequence that acts as an NO donor. The incorporation of NO donating residues into the PA is expected to provide controlled release of NO from the hybrid nanomatrix into the local blood stream, where it will limit smooth muscle cell proliferation and platelet adhesion, while enhancing re-endothelialization.

9. Stents

[0155] Also disclosed herein is a composition comprising a medical device coated with an endothelium mimicking nanomatrix disclosed herein. The medical device can be any device known or identified for use inside the body of a subject. Preferably, the medical device is one that is inserted into the cardiovascular system. The medical device can comprise any material suitable for use as a surgical implant.

[0156] Most stents are crafted from 316L stainless steel. Current examples include the Cordis Palmaz-Schatz stent, the Cordis Crossflex stent, the Guidant MultiLink stent, and the Medtronic Bestent. Disadvantages of steel stents include the high occurrence of subacute thrombosis and restenosis, bleeding complications, corrosion, and re-dilation of the stented vessel segment.

[0157] Gold has long been known as a highly visible, bio-compatible, and usually inert metal. Gold-plated hybrid stents exhibit good visibility and flexibility, but are also quite expensive.

[0158] Currently, Conichrome®, PhynoxTM and Elgiloy® are trademark names for the cobalt-chromium-nickel-molybdenum-iron alloy. This cobalt chromium alloy can be used for manufacturing stents like the Schneider Wallstent.

[0159] Tantalum, element #73, is a shiny, flexible, and highly radio-opaque metal. Though more brittle than stainless steel, tantalum exhibits high ductility and resistance to corrosion. Current examples of tantalum stents include the Wiktor Stent by Medtronic and the Tantalum Cordis Stent.

[0160] Nitinol (from the "Nickel Titanium Naval Ordinance Laboratory") is an example of a biocompatible, super-elastic shape-memory alloy. As a shape-memory alloy consisting of 55% nickel and 45% titanium, nitinol has the ability to return to a specific shape upon heating to a certain temperature after its phase transition. Shape-memory alloys undergo a phase transition in their crystal structure when cooled from their stronger, higher temperature form in the Austenitic phase to their weaker, lower temperature form in the Martensitic phase. Nitinol also has a springy, "rubber-like" behavior that allows it to be super-elastic and contorted at its austenitic temperature. The strong intermetallic bond between nickel and titanium has a very low reaction rate, even in patients with increased sensitivity to nickel. This prevents a strong immunological response and decreases corrosion. Present examples include Boston Scientific's Nitinol-self-expanding Radius stent. Boston Scientific's Symbiot stent, availablein Europe, is comprised of nitinol covered on both sides by 16-micron thick layers of ePTFE.

[0161] Materials for polymer stents include biodegradable stents coupled with polymeric endoluminal paving, and shape-memory polymers. Silicone was the first organic material chosen for stenting. Silicone is a condensation polymer derived from alternating silicone and oxygen atoms which induces low rates of tissue trauma. However, silicone has poor biodurability, tensile and coil strength, and inner to outer diameter ratio.

[0162] Pure plastic biliary stents using polyethylene or polyurethane have also been used in patients. However, polyethylene induces sludge in 20-30% of patients, encourages protein adherence and biofilm formation, and entraps bile crystals and food particles. In contrast, polyurethane has good tensile and coil strength, and good biodurability, but it is also one of the most reactive materials available.

[0163] Biodegradable and bioabsorbable stents are also viable materials for stenting. Though biodegradation, bioabsorption, and bioerosion are often used incorrectly as synonyms, they have different definitions. In biodegradation, a biological agent like an enzyme or a microbe is the dominant component in the degradation process. Biodegradable implants are usually useful for short-term or temporary applications. Bioresorption and bioabsorption imply that the degradation products are removed by cellular activity, such as phagocytosis, in a biological environment. By contrast, a bioerodible polymer is a water-insoluble polymer that has been converted under

physiological conditions into water-soluble materials. This occurs regardless of the physical mechanism involved in the erosion process. The prefix "bio" in this case refers to erosion occurring in physiological conditions, as opposed to erosion via high temperature, strong acids or bases, or weather.

[0164] Because of a stent's temporary structural support to damaged blood vessels, biodegradable polymers can be viewed as a biocompatible, yet easily disposable material, perfect for drug delivery systems. Some biodegradable polymers, such as polyesters, polyorthoesters, and polyanhydrides, may be able to modulate the local delivery of drugs and also degrade "safely" via hydrolytic and other mechanisms. Biodegradable drug delivery systems require steady degradation, permeability, and moderate tensile strength. In a stent, structural support must be accompanied by biocompatibility, hemocompatibility, and good hemodynamics. Currently, biodegradable stents usually induce thrombosis and vascular injury.

[0165] The Duke Bioabsorbable Stent was the first biodegradable stent. Others have also tried incorporating natural polymers by forming Type I collagen from purified bovine Achilles' tendons into a tube without slotted sides which was chemically cross-linked for structural stability. Collagen is quite hemocompatible because it carries an inherently negative charge. Collagen products are biocompatible throughout their lifecycle, and have shown a decrease in thromobosis. Also, anticoagulants and fibrinolytic agents can be bound directly to collagen, which aids in its capacity for drug delivery. Cordis Corporation has also developed a biodegradable stent prototype crafted from a blend of polylactide and trimethylene carbonate.

[0166] Some factors that accelerate polymer degradation include providing the product with a more hydrophilic backbone, more hydrophilic endgroups, less crystallinity, more porosity, and a smaller overall size. The most common chemical functional groups used are esters, anhydrides, orthoesters, and amides.

[0167] A final polymeric possibility is shape-memory polymers. Once the polymer is synthesized, it can be heated or cooled into myriad shapes. Upon introducing a suitable stimulus, the polymer will transition from its temporary state to a memorized, permanent shape. Most of these polymers are created from suitable segments, primarily determined

by screening the qualities of existing aliphatic polyesters, especially poly(etherester)s, as well as L,L-dilactide, diglycolid, and p-dioxanone. Macrodiols can be synthesized based on these already-approved monomers.

[0168] Thus, the herein disclosed medical device, such as stent, can comprise titanium alloy. The medical device can comprise cobalt-cromium. The medical device can comprise nickle-titanium. The medical device can comprise a biodegradable polymer.

[0169] In some aspects, the medical device is a vascular stent. In some aspects, the stent is a drug eluting stent. For example, the stent can be a sirolimus-eluting stent or a paclitaxel-eluting stent.

[0170] The skilled artisan can appreciate additional medical devices for use with the disclosed endothelium mimicking nanomatrix. Preferably, the medical device is one administered to a tissue or organ of the body normally comprising a natural endothelium. For example, in some aspects, the medical device is a vascular graft. In some aspects, the medical device is a catheter. In some aspects, the medical device is a pacemaker. In some aspects, the medical device is a heart valve.

[0171] Also disclosed are methods of implanting the disclosed coated medical devices into a subject. Thus, in one aspect, a method comprises the steps of providing a composition comprising a medical device (e.g., stent, vascular graft, catheter, pacemaker, or heart valve) coated with an endothelium mimicking nanomatrix and implanting the coated substrate into a subject. In a further aspect, providing is coating a medical device with an endothelium mimicking nanomatrix. In a further aspect, a method comprises the step of coating an endothelium mimicking nanomatrix onto a medical device before implantation into a subject. In a further aspect, a method comprises the step of coating an endothelium mimicking nanomatrix onto a medical device after implantation into a subject.

10. Methods of Treatment

[0172] The peptide amphiphiles disclosed herein can be used as part of a nanomatrices to coat stents or other medical devices such as heart valves. Similarly, the peptide amphiphiles can be used in conjunction with vascular grafts such as artial or venous grafts to prevent restenosis of the graft. Thus, disclosed herein are methods of treating a

cardiovascular disease comprising administering to a subject with cardiovascular disease the peptide amphiphiles disclosed herein.

[0173] It is understood that the peptide amphiphiles disclosed herein can be administered by the application of a medical device such as a vascular stent, vascular graft, catheter, pacemaker, or heart valve coated with the peptide amphiphiles. It is further understood that the peptide amphiphiles can be used as part of a nanomatrix such as a polycaprolactone nanofiber in its use on the medical device. Thus, for example, disclosed herein are methods of treating atherosclerosis by administering to a subject a stent comprising a nanofiber such as a polyester nanofiber (e.g., polycaprolactone nanofibers) coated with one or more peptide amphiphiles. It is understood, that any of the peptide amphiphiles disclosed herein can be used with the disclosed methods, such as, for example, one or more peptide amphiphiles wherein each amphiphile comprises a hydrophilic peptide sequence and a hydrophobic tail, wherein the hydrophilic peptide sequence comprises a degradation sequence and one or more of a first cell adhesive sequence and a nitric oxide producing donor sequence.

[0174] Similarly, one of the challenges with treatments of cardiovascular disease is the prevention of further occlusion if a graft is used. Specifically, occlusion of the graft. Graft patency refers to the ability of a graft to remain open or unoccluded. Thus, disclosed herein are methods of increasing graft patency comprising administering to a subject a graft coated with one or more of the peptide amphiphiles disclosed herein.

11. Peptides

[0175] As discussed herein there are numerous variants of the functional peptides/ proteins that are known and herein contemplated. Protein variants and derivatives are well understood to those of skill in the art and in can involve amino acid sequence modifications. For example, amino acid sequence modifications typically fall into one or more of three classes: substitutional, insertional or deletional variants. Insertions include amino and/or carboxyl terminal fusions as well as intrasequence insertions of single or multiple amino acid residues. Insertions ordinarily will be smaller insertions than those of amino or carboxyl terminal fusions, for example, on the order of one to four residues. Immunogenic fusion protein derivatives, such as those described in the examples, are made by fusing a polypeptide sufficiently large to confer immunogenicity to the target

sequence by cross-linking in vitro or by recombinant cell culture transformed with DNA encoding the fusion. Deletions are characterized by the removal of one or more amino acid residues from the protein sequence. Typically, no more than about from 2 to 6 residues are deleted at any one site within the protein molecule. These variants ordinarily are prepared by site specific mutagenesis of nucleotides in the DNA encoding the protein, thereby producing DNA encoding the variant, and thereafter expressing the DNA in recombinant cell culture. Techniques for making substitution mutations at predetermined sites in DNA having a known sequence are well known, for example M13 primer mutagenesis and PCR mutagenesis. Amino acid substitutions are typically of single residues, but can occur at a number of different locations at once; insertions usually will be on the order of about from 1 to 10 amino acid residues; and deletions will range about from 1 to 30 residues. Deletions or insertions preferably are made in adjacent pairs, i.e. a deletion of 2 residues or insertion of 2 residues. Substitutions, deletions, insertions or any combination thereof may be combined to arrive at a final construct. The mutations must not place the sequence out of reading frame and preferably will not create complementary regions that could produce secondary mRNA structure. Substitutional variants are those in which at least one residue has been removed and a different residue inserted in its place. Such substitutions generally are made in accordance with the following Table 2 and are referred to as conservative substitutions.

Table 2: Amino Acid Substitutions

Original Residue	Exemplary Conservative Substitutions, others are known in the art.
Ala	Ser
Arg	Lys; Gln
Asn	Gln; His
Asp	Glu
Cys	Ser
Gln	Asn, Lys
Glu	Asp
Gly	Pro
His	Asn;Gln
Ile	Leu; Val
Leu	Ile; Val
Lys	Arg; Gln
Met	Leu; Ile
Phe	Met; Leu; Tyr
Ser	Thr

Thr	Ser
Trp	Tyr
Tyr	Trp; Phe
Val	Ile; Leu

[0176] Substantial changes in function or immunological identity are made by selecting substitutions that are less conservative than those in Table 2, i.e., selecting residues that differ more significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site or (c) the bulk of the side chain. The substitutions which in general are expected to produce the greatest changes in the protein properties will be those in which (a) a hydrophilic residue, e.g. seryl or threonyl, is substituted for (or by) a hydrophobic residue, e.g. leucyl, isoleucyl, phenylalanyl, valyl or alanyl; (b) a cysteine or proline is substituted for (or by) any other residue; (c) a residue having an electropositive side chain, e.g., lysyl, arginyl, or histidyl, is substituted for (or by) an electronegative residue, e.g., glutamyl or aspartyl; or (d) a residue having a bulky side chain, e.g., phenylalanine, is substituted for (or by) one not having a side chain, e.g., glycine, in this case, (e) by increasing the number of sites for sulfation and/or glycosylation.

[0177] For example, the replacement of one amino acid residue with another that is biologically and/or chemically similar is known to those skilled in the art as a conservative substitution. For example, a conservative substitution would be replacing one hydrophobic residue for another or replacing one polar residue for another. The substitutions include combinations such as, for example, Gly, Ala; Val, Ile, Leu; Asp, Glu; Asn, Gln; Ser, Thr; Lys, Arg; and Phe, Tyr. Such conservatively substituted variations of each explicitly disclosed sequence are included within the mosaic polypeptides provided herein.

[0178] Substitutional or deletional mutagenesis can be employed to insert sites for N-glycosylation (Asn-X-Thr/Ser) or O-glycosylation (Ser or Thr). Deletions of cysteine or other labile residues also may be desirable. Deletions or substitutions of potential proteolysis sites, e.g. Arg, can be accomplished for example by deleting one of the basic residues or substituting one by glutaminyl or histidyl residues.

[0179] Certain post-translational derivatizations are the result of the action of recombinant host cells on the expressed polypeptide. Glutaminyl and asparaginyl residues are frequently post-translationally deamidated to the corresponding glutamyl and asparyl residues. Alternatively, these residues are deamidated under mildly acidic conditions. Other post-translational modifications include hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the o-amino groups of lysine, arginine, and histidine side chains (T.E. Creighton, Proteins: Structure and Molecular Properties, W. H. Freeman & Co., San Francisco pp 79-86 [1983]), acetylation of the N-terminal amine and, in some instances, amidation of the C-terminal carboxyl.

[0180] It is understood that one way to define the variants and derivatives of the disclosed proteins herein is through defining the variants and derivatives in terms of homology/identity to specific known sequences. Specifically disclosed are variants of these and other proteins herein disclosed which have at least, 70% or 75% or 80% or 85% or 90% or 95% homology to the stated sequence. Those of skill in the art readily understand how to determine the homology of two proteins. For example, the homology can be calculated after aligning the two sequences so that the homology is at its highest level.

[0181] Another way of calculating homology can be performed by published algorithms. Optimal alignment of sequences for comparison may be conducted by the local homology algorithm of Smith and Waterman Adv. Appl. Math. 2: 482 (1981), by the homology alignment algorithm of Needleman and Wunsch, J. MoL Biol. 48: 443 (1970), by the search for similarity method of Pearson and Lipman, Proc. Natl. Acad. Sci. U.S.A. 85: 2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by inspection.

[0182] The same types of homology can be obtained for nucleic acids by for example the algorithms disclosed in Zuker, M. *Science* 244:48-52, 1989, Jaeger et al. *Proc. Natl. Acad. Sci. USA* 86:7706-7710, 1989, Jaeger et al. *Methods Enzymol.* 183:281-306, 1989 which are herein incorporated by reference for at least material related to nucleic acid alignment.

[0183] It is understood that the description of conservative mutations and homology can be combined together in any combination, such as embodiments that have at least 70% homology to a particular sequence wherein the variants are conservative mutations.

[0184] As this specification discusses various proteins and protein sequences it is understood that the nucleic acids that can encode those protein sequences are also disclosed. This would include all degenerate sequences related to a specific protein sequence, i.e. all nucleic acids having a sequence that encodes one particular protein sequence as well as all nucleic acids, including degenerate nucleic acids, encoding the disclosed variants and derivatives of the protein sequences. Thus, while each particular nucleic acid sequence may not be written out herein, it is understood that each and every sequence is in fact disclosed and described herein through the disclosed protein sequence.

[0185] It is understood that there are numerous amino acid and peptide analogs which can be incorporated into the disclosed compositions. For example, there are numerous D amino acids or amino acids which have a different functional substituent than natural amino acids. The opposite stereo isomers of naturally occurring peptides are disclosed, as well as the stereo isomers of peptide analogs. These amino acids can readily be incorporated into polypeptide chains by charging tRNA molecules with the amino acid of choice and engineering genetic constructs that utilize, for example, amber codons, to insert the analog amino acid into a peptide chain in a site specific way (Thorson et al., Methods in Molec. Biol. 77:43-73 (1991), Zoller, Current Opinion in Biotechnology, 3:348-354 (1992); Ibba, Biotechnology & Genetic Engineering Reviews 13:197-216 (1995), Cahill et al., TIBS, 14(10):400-403 (1989); Benner, TIB Tech, 12:158-163 (1994); Ibba and Hennecke, Bio/technology, 12:678-682 (1994) all of which are herein incorporated by reference at least for material related to amino acid analogs).

[0186] Molecules can be produced that resemble peptides, but which are not connected via a natural peptide linkage. For example, linkages for amino acids or amino acid analogs can include CH_2NH--, --CH_2S--, --CH_2--CH_2 --, --CH=CH-- (cis and trans), --$COCH_2$ --, --$CH(OH)CH_2$--, and --CHH_2SO—(These and others can be found in Spatola, A. F. in Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins, B. Weinstein, eds., Marcel Dekker, New York, p. 267 (1983); Spatola, A. F., Vega Data (March 1983), Vol. 1, Issue 3, Peptide Backbone Modifications (general review); Morley, Trends Pharm Sci

(1980) pp. 463-468; Hudson, D. et al., Int J Pept Prot Res 14:177-185 (1979) (--CH_2NH--, CH_2CH_2--); Spatola et al. Life Sci 38:1243-1249 (1986) (--CH H_2--S); Hann J. Chem. Soc Perkin Trans. I 307-314 (1982) (--CH--CH--, cis and trans); Almquist et al. J. Med. Chem. 23:1392-1398 (1980) (--$COCH_2$--); Jennings-White et al. Tetrahedron Lett 23:2533 (1982) (--$COCH_2$--); Szelke et al. European Appln, EP 45665 CA (1982): 97:39405 (1982) (--$CH(OH)CH_2$--); Holladay et al. Tetrahedron. Lett 24:4401-4404 (1983) (--$C(OH)CH_2$--); and Hruby Life Sci 31:189-199 (1982) (--CH_2--S--); each of which is incorporated herein by reference. A particularly preferred non-peptide linkage is --CH_2NH--. It is understood that peptide analogs can have more than one atom between the bond atoms, such as b-alanine, g-aminobutyric acid, and the like.

[0187] Amino acid analogs and analogs and peptide analogs often have enhanced or desirable properties, such as, more economical production, greater chemical stability, enhanced pharmacological properties (half-life, absorption, potency, efficacy, etc.), altered specificity (e.g., a broad-spectrum of biological activities), reduced antigenicity, and others.

[0188] D-amino acids can be used to generate more stable peptides, because D amino acids are not recognized by peptidases and such. Systematic substitution of one or more amino acids of a consensus sequence with a D-amino acid of the same type (e.g., D-lysine in place of L-lysine) can be used to generate more stable peptides. Cysteine residues can be used to cyclize or attach two or more peptides together. This can be beneficial to constrain peptides into particular conformations. (Rizo and Gierasch Ann. Rev. Biochem. 61:387 (1992), incorporated herein by reference).

12. Nucleic Acids

[0189] There are a variety of molecules disclosed herein that are nucleic acid based, including for example the nucleic acids that encode, for example, the peptide amphiphiles disclosed herein. The disclosed nucleic acids can be made up of for example, nucleotides, nucleotide analogs, or nucleotide substitutes. Non-limiting examples of these and other molecules are discussed herein. It is understood that for example, when a vector is expressed in a cell, the expressed mRNA will typically be made up of A, C, G, and U. Likewise, it is understood that if, for example, an antisense molecule is introduced into a cell or cell environment through, for example exogenous delivery, it is advantageous that

the antisense molecule be made up of nucleotide analogs that reduce the degradation of the antisense molecule in the cellular environment.

[0190] A nucleotide is a molecule that contains a base moiety, a sugar moiety and a phosphate moiety. Nucleotides can be linked together through their phosphate moieties and sugar moieties creating an internucleoside linkage. The base moiety of a nucleotide can be adenin-9-yl (A), cytosin-1-yl (C), guanin-9-yl (G), uracil-1-yl (U), and thymin-1-yl (T). The sugar moiety of a nucleotide is a ribose or a deoxyribose. The phosphate moiety of a nucleotide is pentavalent phosphate. A non-limiting example of a nucleotide would be 3'-AMP (3'-adenosine monophosphate) or 5'-GMP (5'-guanosine monophosphate). There are many varieties of these types of molecules available in the art and available herein.

[0191] A nucleotide analog is a nucleotide which contains some type of modification to the base, the sugar, and/or the phosphate moieties. Modifications to nucleotides are well known in the art and would include for example, 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, and 2-aminoadenine as well as modifications at the sugar or phosphate moieties. There are many varieties of these types of molecules available in the art and available herein.

[0192] Nucleotide substitutes are molecules having similar functional properties to nucleotides, but which do not contain a phosphate moiety, such as peptide nucleic acid (PNA). Nucleotide substitutes are molecules that will recognize nucleic acids in a Watson-Crick or Hoogsteen manner, but which are linked together through a moiety other than a phosphate moiety. Nucleotide substitutes are able to conform to a double helix type structure when interacting with the appropriate target nucleic acid. There are many varieties of these types of molecules available in the art and available herein.

[0193] It is also possible to link other types of molecules (conjugates) to nucleotides or nucleotide analogs to enhance for example, cellular uptake. Conjugates can be chemically linked to the nucleotide or nucleotide analogs. Such conjugates include but are not limited to lipid moieties such as a cholesterol moiety. (Letsinger et al., Proc. Natl. Acad. Sci. USA, 1989, 86, 6553-6556). There are many varieties of these types of molecules available in the art and available herein.

[0194] A Watson-Crick interaction is at least one interaction with the Watson-Crick face of a nucleotide, nucleotide analog, or nucleotide substitute. The Watson-Crick face of a nucleotide, nucleotide analog, or nucleotide substitute includes the C2, N1, and C6 positions of a purine based nucleotide, nucleotide analog, or nucleotide substitute and the C2, N3, C4 positions of a pyrimidine based nucleotide, nucleotide analog, or nucleotide substitute.

[0195] A Hoogsteen interaction is the interaction that takes place on the Hoogsteen face of a nucleotide or nucleotide analog, which is exposed in the major groove of duplex DNA. The Hoogsteen face includes the N7 position and reactive groups (NH2 or O) at the C6 position of purine nucleotides.

[0196] There are a variety of sequences related to the protein molecules involved in the signaling pathways disclosed herein, all of which are encoded by nucleic acids or are nucleic acids. The sequences for the human analogs of these genes, as well as other anlogs, and alleles of these genes, and splice variants and other types of variants, are available in a variety of protein and gene databases, including Genbank. Those sequences available at the time of filing this application at Genbank are herein incorporated by reference in their entireties as well as for individual subsequences contained therein. Genbank can be accessed at http://www.ncbi.nih.gov/entrez/query.fcgi. Those of skill in the art understand how to resolve sequence discrepancies and differences and to adjust the compositions and methods relating to a particular sequence to other related sequences. Primers and/or probes can be designed for any given sequence given the information disclosed herein and known in the art.

C. Methods of Making ECM Mimicking Nanomatrix

[0197] Also disclosed is a method of making an endothelium mimicking nanomatrix, comprising inducing self-assembly of one or more peptide amphiphiles disclosed herein into nanofibers. The self-assembly can be induced by, for example, drying a liquid composition comprising the one or more peptide amphiphiles on a solid surface. Other methods of inducing self-assembly of peptide amphiphiles are known in the art and can be used in the disclosed methods. For example, assembly can be induced by divalent ions (calcium choloride) or pH.

[0198] The disclosed method can further comprise reacting the one or more peptide amphiphiles with nitric oxide to form a diazeniumdiolate-modified peptide. For example, diazeniumdiolate-modified peptide can comprise the sequence $[K[N(O)NO^-]]_n$. In some aspects, n is from 1 to 20. In some aspects, n is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or higher. Thus, the diazeniumdiolate-modified peptide can comprise the sequence $[K[N(O)NO^-]]_5$.

D. Methods of Using ECM Mimicking Nanomatrix

[0199] Also disclosed is a method comprising coating a medical device with an endothelium mimicking nanomatrix disclosed herein. The method can comprise inducing self-assembly of one or more peptide amphiphiles disclosed herein into nanofibers on the medical device. For example, the method can comprise drying a liquid composition comprising the one or more peptide amphiphiles on the medical device.

[0200] The medical device can be any device known or identified for use inside the body of a subject. Preferably, the medical device is one that is inserted into the cardiovascular system.

[0201] The medical device can comprise any material suitable for use as a surgical implant. For example, the medical device can comprise titanium alloy. The medical device can comprise cobalt-cromium. The medical device can comprise nickle-titanium. The medical device can comprise a biodegradable polymer.

[0202] In some aspects, the medical device is a vascular stent. For example, the stent can be a bare medal stent. In some aspects, the stent is a drug eluting stent. For example, the stent can be a sirolimus-eluting stent or a paclitaxel-eluting stent.

[0203] In some aspects, the medical device is a vascular graft. In some aspects, the medical device is a catheter. In some aspects, the medical device is a pacemaker. In some aspects, the medical device is a heart valve.

[0204] It is also contemplated that the disclosed endothelium mimicking nanomatrix compositions can further comprise one or more biologically active agent(s). For example,

in one aspect, an endothelium mimicking nanomatrix can include an effective amount of one or more biologically active agent(s).

[0205] It is also contemplated that the disclosed endothelium mimicking nanomatrix compositions can further comprise one or more pharmaceutically active agent(s). For example, in one aspect, an endothelium mimicking nanomatrix can include an effective amount of one or more pharmaceutically active agent(s).

E. Methods of Making the Hydrophilic Peptides

[0206] The compositions disclosed herein and the compositions necessary to perform the disclosed methods can be made using any method known to those of skill in the art for that particular reagent or compound unless otherwise specifically noted.

1. Peptide Synthesis

[0207] One method of producing the disclosed proteins, such as SEQ ID NO:1 to SEQ ID NO:11, is to link two or more peptides or polypeptides together by protein chemistry techniques. For example, peptides or polypeptides can be chemically synthesized using currently available laboratory equipment using either Fmoc (9-fluorenylmethyloxycarbonyl) or Boc (*tert* -butyloxycarbonoyl) chemistry. (Applied Biosystems, Inc., Foster City, CA). One skilled in the art can readily appreciate that a peptide or polypeptide corresponding to the disclosed proteins, for example, can be synthesized by standard chemical reactions. For example, a peptide or polypeptide can be synthesized and not cleaved from its synthesis resin whereas the other fragment of a peptide or protein can be synthesized and subsequently cleaved from the resin, thereby exposing a terminal group which is functionally blocked on the other fragment. By peptide condensation reactions, these two fragments can be covalently joined via a peptide bond at their carboxyl and amino termini, respectively, to form an antibody, or fragment thereof. (Grant GA (1992) Synthetic Peptides: A User Guide. W.H. Freeman and Co., N.Y. (1992); Bodansky M and Trost B., Ed. (1993) Principles of Peptide Synthesis. Springer-Verlag Inc., NY (which is herein incorporated by reference at least for material related to peptide synthesis). Alternatively, the peptide or polypeptide is independently synthesized *in vivo* as described herein. Once isolated, these independent peptides or polypeptides may be linked to form a peptide or fragment thereof via similar peptide condensation reactions.

[0208] For example, enzymatic ligation of cloned or synthetic peptide segments allow relatively short peptide fragments to be joined to produce larger peptide fragments, polypeptides or whole protein domains (Abrahmsen L et al., Biochemistry, 30:4151 (1991)). Alternatively, native chemical ligation of synthetic peptides can be utilized to synthetically construct large peptides or polypeptides from shorter peptide fragments. This method consists of a two step chemical reaction (Dawson et al. Synthesis of Proteins by Native Chemical Ligation. Science, 266:776-779 (1994)). The first step is the chemoselective reaction of an unprotected synthetic peptide--thioester with another unprotected peptide segment containing an amino-terminal Cys residue to give a thioester-linked intermediate as the initial covalent product. Without a change in the reaction conditions, this intermediate undergoes spontaneous, rapid intramolecular reaction to form a native peptide bond at the ligation site (Baggiolini M et al. (1992) FEBS Lett. 307:97-101; Clark-Lewis I et al., J.Biol.Chem., 269:16075 (1994); Clark-Lewis I et al., Biochemistry, 30:3128 (1991); Rajarathnam K et al., Biochemistry 33:6623-30 (1994)).

[0209] Alternatively, unprotected peptide segments are chemically linked where the bond formed between the peptide segments as a result of the chemical ligation is an unnatural (non-peptide) bond (Schnolzer, M et al. Science, 256:221 (1992)). This technique has been used to synthesize analogs of protein domains as well as large amounts of relatively pure proteins with full biological activity (deLisle Milton RC et al., Techniques in Protein Chemistry IV. Academic Press, New York, pp. 257-267 (1992)).

2. Nucleic Acid Synthesis

[0210] Another method of producing the disclosed proteins, such as SEQ ID NO:1 to SEQ ID NO:11, is to produce a nucleic acid encoding the disclosed proteins operably linked to an expression control sequence. Such nucleic acids can be made using standard chemical synthesis methods or can be produced using enzymatic methods or any other known method. Such methods can range from standard enzymatic digestion followed by nucleotide fragment isolation (see for example, Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Edition (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989) Chapters 5, 6) to purely synthetic methods, for example, by the cyanoethyl phosphoramidite method using a Milligen or Beckman System 1Plus DNA

synthesizer (for example, Model 8700 automated synthesizer of Milligen-Biosearch, Burlington, MA or ABI Model 380B). Synthetic methods useful for making oligonucleotides are also described by Ikuta et al., Ann. Rev. Biochem. 53:323-356 (1984), (phosphotriester and phosphite-triester methods), and Narang et al., Methods Enzymol., 65:610-620 (1980), (phosphotriester method). Protein nucleic acid molecules can be made using known methods such as those described by Nielsen et al., Bioconjug. Chem. 5:3-7 (1994).

F. Methods of Making Peptide Amphiphiles

[0211] Also disclosed herein is a method of making a peptide amphiphile, comprising the steps: a) providing a hydrophilic peptide comprising a degradation sequence and one or more of an endothelial cell adhesive sequence and a nitric oxide producing donor sequence; and b) alkylating the N-terminus of the hydrophilic peptide with a hydrophobic moiety. In a further aspect, the alkylation can comprise amidation with a hydrophobic carboxylic acid. The hydrophobic carboxylic acid can be a fatty acid. The fatty acid can be palmitic acid.

[0212] It is contemplated that a disclosed amphiphile can be prepared by attachment of a hydrophobic moiety via conventional synthetic techniques. For example, a hydrophobic moiety can be attached at the N-terminus of the hydrophilic peptide. That is, hydrophobic electrophilic compounds (e.g., alkyl halide, carboxylic compound) can be reacted with the amine function present at the N-terminus to provide a covalent linkage (e.g., secondary or tertiary amine, amide).

[0213] In further examples, a hydrophobic moiety can be attached at the C-terminus of the hydrophilic peptide. That is, hydrophobic nucleophilic compounds (e.g., alcohol, amine, thiol) can be reacted with the carboxylic function present at the C-terminus to provide a covalent linkage (e.g., ester, amide, thioesters). It is further contemplated that the carboxylic function present at the C-terminus can be derivatized or reduced prior to reaction. For example, the carboxylic function can be reduced to form an alcohol and subsequently reacted with one or more hydrophobic electrophilic compounds (e.g., alkyl halide, carboxylic compound) to provide a covalent linkage (e.g., ether, ester).

[0214] As readily understood by those of skill in the art, peptide sequences can comprise peptide residues having one or more pendant groups. The pendant groups, in various aspects, can comprise one or more nucleophilic moieties (e.g., amine, hydroxyl, thiol) or one or more electrophilic moieties (e.g., carboxylic function). Such moieties can be reacted in a manner analogous to that disclosed above with respect to N-terminus and C-terminus of a disclosed hydrophilic peptide.

G. EXAMPLES

[0215] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how the compounds, compositions, articles, devices and/or methods claimed herein are made and evaluated, and are intended to be purely exemplary and are not intended to limit the disclosure. Efforts have been made to ensure accuracy with respect to numbers (e.g., amounts, temperature, etc.), but some errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, temperature is in °C or is at ambient temperature, and pressure is at or near atmospheric.

1. Example 1:

i. Materials and Methods

a. Synthesis of Peptide Amphiphile

[0216] Two thirteen-amino acid peptides consisting of MMP-2 sensitive sequences (GTAGLIGQ; SEQ ID NO:1) with cell-adhesive sequence YIGSR (SEQ ID NO:2) ("PA-YIGSR") or NO donor sequence KKKKK (SEQ ID NO:3) ("PA-KKKKK") were synthesized using standard Fmoc-chemistry on an Advanced Chemtech Apex 396 peptide synthesizer. Alkylation was obtained by reacting N-termini of the peptides with 2 equivalents of palmitic acid, 2 equivalents of o-benzotriazole-N,N,N',N'-tetramethyluroniumhexafluorophosphate (HBTU), and 4 equivalents of diisopropylethylamine (DiEA) in dimethylformamide (DMF) for 12 h at room temperature. After repeating the alkylation reaction, cleavage and deprotection of PAs were performed using a mixture of trifluoroacetic acid (TFA), deionized (DI) water, triisopropylsilane, and anisole in the ratio of 90:1:1:1 for 3 hours at room temperature. The solution was concentrated using a rotary evaporator. PAs were precipitated in cold ether, collected, and dried under vacuum. The crude PA was dissolved in DI water at a

concentration of 2 wt %. The PA was analyzed by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry.

b. Transmission Electron Microscope (TEM) Imaging

[0217] For TEM samples, 5 µl of each 0.1 wt % PA aqueous solution were cast on a carbon coated formvar copper grid (400 mesh). This grid was dried overnight. Before imaging, the dried samples were negatively stained with 10 µl of 20% phosphotungstenic acid (PTA) for 30s. The samples were imaged (42000x, 52000x) on a FEI Tecnai T12 TEM microscope at 60 kV accelerating voltage.

c. Self-Assembly of Peptide Amphiphiles into Nanofibers

[0218] 0.1 wt % stock solutions for PA-YIGSR and PA-KKKKK were prepared in DI water (pH 7.4) and mixed in a molar ratio of 9:1 ("PA-YK"). 50 µl of PA-YK solution per well were placed in 12-well silicone flexiPERM cell-culture chambers attached to glass coverslips. The chambers were placed in a chemical fume hood for 24 hours to induce self-assembly by solvent evaporation. The chambers were further dried for another 48 hours in a 37 °C incubator.

d. Cell Maintenance

[0219] Human umbilical vein endothelial cells (HUVECs) were grown in endothelium growth medium (EGM) complete medium (0.1% Gentamycin/ Amphotericin B). This cell culture medium was used in all HUVEC experiments. Cells were passaged by trypsinizing (0.05% trypsin/EDTA) and subcultured at a density of 2500-5000 cells/cm^2. Human aortic smooth muscle cells (AoSMCs) were grown in smooth muscle cell basal medium (SmBM) SingleQuot® Kit complete culture medium (0.1 % Gentamycin/ Amphotericin B). This cell culture medium was used in all AoSMC experiments. Cells were passaged by trypsinizing (0.05% trypsin/EDTA) and subculturing at a density of 3500 cells/cm^2. All cell cultures were maintained under standard culture conditions (37°C, 95% relative humidity, and 5% CO_2). All cells and media were purchased from Lonza Inc. (Walkersville, MD).

e. Initial Attachment and Spreading of HUVECs and AoSMCs on PA-YK Nanomatrix

[0220] PA-YK nanomatrix coated culture chambers were prepared as disclosed herein. For initial cell attachment, HUVECs and AoSMCs were seeded on PA-YK nanomatrix coated culture chamber at densities of 30,000 cells/cm^2 and 15,000 cells/cm^2, respectively. After 2 hours of incubation cells were stained with Calcein AM green fluorescent dye and Ethidium homodimer-1 red fluorescent dye using LIVE/DEAD Viability/Cytotoxicity Kit (Molecular Probes, Eugene, OR). The number of attached cells per field of view (20 x) was determined using fluorescent microscopy (Nikon Eclipse E2000), and by averaging five random fields were averaged per sample. The individual cell spreading was analyzed by image processing software (NIS-elements AR 2.30).

f. Platelet Adhesion on PA-YK and PA-YK-NO Nanomatrix

[0221] PA-YK and PA-YK-NO solution was prepared as described herein and cast into films by dropping 150 µl of the solution on 13 mm circular glass cover slips. A solution of 2.5 mg/ml collagen I was prepared in 3% glacial acetic acid and cast into films in the same manner to serve as the control surface. Whole blood from a healthy volunteer was collected in BD Vacutainer® Heparin Tubes (BD, NJ) and mixed with 10uM mepacrine to fluorescently label the platelets. Before the experiment, PA-YK, PA-YK-NO, and collagen films were rinsed with PBS. Thereafter, collagen I, PA-YK, PA-YK-NO films were separately incubated with mepacrine-labeled blood at 37°C for 15 minutes and then rinsed with PBS. The number of adherent platelets per field of view (40x) was determined using a fluorescent microscope (Nikon Eclipse E2000) by averaging five random fields per sample.

g. Preparation of scrubbed NO

[0222] First, the NO solution can be scrubbed. Scrubbing is a process by which a gas is passed through a large surface area of a liquid to remove the unwanted impurities from the gas stream. In this case, the commercially available nitric oxide is passed through an alkaline solution which dissolves the unwanted higher nitrogen oxide species. The apparatus shown in Figure 4 was first degassed with Argon. NO was scrubbed through a 5 M NaOH solution and collected in a reaction vessel containing PA-YK solution.

h. Synthesis of NO Releasing Nanomatrix (PA-YK-NO)

[0223] "PA-YK-NO" was synthesized by reacting PA-YK with scrubbed NO under argon gas. 0.1 wt % PA-YK aqueous solution was reacted with scrubbed NO solution at room temperature under argon gas in 100 mL round bottom flask overnight. The resulting PA-YK solution was cast into films by dropping 130 µl on 13mm glass cover slips. The films were dried in a chemical fume hood for first 24 hours and at 37°C for next 48 hours. In order to determine NO release profile, each PA-YK-NO film was incubated in 500µl of HBS in a 24 well tissue culture plate (Corning Inc., Corning, NY). The HBS was collected, frozen (-20°C) and replaced by fresh HBS at different time points over 1 months. NO release from the PA-YK-NO nanomatrix was then confirmed and quantified using the Greiss assay, containing sulfanilamide and N-1 napthylethylenediamine dihydrochloride (Promega, WI) to measure the nitrite content, which is the principle degradation product of NO.56. At the end of day 5 each collected sample was mixed with 100 µl of the Griess reagent. After incubation for 15 minutes at room temperature, the samples were read at 540 nm using an absorbance microplate reader (EL x 800, BIO-TEK Instrument, VT).

i. Evaluation of Proliferation of HUVECs and AoSMCs on PA-YK-NO Nanomatrix

[0224] PA-YK-NO and PA-YK films were prepared as describe herein, and sterilized under UV for 4 hours. Proliferation of HUVECs and AoSMCs was evaluated by proliferating cell nuclear antigen (PCNA) staining. PCNA is a 36 kDa non- histone protein found in the nucleus that plays a role in the initiation of cell proliferation. Its prominent presence in nucleoli during the late S phase of the cell cycle makes it an ideal marker for cell proliferation. HUVECs and AoSMCs were seeded at densities of 30,000 cells/cm^2 and 15,000 cells/cm^2, respectively. Cells were incubated under standard culture conditions (37°C, 95% relative humidity, and 5% CO_2). After 48 hrs of incubation, cells were fixed in a 10% neutral buffered formalin solution (Sigma Chemical Co., St. Louis, MO) and rinsed with PBS. The cells were then permeabilized by incubating in histological grade methanol (Sigma Chemical Co., St. Louis, MO), followed by rinsing with PBS. A 3% hydrogen peroxide solution was used to block endogenous peroxidases. After rinsing with PBS, the cells were then incubated with tris-buffered saline, followed by incubation with mouse IgG anti-PCNA primary antibody (Dako Corp., Carpinteria, CA) diluted 1:100 in

phosphate-buffered saline (PBS) with 3% FBS. After aspirating the primary antibody and rinsing with PBS, the cells were incubated with anti-mouse IgG HRP (Dako Corp., Carpinteria, CA) diluted 1:100 in PBS with 3% FBS, followed by incubation with aminoethylcarbazole chromogen (Dako Corp., Carpinteria, CA). The chromogen generates a red precipitate representing the proliferating cells. The cells were then rinsed with PBS and counterstained with Mayer's hematoxylin. Excess hemotoxylin was washed away by rinsing the samples with 37mM ammonium hydroxide 2-3 times. The percentage of proliferating cells per field of view (20 X) was determined by counting the red proliferating cells and hemotoxylin stained blue non-proliferating cells using phase contrast microscopy (Nikon Eclipse E2000) after averaging five fields per sample.

j. Statistical Analysis

[0225] All data were compared with one-way ANOVA tests using SPSS software. A p value less than 0.05 was considered to be statistically significant.

ii. Results

a. Self-Assembly of Peptide Amphiphiles into Nanofibers

[0226] PA-YIGSR and PA-KKKKK were successfully synthesized, and their molecular weights were confirmed by MALDI-Tof mass spectrometry. A hybrid peptide amphiphile of PA-YIGSR and PA-KKKK ("PA-YK") was synthesized by mixing 0.1 wt % solutions of PA-YIGSR and PA-KKKKK in a molar ratio of 9:1. The self-assembly of the PAs into the nanofibers was induced by evaporating the solvent as described earlier. NO releasing PA-YK-NO was synthesized by further reacting PA-YK with NO under argon. The TEM images (Figure 4) demonstrate successful self-assembly of PAs into nanofibers by solvent evaporation. The nanofibers obtained were similar in dimension to those previously reported in self-assembly studies using divalent ion or pH change.

b. Evaluation of Initial Cell Attachment and Spreading

[0227] Both HUVECs and AoSMCs were separately seeded on the PA-YK nanomatrix and cell attachment was evaluated to determine whether endothelial cells recognize the adhesive ligand YIGSR in the nanomatrix. Initial attachment of HUVECs was found to be slightly higher than compared to AoSMCs (Figure 5). This was confirmed by evaluating the spreading of HUVECs and AoSMCs on the PA-YK nanomatrix after 2 hours (Figure 6 and 7). After 2 hours, HUVECs were found to spread three fold more than AoSMCs. The

results indicate that HUVECs can recognize the YIGSR incorporated into the PA-YK, signifying that PA-YK nanomatrix promotes HUVEC attachment and spreading.

c. Evaluation of Attachment of Platelets and on PA-YK Nanomatrix

[0228] Platelet attachment on PA-YK-NO and PA-YK nanomatrix was evaluated using mepacrine-labeled whole blood. Platelet adhesion was approximately 50-fold lower on PA-YK nanomatrices compared to the positive control, Collagen I. Furthermore, exposure of blood to NO-releasing PA-YK-NO nanomatrices resulted into virtually no platelet attachment. (Figure 8)

d. NO Release from PA-YK-NO Nanomatrix

[0229] The NO release profile from the PA-YK-NO nanomatrix over a period of one month is shown in Figure 10. Most of the NO was released in the first 24 hours, followed by a slow sustained release over a period of two weeks, followed by another burst release resulting in recovery of about 53% NO. The value of 100% NO (8.6 μmoles) is calculated by assuming that every lysine residue in PA-YK reacts with two molecules of NO.

e. Evaluation of Proliferation of HUVECs and AoSMCs on PA-YK-NO Nanomatrix

[0230] To examine the effect of NO on HUVECs and AoSMCs, the cells were seeded on PA-YK-NO nanomatrix coated culture chambers. The proliferation of the cells was evaluated using PCNA staining after 48 hours of incubation. Parallel proliferation experiments were also conducted on PA-YK nanomatrix as a control. As shown in Figure 9, the percentage of PCNA positive HUVECs ((66.8±1.94)%) on PA-YK-NO was found to be significantly greater as compared to PA-YK ((50.29±3.4)%). Conversely, the percentage of PCNA positive AoSMCs on PA-YK-NO ((16.4±2.8)%) was significantly lower than that on PA-YK ((34.8±1.9)%).

2. Example 2: In vivo evaluation of native endothelium mimicking self-assembled nanomatrix

[0231] Self-assembled nanomatrix coated stents were implanted in rabbit iliac arteries and evaluated by histomorphometry for evidence of stenosis and thrombosis.

i. Materials and Methods

a. Stent coating and Characterization

[0232] For uniform coating with the PA solution, a commercially available stainless steel stent was mounted on a mandrel (stainless steel wire – 0.018 inches diameter) attached to a motor rotating at a speed of 15 rpm. The rotating stent was immersed in a PA solution contained in an open-top reservoir as shown in Figure 11. The open-top reservoir facilitates evaporation induced self-assembly of PA into nanomatrix on the surface of the stent. Rotation of the stent ensures uniform coating of PA nanofibers throughout the outer and inner surfaces of the stent. The stoppers at both ends of the stents prevent the stent from sliding on the mandrel. The stent was rotated for 12 hours in the PA solution and then allowed to dry for another 24 hours. Figure 12 shows the SEM image of 0.1 wt% PAYK coated stent after handling by the clinician. It is noted that the smooth uniform coating surface stays undisturbed after handling (mounting on angioplasty balloon and expansion). This result indicates that the PA nanomatrix can be uniformly coated onto stents and remain stable during the handling process.

b. Study groups for in vivo assessment

[0233] Male white New Zealand Rabbits were used for this study. One rabbit was used per group with two stents implanted per rabbit. There were 2 different nanomatrix coatings and 1 uncoated bare metal stainless steel stent evaluated. Each stent type was evaluated at 2 weeks and 4 weeks as follows: Control (bare metal stent) 2 weeks, 4 weeks; Low dose (0.1 wt% PAYKNO coated) 2 weeks, 4 weeks; and High dose (1 wt% PAYKNO coated) 2 weeks, 4 weeks. For the two week time points, all the stents were implanted without prior balloon injury. All rabbits were housed for at least two days before the surgery. Surgery protocol was approved by Institutional Animal Care and Use Committee (IACUC) at University of Alabama at Birmingham, and is described below.

c. Stent Implantation

[0234] The day of the procedure, the rabbits were anesthetized with ketamine/xylazine 35/5 mg/kg. An endotracheal tube was inserted and connected to a respirator operated with a tidal volume of 400 ml at a rate of 16 breaths per minute. Anesthesia was sustained with isoflurane 2%. Heart rate and blood oxygen saturation was monitored using a pulse-oxymeter placed on the animal's tongue. Blood pressure was continuously monitored

using a cuff on a hind limb. The rabbit was secured to the table in the dorsal recumbent position. 81 mg/day of aspirin by mouth was given daily, starting the day before the procedure, until the time of euthanasia.

[0235] The right carotid artery was surgically exposed and vascular access was obtained. A 6 French Sheath was inserted into the carotid artery and heparin (150 units/kg) was administered intravenously. Under fluoroscopic guidance, a 6 French JR4 coronary guide catheter was advanced over a 0.014" coronary guide wire to the descending aorta. Angiography was performed using approximately 8 ccs of Meglumine diaztroate contrast injected via the catheter. The baseline arteriogram was recorded digitally. Following angiography, a stent mounted on a delivery balloon was advanced over the guide wire into one iliac artery. The stent was deployed to be slightly oversized, with a stent to artery ratio of 1.1 to 1. A second stent was deployed in a similar manner in the other iliac artery. After stent implantation, the catheter, guidewire, and arterial sheath were removed. The carotid artery was ligated. The wound was sutured closed. The animal was then allowed to recover under observation. Buprenex (0.05 mg/kg) was administered intramuscularly every 12 hours, starting at the completion of the procedure.

[0236] Animals were monitored daily for any significant appetite loss/weight loss (more than 20% body weight loss), and lack of blood circulation in the lower limbs.

d. Stent Harvest

[0237] 2 weeks and 4 weeks after stent implantation euthanasia was performed and the stented iliac arteries were pressure perfusion fixed with formalin. The stents were removed and placed in 10% buffered formalin for histological studies.

e. Tissue Processing

[0238] All fixed stents were dehydrated and embedded in methylmethacrylate resin. After complete polymerization, the areas of interest were brought closer to the surface by preliminary grinding. The opposite side of the block was mounted on a slide using Technovit 4000 (Exakt Technologies, Inc., Oklahoma City, OK). Sections (about 100 microns thick) are cut from each specimen using the Exakt Diamond Saw (Exakt Technologies, Inc., Oklahoma City, OK), which is somewhat like a large band saw that utilizes a diamond-coated cutting band with a water-cooling and flushing system. The

sections were grounded to about 20-30μm with the Exakt Grinding System (Exakt Technologies, Inc., Oklahoma City, OK), which produces precision parallel surfaces, and was smoothed using increasing grits of sandpaper. After the surface of the specimen to be studied was reached, it was polished with 4000 grit sandpaper to create as smooth a surface a possible. Sections were made at 25%, 50% and 75% regions of the stent. All sections were stained with Methylene Blue/Basic Fuchsin stain.

[0239] Histological analysis included injury, thrombus formation, inflammation, and the presence of neointima. The stented segments were analyzed and graded for arterial injury, using the Schwartz injury score. Inflammation and thrombus was also assessed around each strut, with a grading scale. Neointimal thickness at each strut was measured in microns. Computer-guided morphometric measurements were performed using digital images and Bioquant image analysis software (Bioquant Image Analysis Corp, Nashville, TN). Computerized planimetry was performed.

ii. Results

[0240] Figure 13A shows that balloon inflation successfully deployed stents in rabbit iliac artery. As shown in Figure 13B, the stents were overall deployed fully and there was minimal underlying tissue injury. Blood vessels were intact and patent. No flaking or peel off of the nanomatrix coating was noted. Minimal inflammation was found around the stent struts. Notably, there was very little neointimal hyperplasia and no thrombus found on the surface of the nanomatrix coated stents.

[0241] Injury scores were assigned at each stent strut from 0 to three as follows: 0 = no injury; 1 = break in the internal elastic membrane; 2 = perforation of the media; and 3 = perforation of the external elastic membrane to the adventitia.

[0242] The average injury score for each segment was calculated by dividing the sum of injury scores by the total number of struts at the examined section. With the exception of control stents in 4 weeks group (average injury score ~0.02), no evidence of injury was seen in any stent.

[0243] Neointimal Thickness (NI) was measured in microns. At 2 weeks as shown in Figure 14, there appeared to be a trend towards less NI thickness in the high dose stents compared to the control. At 4 weeks NI as shown in Figure 15, was greater than at 2

weeks, but the trend towards less NI in both the low dose and high dose groups appeared to persist, compared with controls.

[0244] Inflammation around each strut was assessed using the following grading scale: 0 = no inflammatory cells surrounding the strut; 1 = light, noncircumferential lymphohistocytic infiltrate surrounding the strut; 2 = localized, moderate to dense cellular aggregate surrounding the strut noncircumferentially; and 3 = circumferential dense lymphohistiocytic cell infiltration of the strut.

[0245] Average inflammation scores for all study groups were less than 0.5 with no significant differences in any of the 2 weeks for 4 weeks stent groups. Also, inflammation scores were found to be similar for both 2 weeks and 4 weeks groups as shown in Figure 16 and 17.

[0246] Thrombus scores around each strut were scaled from zero to three. At 2 weeks there was minimal or no thrombus found in all stents. At 4 weeks the average thrombus score was less than 0.1 for all stent groups with no significant difference between the groups. One of the two high dose stent displayed no thrombus at 4 weeks as shown in Figure 18.

iii. Conclusions

[0247] Overall, all animals survived and there was a minimal tissue injury associated with the stent deployment in all groups. Stent coating seems to be stable as no flaking or peel off of the nanomatrix coating was noted. There was minimal inflammation and almost no thrombus observed at both the 2 week and 4 week time points. A comparison of neointimal thickness across the groups showed no appreciable differences among any group, although there was a suggestion of a decreasing trend in the high dose and low dose stent groups compared to the uncoated control stent groups. Endothelial cells were observed on the histology sections including the high dose stents. The absence of fibrin deposition and no consequent thrombus around the struts can be due to presence of endothelial cell lining on the surfaces.

3. **Example 3: A hybrid biomimetic nanomatrix by combination of electrospun PCL nanofibers and native endothelium mimicking peptide amphiphiles (PAs)**

i. Materials and methods

a. Synthesis of ePCL nanofibers

[0248] PCL pellets (Sigma Aldrich, St. Louis, MO; M_n = 80,000) were dissolved in a solvent system of 1:1 (v/v) chloroform: methanol to obtain a 22.5 wt% viscous polymer solution and transferred to a syringe capped with a 25-G blunt tipped needle. The syringe was placed in a syringe pump (KD Scientific, Holliston, MA) that was set at a flow rate of 1 ml/hr. The needle tip was connected to a high voltage source (Gamma High-Voltage Research, Ormond Beach, FL) through which a voltage of +21 kV was applied to the needle tip. The resulting electrospun PCL (ePCL) nanofibers were deposited on a grounded aluminum collector that was placed 28 cm from the needle tip. The collectors with ePCL sheets on them were then stored in a vacuum dessicator for 2-3 days to remove any residual solvents. Morphology of the ePCL nanofibers was characterized using scanning electron microscopy (SEM). The nanofibers were sputter coated with gold/palladium and their morphology was observed under a Philips SEM 510 at an accelerating voltage of 20 kV. SEM images were analyzed using an image analyzer (Image-proplus, Media Cybernetics Co., Silver Spring, MD, USA) for measurement of fiber diameters.

b. Synthesis of peptide amphiphiles

[0249] The peptides can be synthesized using Fmoc chemistry in an Aapptech Apex 396 Peptide synthesizer, as described before (Jun *et al.* 2005).Two thirteen-amino acid peptides consisting of MMP-2 sensitive sequences (GTAGLIGQ) with cell-adhesive sequence YIGSR or NO donating residue KKKKK were synthesized. These peptides were alkylated to be linked to a 16 carbon palmityl chain, thereby creating an amphiphiles. Therefore, two different PAs, C_{16}-GTAGLIGQ-YIGSR (PA-YIGSR) and C_{16}-GTAGLIGQ-KKKKK (PA-KKKKK) were synthesized.

c. Cell maintenance

[0250] Human umbilical vein endothelial cells (HUVECs) were grown in endothelium basal medium (EBM) supplemented with endothelial growth media (EGM) (2% FBS,

0.1% hEGF, 0.1% hydrocortisone, 0.1% gentamycin A, 0.4% bovine brain extract). This cell culture medium was used in all HUVEC experiments. Cells were passaged by trypsinizing (0.05% trypsin/EDTA) and subcultured at a density of 2500-5000 cells/cm^2. Human aortic smooth muscle cells (AoSMCs) were grown in smooth muscle cell basal medium (SmBM) supplemented with Smooth muscle cell growth media (SmGM-2) SingleQuot® Kit (5% FBS, 0.1% Insulin, 0.2% hFGF-B, 0.1% gentamycin A, 0.1% hEGF). This cell culture medium was used in all AoSMC experiments. Cells were passaged by trypsinizing (0.05% trypsin/EDTA) and subculturing at a density of 3500 cells/cm^2. All cell cultures were maintained under standard culture conditions (37°C, 95% relative humidity, and 5% CO_2). All cells and media were purchased from Lonza Inc. (Walkersville, MD).

d. Optimization of ratio of PA-YIGSR and PA-KKKKK

[0251] HUVECs were seeded at density of 30,000 cells/cm^2 on different molar ratios of PA-YIGSR and PA-KKKKK coated onto ePCL, designated as ePCL-YK 90 (90% PA YIGSR, 10% PA KKKKK), ePCL-YK 75 (75% PA YIGSR, 25% PA KKKKK), ePCL-YK 50 (50% PA YIGSR, 50% PA KKKKK), and ePCL-YK 25 (25% PA YIGSR, 75% PA KKKKK). Uncoated ePCL was used as a control. After a 2 hour incubation period, the media was aspirated; cells were trypsinized using 400 µl of 0.25% clear trypsin for 30 minutes. The trypsinized cells were collected at 1:1 Dilutions with PBS into 1.5 ml eppendorf tubes and store in -80°C. The samples thus collected were subjected to a Picogreen DNA assay to evaluate the DNA content which is proportional to the cell number.

[0252] The cells were permeabilized by repeated freeze-thaw cycles. Then, PicoGreen dye was added to the cell sample. The PicoGreen dye binds to double stranded DNA in cells and allows its quantification. Known volumes of calf thymus dsDNA were used as standards. The fluorescence was measured in a microplate fluorescence reader (Synergy HT, Bio-Tek Instruments, VT). The amount of DNA was correlated to cell number by an empirical value of 8 pg DNA per cell.

e. Preparation and characterization of NO releasing hybrid nanomatrix

[0253] NO releasing PAs were synthesized by reacting PA solutions overnight with pure NO under high pressure. Pure NO was obtained by the process of scrubbing.

Commercially available NO gas is passed through 5 M potassium hydroxide to remove impurities such as higher oxide species. Prior to NO scrubbing, the apparatus is degassed by passing argon through the system.

[0254] A 9:1 ratio of PA-YIGSR and PA-KKKKK was chosen as the best ratio. Hereinafter, this mixture is referred to as PA-YK. PA-YK was therefore reacted with NO to obtain NO releasing PA, which was called PA-YK-NO. Self assembly of PA-YK and PA-YK-NO was characterized by transmission electron microscopy. This was done to ascertain that the NO reaction does not interfere with the self-assembly process. PA-YK-NO was coated onto 16 mm diameter ePCL discs to obtain the NO releasing hybrid nanomatrix, called ePCL-YK-NO. This was carried out as follows.

[0255] Using a Humboldt boring machine (Fisher Scientific), ePCL sheets were cut into 16 mm diameter discs. These discs were sterilized with decreasing concentrations of ethanol. These discs were coated with 200 µl of 0.1 wt. % PAs. These scaffolds were then placed on a shaker for 24 hours and then the PAs were allowed to self assemble onto ePCL nanofibers, via solvent evaporation by drying in a chemical hood for 48 hours. Successful self-assembly of PAs onto ePCL nanofibers was confirmed by TEM. PCL was electrospun onto 600 mesh hexagonal TEM grids and dessicated in a vacuum dessicator for 48 hours. They were then coated with 5 µl 0.1 wt % PAs. The grids were then allowed to dry overnight in a chemical hood. These grids were then stained with 2% PTA for 30 seconds. The grids were then imaged using a FEI Technai T12 TE microscope at 60 kV accelerating voltage. Also, to ensure that PAs do not interfere with ePCL networks, PA coated ePCL were imaged using SEM.

[0256] For NO release studies, the 16 mm ePCL scaffolds were coated with 200 µl 1 wt% PA-YK-NO. These discs were then placed in a shaker for 24 hours, followed by 48 hours in a chemical hood to allow self-assembly of PAs on to ePCL nanofibers. These scaffolds were then incubated in 400 µl HEPES buffered saline (HBS) in a 48 well tissue culture plate. Samples were collected at 0h, 2h, 4h, 6h, 24h, 48h, 4 day, 6 day, 10 day, 14 day, 21 day and 28 day time points. As a control, ePCL scaffolds were coated with PA-YIGSR-NO. PA-YIGSR-NO was obtained by reacting PA-YIGSR with pure NO. This accounts for non-specific binding of NO to PAs. After samples were collected, the nitrite content was measured by using Greiss Assay (Promega), which uses sulfanilamide and N-1

napthylethylenediamine dihydrochloride. Nitrite is the primary degradation product of NO. 50 µl of sample was treated with 50 µl sulfanilamide and 50 µl of N-1 napthylethylenediamine dihydrochloride. After incubation for 15 minutes, absorbance was read at 540 nm using a microplate reader (EL x 800, BIO-TEK Instrument, VT). The difference in nitrite content between ePCL-PA-YK-NO and ePCL-PA-YIGSR-NO accounts for the NO released from the lysine residues of PA-KKKKK. This was plotted as a measure of NO released against time.

f. Evaluation of cellular behaviors on hybrid nanomatrix

[0257] HUVECs and AoSMCs were seeded on ePCL-PA-YK-NO and ePCL-PA-YK at densities of 30,000 cells/cm^2 and 15,000 cells/cm^2, respectively. After 2 hours of incubation cells were morphologically stained with the LIVE/DEAD Viability/Cytotoxicity Kit (Molecular Probes, Eugene, OR). Initial cell adhesion was analysed by seeding HUVECs and AoSMCs on 3 different substrates: ePCL-PA-YK-NO; ePCL-PA-YK and ePCL. HUVECs were seeded at 30,000 cells/cm^2 and AoSMCs were seeded at 15,000 cells/cm^2 onto the substrates that were held in the wells of a 48 well tissue culture plate. After a 2 hour incubation period, the media was aspirated; cells were trypsinized using 400 µl of 0.25% clear trypsin for 30 minutes. The trypsinized cells were collected at 1:1 Dilutions with PBS into 1.5 ml eppendorf tubes and store in -80°C. The samples thus collected were subjected to a Picogreen DNA assay to evaluate the DNA content which is proportional to the cell number.

[0258] To evaluate the effect of NO on cell proliferation, 0.1 wt% PA-YK-NO and PA-YK were coated onto 16mm ePCL discs. HUVECs and AoSMCs were seeded at densities of 30,000 cells/cm^2 and 15,000 cells/cm^2, respectively. Proliferation of HUVECs and AoSMCs was evaluated by proliferating cell nuclear antigen (PCNA) staining. After 48 hrs of incubation, cells were fixed in 10% formalin, permeabilized in methanol, and blocked using a 3% hydrogen peroxide solution. Cells were then incubated with tris-buffered saline, followed by incubation with mouse IgG anti-PCNA primary antibody (Dako Corp., Carpinteria, CA) diluted 1:100 in PBS with 3% FBS. Then, cells were incubated with anti-mouse IgG HRP (Dako Corp., Carpinteria, CA) diluted 1:100 in PBS with 3% FBS, followed by incubation with aminoethylcarbazole chromogen (Dako Corp., Carpinteria, CA). The cells were counterstained with Mayer's hematoxylin and rinsed with

37mM ammonium hydroxide. The percentage of proliferating cells per field of view (20 X) was determined by counting the red proliferating cells and blue non-proliferating cells using phase contrast microscopy. For all cell quantifications, five random fields were imaged and averaged for each well. Furthermore, four samples were tested for each condition making up the experiments. The results shown in the graphs depict an average of over 120 images from 3 independent experiments (n=12).

g. Statistical Analysis

[0259] All experiments were performed at least three independent times. All data were compared with one-way ANOVA tests to evaluate statistical significance using SPSS software. Within the ANOVA analysis, Tukey multiple comparisons test was performed to find significant differences between pairs. A value of $p<0.05$ was considered to be statistically significant.

ii. Results and Discussions

[0260] Disclosed herein are hybrid biomimetic nanomatrices designed to reconstitute the properties of native endothelium onto the cardiovascular implant surface. ePCL nanofibers, known to possess the characteristics desired for biomedical applications, were successfully fabricated. As the SEM image in *Figure 20a* shows, the ePCL fibers have relatively uniform morphologies, possess nanoscale diameters between 200 nm – 700 nm, and are free from beads. The random, interwoven nature that is typical of all ePCL nanofibers is also clearly evident from the image. Additionally, the majority of the fibers had diameters measured to be within the range of 300 nm – 400 nm, which is similar to collagen fiber bundles found in native ECM (Elsdale T and Bard J: 1972). This aspect of ECM mimicking nanofibrous topography is favored by cells. However, this attractive morphological feature is negated by the lack of bioactivity in ePCL. This lack of cell recognition sites on ePCL prevents the scaffold from actively interacting with cells, and therefore, is unable to effectively integrate with host tissues. Thus, also disclosed herein, these ePCL nanofibers were endowed with bioactivity.

[0261] This bioactivity was introduced to ePCL nanofibers through the use of PAs. Two different PAs, C_{16}-GTAGLIGQ-YIGSR (PA-YIGSR) and C_{16}-GTAGLIGQ-KKKKK (PA-KKKKK) were successfully synthesized. The PAs contain enzyme-mediated degradable MMP-2 sensitive sequences (GTAGLIGQ), along with YIGSR or a polylysine

(KKKKK) group to form NO donating residues. PA-YKs were also designed by mixing different molar ratios of PA-YIGSR and PA-KKKKK. Thus, densities of cell adhesive ligands and NO can be tuned by varying the ratios of PA-YIGSR and PA-KKKKK. These PA-YKs were coated onto ePCL to create a hybrid nanomatrix (ePCL-PA-YK), which was then characterized by SEM (*Figure 20b*). It is clear that coating with PA-YK does not affect ePCL nanofiber morphology. ePCL-PA-YK was also characterized by TEM (*Figure 21a*). From the TEM image, a central ePCL nanofiber (approximately 300 nm in diameter) was observed, and it was coated on either side with PAs (7-8 nm in diameter). Due to a lack of depth in TEM imaging, uniform coating was confirmed by tilting the stage of the electron microscope in the transverse axis to obtain an image (*Figure 21b*). This image is similar to the untilted image, and therefore it can be reasonably inferred that the coating of PAs on ePCL nanofibers is uniform. To determine the optimal matrix composition, endothelial cells were seeded on various ratios of ePCL-PA-YKs and cell adhesion was found to be significantly greater with increasing PA-YIGSR concentration (*Figure 22*). Thus, ePCL-PA-YK90 (9:1 mol/mol), henceforth referred to as ePCL-PA-YK, was used for all further studies.

[0262] PA-YK was reacted with NO to obtain PA-YK-NO. This was then coated onto ePCL to fabricate ePCL-PA-YK-NO, which is the NO releasing, biomimetic hybrid nanomatrix. ePCL-YK-NO was characterized by SEM (*Figure 19c*), and it was confirmed that PA-YK-NO does not affect the morphology of ePCL nanofibers. To confirm uniform coating, ePCL-PA-YK-NO was then imaged by TEM (*Figure 21c*). Uniform coating of PA-YK-NO is visible on either side of ePCL nanofibers, and this was confirmed, again, by imaging after tilting the stage in the transverse axis (*Figure 21d*).

[0263] The NO release profile from the ePCL-PA-YK-NO hybrid nanomatrix was evaluated using the Greiss assay. Successful NO release was observed over 28 days. An initial burst release occurred in first 48 hours, followed by a slow sustained release over a period of 4 weeks resulted in a 48% recovery of NO (*Figure 23*). The initial burst release can be explained as NO release from the surface of the ePCL-PA-YK-NO nanofibrous matrix, which was easily accessible for local delivery. This is especially important for limiting the proliferation of smooth muscle cells, which is one of the key events in restenosis, begins as early as one day after injury (Weintraub WS: 2007). Therefore, the 48

hour burst release of NO is critical to arrest neointimal hyperplasia. Subsequent sustained release can be attributed to NO released from the bulk of the hybrid nanofibrous matrix by a combination of diffusion and enzyme degradation. Over time, due to the presence of MMP-2 degradable sites, ePCL-PA-YK-NO degrades slowly, and this is believed to aid in the sustained release of NO from the bulk of the matrix. The presence of enzyme mediated degradable sites is also expected to create a concentration gradient of NO in the hybrid nanomatrix. From the study, it can be observed that 3.8 μmoles of NO was released over the period of 4 weeks from an ePCL-PA-YK-NO disc that was 16 mm in diameter. This amount is comparable to the cumulative NO released by endothelial cells at a rate of $1x10^{-10}$ mol cm^{-2} min^{-1} (Vaughn MW, *et al.* 1998).The slow sustained release over a longer period is required to maintain the non-proliferative state of smooth muscle cells, anti-thrombogenic nature of the vessel wall, and promote endothelialization during the recovery period, which typically takes several weeks. Another feature of the hybrid nanomatrix is that the amount of NO released can be easily tuned in future applications by changing the number of lysine moieties in PA-KKKKK. Thus, increasing the number of lysines by extending the number of lysines in PA-KKKKK (e.g., PA-KKKKKKK) or by increasing the proportion of PA-KKKKK in PA-YK (e.g., a PA-YIGSR to PA-KKKKK ratio of 9:2, 3:1, 9:4, 2:1, 9:5, 3:2, 9:7, 9:8, 1:1, 3:4) can increase the rate of NO release and increase the duration of NO release.

[0264] Initial cell adhesion was studied by seeding HUVECs and AoSMCs on ePCL-PA-YK-NO and cPCL-PA-YK. Uncoated ePCL was used as a control. These cells were imaged morphologically, and it can be seen that ePCL-PA-YK-NO and ePCL-PA-YK improve the adhesion and spreading of HUVECs when compared to ePCL (Figure 24), while ePCL-PA-YK-NO and ePCL-PA-YK do not support AoSMC spreading (Figure 25). From Figure 26a, it can be seen that ePCL-PA-YK-NO (15576±2414) and ePCL-PA-YK (16685±808) show significantly greater HUVEC adhesion when compared to uncoated ePCL (12490±639). From figure 26b, it is evident that ePCL-PA-YK-NO (9123±1344) and ePCL-PA-YK (9404±1259) show similar adhesion as ePCL (9310±561), and therefore, do not support AoSMC adhesion. These results clearly show that the hybrid nanomatrices (ePCL-PA-YK-NO and ePCL-PA-YK) promote endothelial cell adhesion and spreading, while simultaneously not supporting smooth muscle cell adhesion and

spreading. This is due to PAs endowing the ePCL nanofibers with endothelial cell specific bioactivity in the form laminin derived YIGSR cell adhesive moiety.

[0265] To effect of NO on proliferation of HUVECs and AoSMCs was studied by using the Greiss assay after 48 hours of incubation. As shown in Figure 27, the percentage of PCNA positive HUVECs on the ePCL-PA-YK-NO nanomatrix (70±2%) was found to be significantly greater compared to the ePCL-PA-YK nanomatrix (57 ± 3 %). However, the percentage of PCNA positive AoSMCs on ePCL-PA-YK-NO (41±3%) was significantly lower than on ePCL-PA-YK (58±4%). These results indicate that the ePCL-PA-YK-NO nanomatrix enhances endothelial cell growth but limits smooth muscle cell growth. Enhancement of endothelialization, while preventing smooth muscle growth, is a crucial step towards improving graft patency by preventing intimal hyperplasia and restenosis.

[0266] Therefore, this hybrid nanomatrix has great potential for use as biomimetic cardiovascular implants. Conventional cardiovascular implants are limited to providing structural support, and are unable to effectively integrate with the host tissue due to their inability to closely mimic the native endothelium. Additionally, they are characterized by a lack of re-endothelialization, restenosis, and thrombosis. As disclosed herein, hybrid biomimetic scaffolds consisting of electrospun PCL and PA nanofibers were developed. ePCL provides the mechanical strength and topographical structure that is desirable for a cardiovascular graft that is exposed to regular pulsatile forces. The PA nanofibers consist of endothelial cell adhesive YIGSR moiety, which is expected to recruit and promote the adhesion of endothelial cells against the shear flow. NO released from the hybrid nanomatrix smooth muscle cell proliferation and endows the nanomatrix with an anti-thrombotic nature, while simultaneously promoting endothelial cell proliferation. The enzyme mediated degradable sites present in the PAs are expected to cause slow cell directed degradation of the matrix, and this aids in the sustained release of NO. Therefore, as an example of the applications for this hybrid nanomatrix, the nanomatrix can be used on implantable cardiovascular devices, such as vascular grafts and heart valves.

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I. SEQUENCES

SEQ ID NO:1
Gly-Thr-Ala-Gly-Leu-Ile-Gly-Gln

SEQ ID NO:2
Tyr-Ile-Gly-Ser-Arg

SEQ ID NO:3
Lys-Lys-Lys-Lys-Lys

SEQ ID NO:4
Gly-Thr-Ala-Gly-Leu-Ile-Gly-Gln-Tyr-Ile-Gly-Ser-Arg

SEQ ID NO:5
Gly-Thr-Ala-Gly-Leu-Ile-Gly-Gln-Lys-Lys-Lys-Lys-Lys

SEQ ID NO:6
Gly-Thr-Ala-Gly-Leu-Ile-Gly-Gln-Tyr-Ile-Gly-Ser-Arg-Lys-Lys-Lys-Lys-Lys

SEQ ID NO:7
Gly-Thr-Ala-Gly-Leu-Ile-Gly-Gln-Lys-Lys-Lys-Lys-Lys-Tyr-Ile-Gly-Ser-Arg

SEQ ID NO:8
Arg-Gly-Asp

SEQ ID NO:9
Arg-Gly-Asp-Ser

SEQ ID NO:10
Asp-Gly-Glu-Ala

SEQ ID NO:11
Val-Ala-Pro-Gly

SEQ ID NO:12
Arg- Glu-Asp-Val

SEQ ID NO:13
Asp-Gly-Glu-Ala

SEQ ID NO:14
Lys-Arg-Ser-Arg

SEQ ID NO:15
Gly-Pro-Gln-Gly-Leu-Leu-Gly

SEQ ID NO:16
Gly-Pro-Gly-Ile-Trp-Gly-Gln

SEQ ID NO:17
Cys-Cys-Cys-Cys-Cys

[0267] It will be apparent to those skilled in the art that various modifications and variations can be made in the present invention without departing from the scope or spirit of the invention. Other embodiments of the invention will be apparent to those skilled in the art from consideration of the specification and practice of the invention disclosed herein. It is intended that the specification and examples be considered as exemplary only, with a true scope and spirit of the invention being indicated by the following claims.

CLAIMS

What is claimed is:

1. A peptide amphiphile, comprising a hydrophilic peptide sequence and a hydrophobic tail, wherein the hydrophilic peptide sequence comprises a degradation sequence and one or more of a first cell adhesive sequence and a nitric oxide producing donor sequence, wherein the first adhesive sequence is an endothelial cell adhesive sequence; wherein the cell adhesive sequence comprises YIGSR (SEQ ID NO:2), RGD (SEQ ID NO:8), RGDS (SEQ ID NO:9), DGEA (SEQ ID NO:10), VAPG (SEQ ID NO:11), REDV (SEQ ID NO:12), DGEA (SEQ ID NO:13), or LRSR (SEQ ID NO:14); and wherein the nitric oxide producing donor sequence comprises polylysine, polycysteine sequence, or modified polylysine.

2. The peptide amphiphile of claim 1, wherein the hydrophilic peptide sequence comprises the formula:

DS---CA,

wherein --- is a direct or indirect covalent linkage,

wherein "DS" is a degradation sequence; and

wherein "CA" is an endothelial cell adhesive sequence.

3. The peptide amphiphile of claim 1, wherein the hydrophilic peptide sequence comprises the formula:

DS---KK,

wherein --- is a direct or indirect covalent linkage,

wherein "DS" is a degradation sequence; and

wherein "KK" is a nitric oxide producing donor sequence.

4. The peptide amphiphile of claim 1, wherein the hydrophilic peptide sequence comprises the formula:

DS---CA---KK,

wherein --- is a direct or indirect covalent linkage,

wherein "DS" is a degradation sequence;

wherein "CA" is an endothelial cell adhesive sequence; and

wherein "KK" is a nitric oxide producing donor sequence.

5. The peptide amphiphile of claim 1, wherein the hydrophilic peptide sequence comprises the formula:

DS---KK---CA,

wherein --- is a direct or indirect covalent linkage,

wherein "DS" is a degradation sequence;

wherein "CA" is an endothelial cell adhesive sequence; and

wherein "KK" is a nitric oxide producing donor sequence.

6. The peptide amphiphile of any one of claims 1-5, wherein the degradation sequence comprises a sequence that undergoes cell-mediated proteolytic degradation.

7. The peptide amphiphile of claim 6, wherein the degradation sequence comprises a matrix metalloprotease (MMP) specific cleavage site.

8. The peptide amphiphile of claim 7, wherein the degradation sequence comprises a matrix metalloprotease-2 (MMP2) specific cleavage site.

9. The peptide amphiphile of any one of claims 1-8, wherein the amphiphile is coated on a nanofiber.

10. The peptide amphiphile of claim 9, wherein the nanofibers are fabricated by electrospinning.

11. A composition comprising a first and second peptide amphiphile, each independently comprising a hydrophilic peptide sequence and a hydrophobic tail, wherein the hydrophilic peptide sequence of the first peptide amphiphile comprises a degradation sequence and an endothelial cell adhesive sequence, wherein the cell adhesive sequence comprises YIGSR (SEQ ID NO:2), RGD (SEQ ID NO:8), RGDS (SEQ ID NO:9), DGEA (SEQ ID NO:10), VAPG (SEQ ID NO:11), REDV (SEQ ID NO:12), DGEA (SEQ ID NO:13), or LRSR (SEQ ID NO:14), and wherein the hydrophilic peptide sequence of the second peptide amphiphile comprises a degradation sequence and a nitric oxide producing donor sequence, wherein the nitric oxide producing donor sequence comprises polylysine, polycysteine sequence, or modified polylysine.

12. The composition of claim 11, wherein the hydrophilic peptide sequence of the first peptide amphiphile comprises the formula:

DS---CA,

wherein each --- is independently a direct or indirect covalent linkage,

wherein "DS" is a degradation sequence; and

wherein "CA" is an endothelial cell adhesive sequence;

and where the hydrophilic peptide sequence of the second peptide amphiphile comprises the formula:

DS---KK,

wherein "KK" is a nitric oxide producing donor sequence.

13. The composition of claim 11 or claim 12, further comprising nanofibers, wherein the first and second amphiphiles are coated on the nanofiber.

14. The peptide amphiphile of claim 9, or composition of claim 13, wherein the nanofiber or nanofibers comprise a polyester.

15. The peptide amphiphile or composition of claim 14, wherein the polyester is a polycaprolactone.

16. An endothelium mimicking nanomatrix, comprising one or more peptide amphiphiles assembled into nanofibers, wherein the peptide amphiphiles each comprise a hydrophilic peptide sequence and a hydrophobic tail, wherein the hydrophilic peptide sequence comprises a degradation sequence and one or more of a first cell adhesive sequence and a nitric oxide producing donor sequence, wherein the first adhesive sequence is an endothelial cell adhesive sequence, wherein the cell adhesive sequence comprises YIGSR (SEQ ID NO:2), RGD (SEQ ID NO:8), RGDS (SEQ ID NO:9), DGEA (SEQ ID NO:10), VAPG (SEQ ID NO:11), REDV (SEQ ID NO:12), DGEA (SEQ ID NO:13), or LRSR (SEQ ID NO:14); and wherein the nitric oxide producing donor sequence comprises polylysine, polycysteine sequence, or modified polylysine.

17. The endothelium mimicking nanomatrix of claim 16, wherein the nanofibers comprise a mixture of peptide amphiphiles having formulas DS---CA and DS---KK.

18. The endothelium mimicking nanomatrix of claim 17, wherein the DS---CA and DS---KK peptide amphiphiles are present in the nanofibers at a ratio of about 1:9 to about 9:1.

19. The endothelium mimicking nanomatix of any of claims 16-18, wherein the nanofibers further comprise polycaprolactone.

20. A composition comprising a medical device coated with an endothelium mimicking nanomatrix, comprising one or more peptide amphiphiles assembled into nanofibers, wherein the peptide amphiphiles each comprise a hydrophilic peptide sequence and a hydrophobic tail, wherein the hydrophilic peptide sequence comprises a degradation sequence and one or more of a first cell adhesive sequence and a nitric oxide producing donor sequence, wherein the first adhesive sequence is an endothelial cell adhesive sequence, wherein the cell adhesive sequence comprises YIGSR (SEQ ID NO:2), RGD (SEQ ID NO:8), RGDS (SEQ ID NO:9), DGEA (SEQ ID NO:10), VAPG (SEQ ID NO:11), REDV (SEQ ID NO:12), DGEA (SEQ ID NO:13), or LRSR (SEQ ID NO:14); and wherein the nitric oxide producing donor sequence comprises polylysine, polycysteine sequence, or modified polylysine.

21. The composition of claim 20, wherein the medical device is a vascular stent, vascular graft, catheter, pacemaker, or heart valve.

22. The composition of claim 20 or claim 21, wherein the nanofibers comprise polycaprolactone nanofibers onto which the amphiphiles are coated forming the nanomatrix.

23. A method of making a peptide amphiphile, comprising the steps:

a) providing a hydrophilic peptide comprising a degradation sequence and one or more of an endothelial cell adhesive sequence and a nitric oxide producing donor sequence, wherein the cell adhesive sequence comprises YIGSR (SEQ ID NO:2), RGD (SEQ ID NO:8), RGDS (SEQ ID NO:9), DGEA (SEQ ID NO:10), VAPG (SEQ ID NO:11), REDV (SEQ ID NO:12), DGEA (SEQ ID NO:13), or LRSR (SEQ ID NO:14); and wherein the nitric oxide producing donor sequence comprises polylysine, polycysteine sequence, or modified polylysine;

b) alkylating the N-terminus of the hydrophilic peptide with a hydrophobic moiety.

24. The method of claim 23, wherein the alkylation comprises amidation with a hydrophobic carboxylic acid.

25. The method of claim 24, wherein the hydrophobic carboxylic acid is a fatty acid.

26. The method of any of claims 23-25, further comprising inducing self-assembly of one or more peptide amphiphiles into nanofibers.

27. The method of any of claims 25-26, further comprising reacting the one or more peptide amphiphiles with nitric oxide to form a diazeniumdiolate-modified peptide $[K[N(O)NO^-]]_5$.

28. A method of treating a subject with cardiovascular disease comprising administering to the subject one or more peptide amphiphiles, wherein at least one peptide amphiphile is the peptide amphiphile of any of claims 1-10.

29. The method of claim 28, wherein the peptide amphiphiles each comprise a hydrophilic peptide sequence and a hydrophobic tail, wherein the hydrophilic peptide sequence

comprises a degradation sequence and one or more of a first cell adhesive sequence and a nitric oxide producing donor sequence.

30. A method of improving graft patency comprising administering to a recipient of a graft one or more peptide amphiphiles, wherein at least one peptide amphiphile is the peptide amphiphile of claim 1.

31. The method of claim 30, wherein the peptide amphiphiles each comprise a hydrophilic peptide sequence and a hydrophobic tail, wherein the hydrophilic peptide sequence comprises a degradation sequence and one or more of a first cell adhesive sequence and a nitric oxide producing donor sequence.

32. Use of the peptide amphiphile of any one of claims 1-10, 14 or 15 for treating a subject with cardiovascular disease or improving graft patency in a recipient of a graft.

33. Use of the peptide amphiphile of any one of claims 1-10, 14 or 15 for the preparation of a medicament for treating a subject with cardiovascular disease, and/or for improving graft patency in a recipient of a graft.

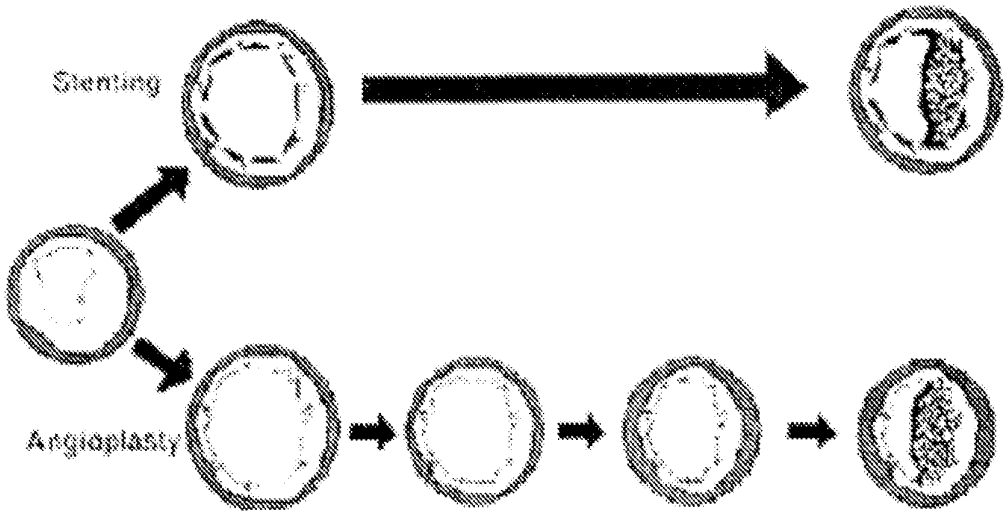
34. The peptide amphiphile of any one of claims 1-10, 14 or 15 for use in treating a subject with cardiovascular disease or in improving graft patency in a recipient of a graft.

35. The use of claim 32 or claim 33 or the peptide amphiphile of claim 34, wherein the peptide amphiphiles each comprise a hydrophilic peptide sequence and a hydrophobic tail, wherein the hydrophilic peptide sequence comprises a degradation sequence and one or more of a first cell adhesive sequence and a nitric oxide producing donor sequence.

36. The method of any of claims 23-31, or the use of any of claims 32, 33, or 35 or the peptide amphiphile of claim 34, wherein the peptide amphiphile are coated on a polyester nanofiber.

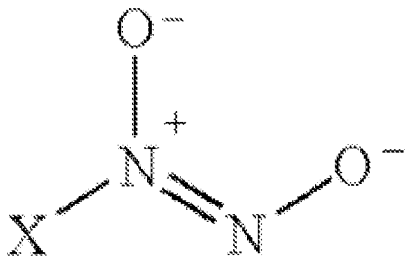
37. The method, use or peptide amphiphile of claim 36, wherein the polyester nanofiber is a polycarbolactone nanofiber.

38. The method, use or peptide amphiphile of claim 36 or claim 37, wherein the graft is an artery, vein, vascular stent, catheter, pacemaker, or heart valve.



Phenomenon	Elastic recoil	Negative remodeling	Neointima formation
Pathophysiology	Passive recoil of elastic media	Increased collagen in extracellular matrix and adventitial thickening	Proliferation and migration of smooth muscle cells and extracellular matrix formation
Time course	Within one hour	One to six months	One to six months

Figure 1

A. 

B. $X^- + 2NO \rightarrow X\text{-}[N(O)NO]^-$

C. $X\text{-}[N(O)NO] \rightarrow X^- + 2NO$

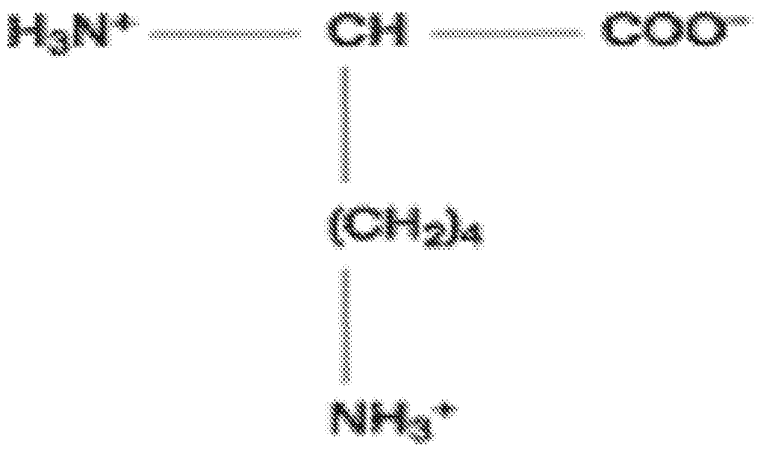
D. 

Figure 2

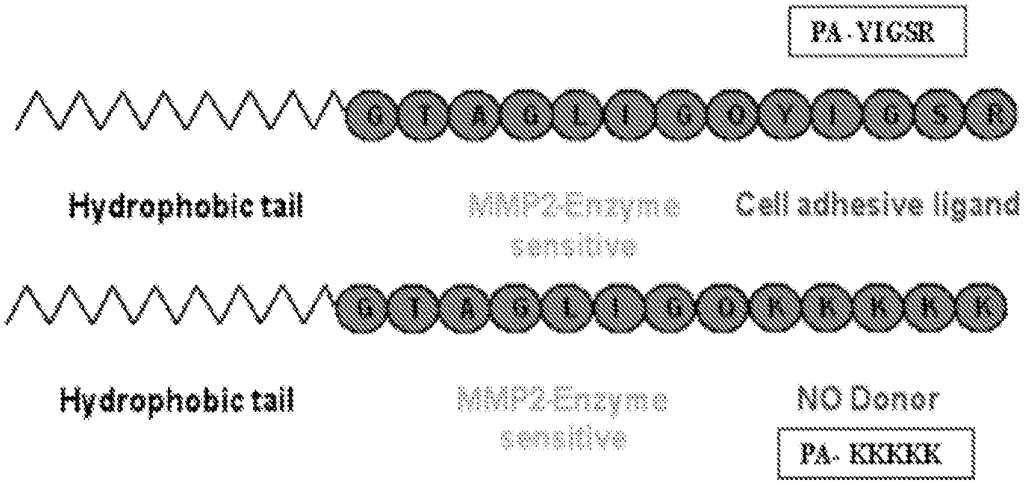

PA-YIGSR
G I A G L I G Q Y I G S R
Hydrophobic tail
MMP2-Enzyme sensitive
Cell adhesive ligand
G I A G L I G Q K K K K K
Hydrophobic tail
MMP2-Enzyme sensitive
NO Donor
PA- KKKKK

Figure 3

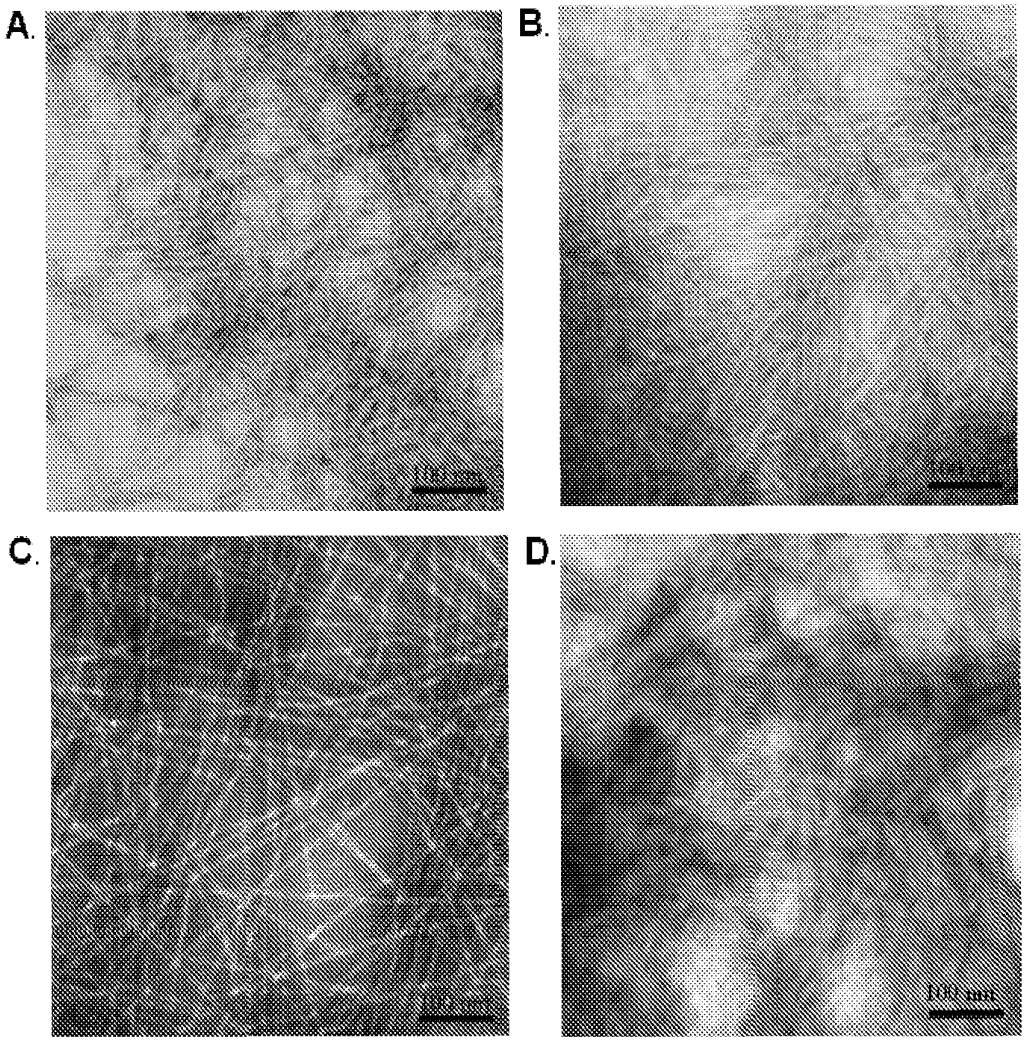


Figure 4

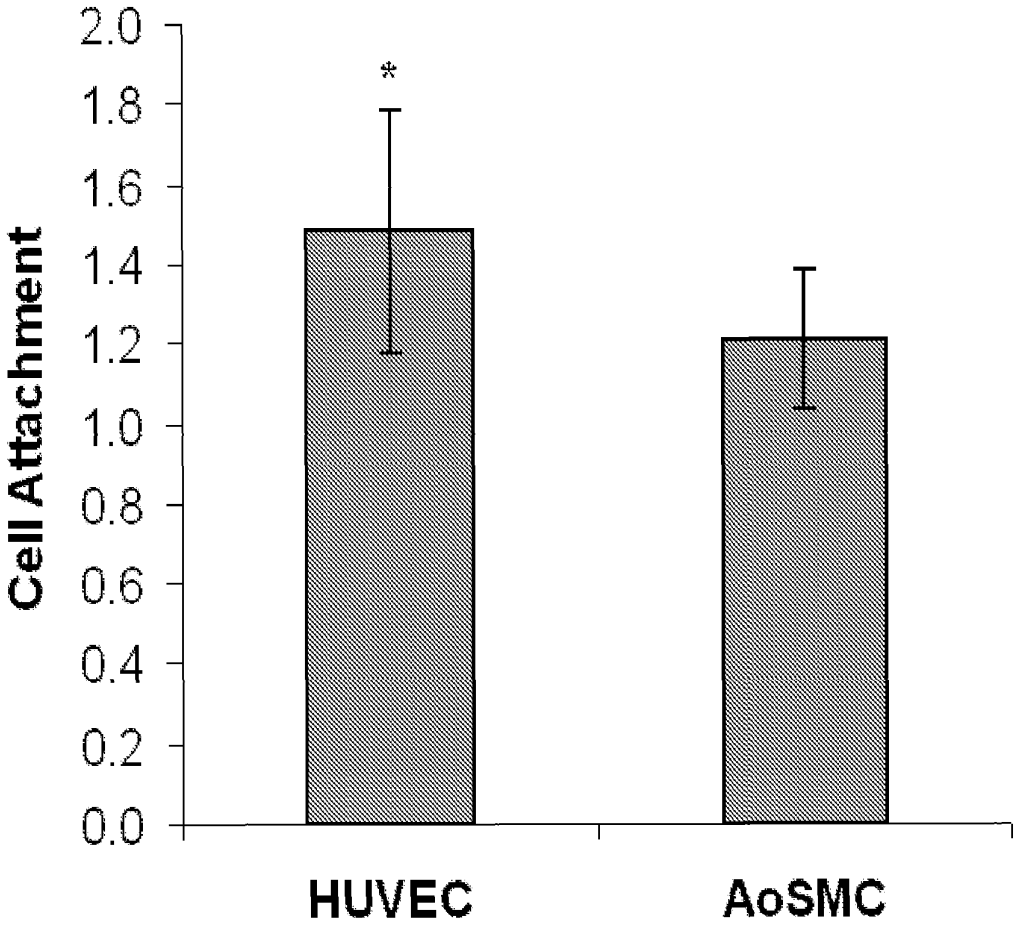


Figure 5

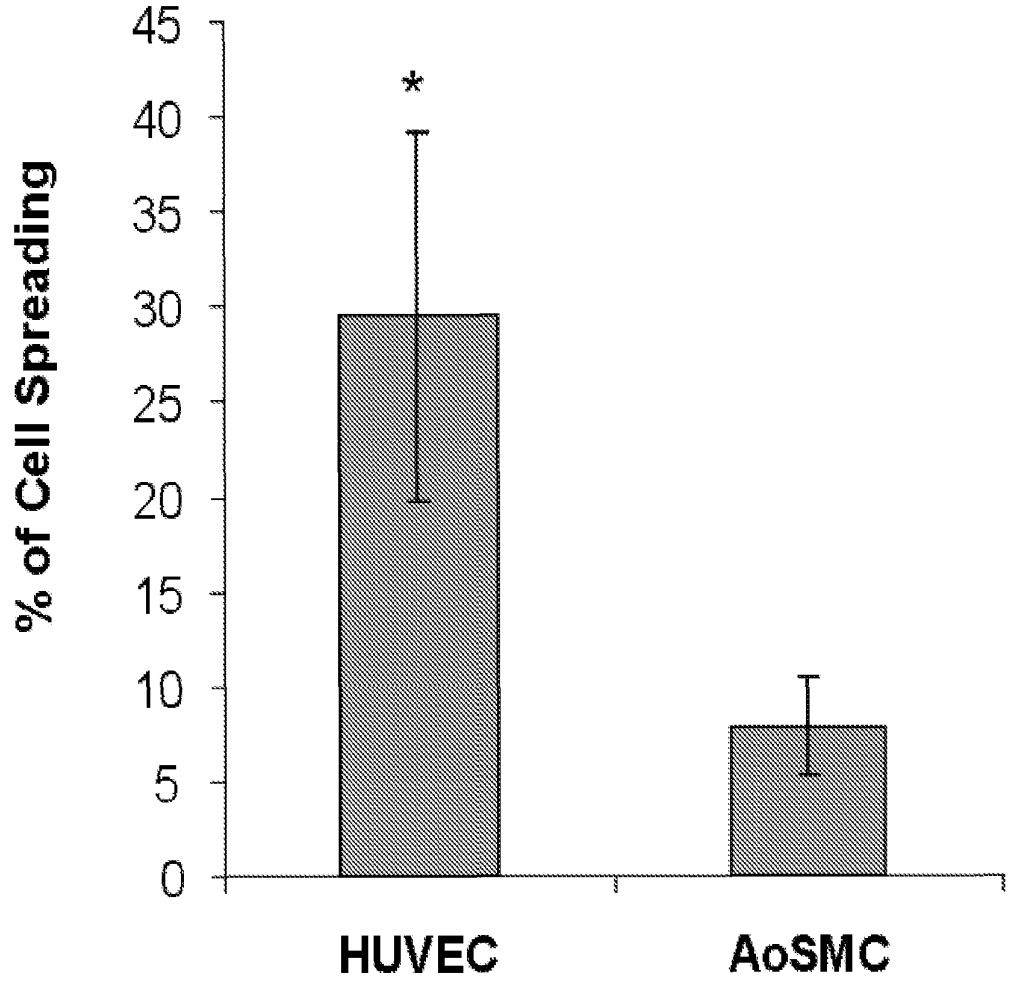


Figure 6

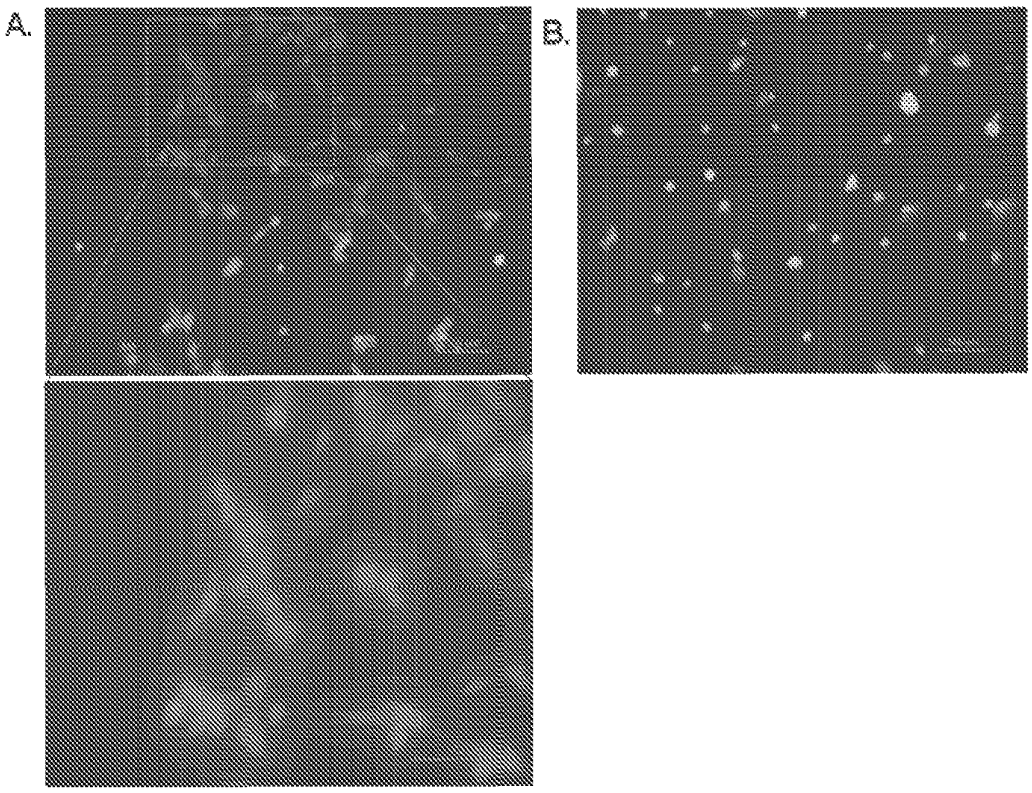


Figure 7

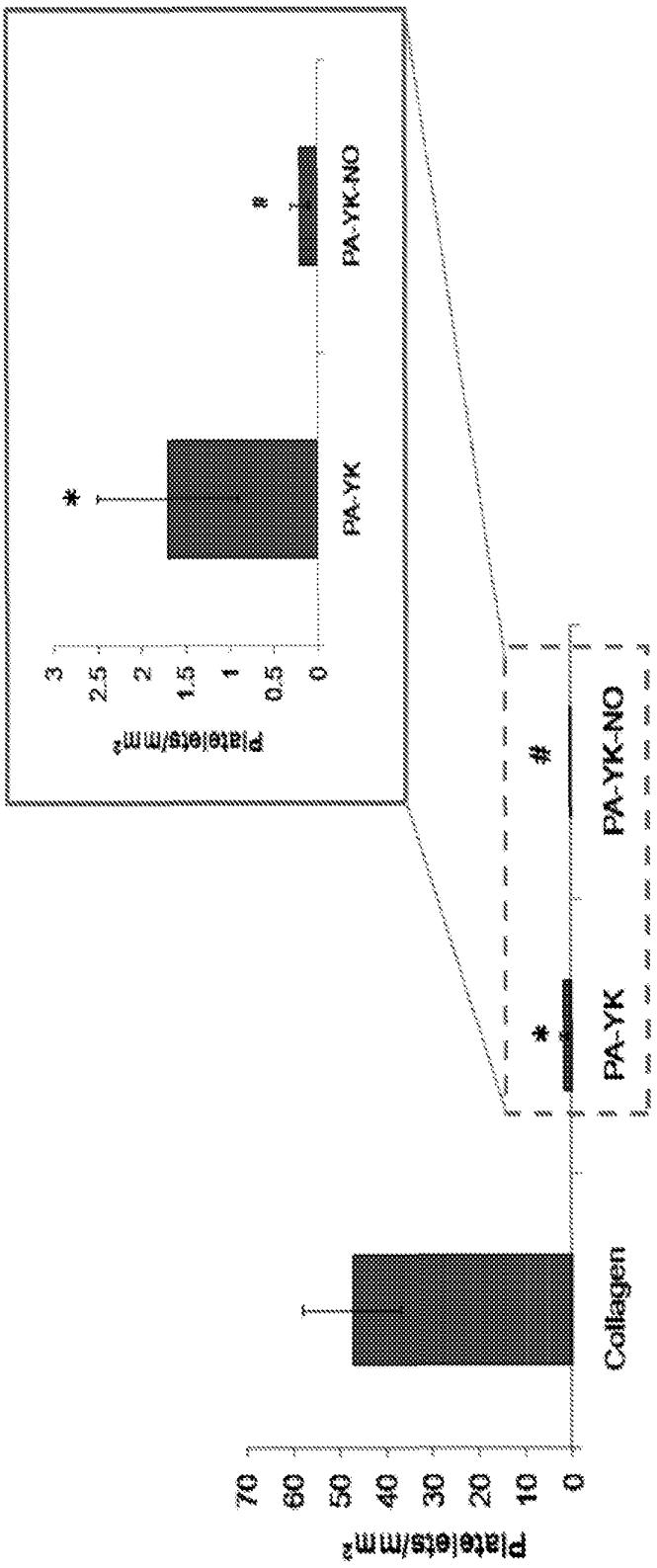

Platelets/mm²
70
60
50
40
30
20
10
0
Collagen
*
PA-YK
#
PA-YK-NO
Platelets/mm²
3
2.5
2
1.5
1
0.5
0
*
PA-YK
#
PA-YK-NO

Figure 8

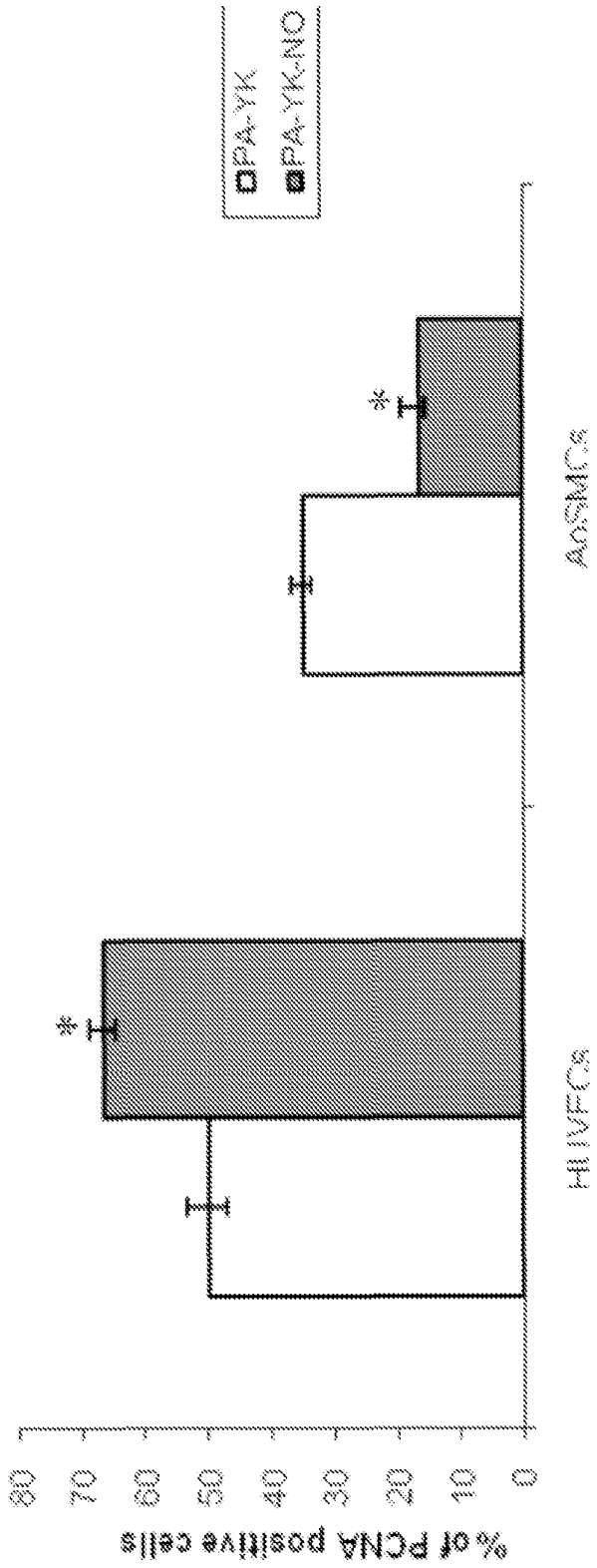

PA-YK
PA-YK-NO
% of PCNA positive cells
0
10
20
30
40
50
60
70
80
HI IVFCs
AoSMCs
*
*

Figure 9

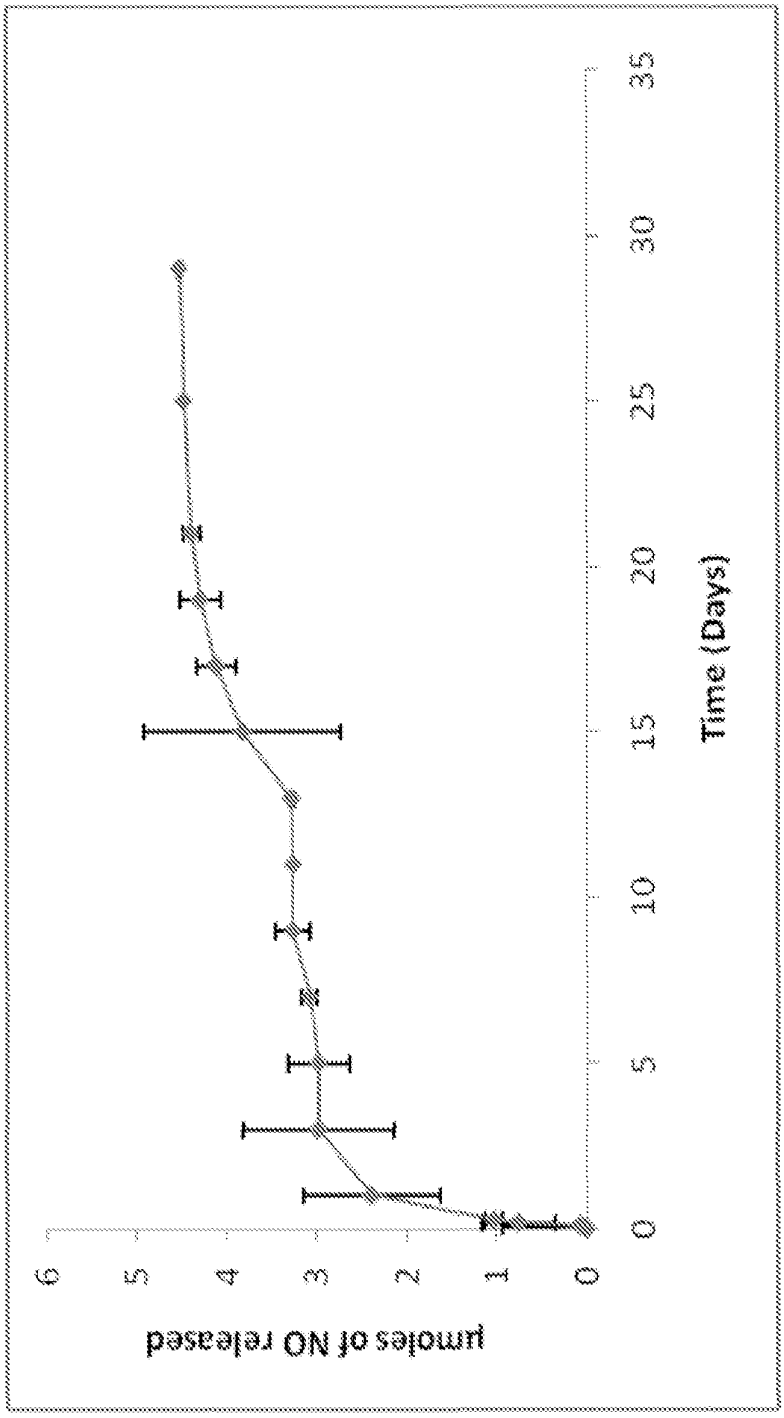


Figure 10

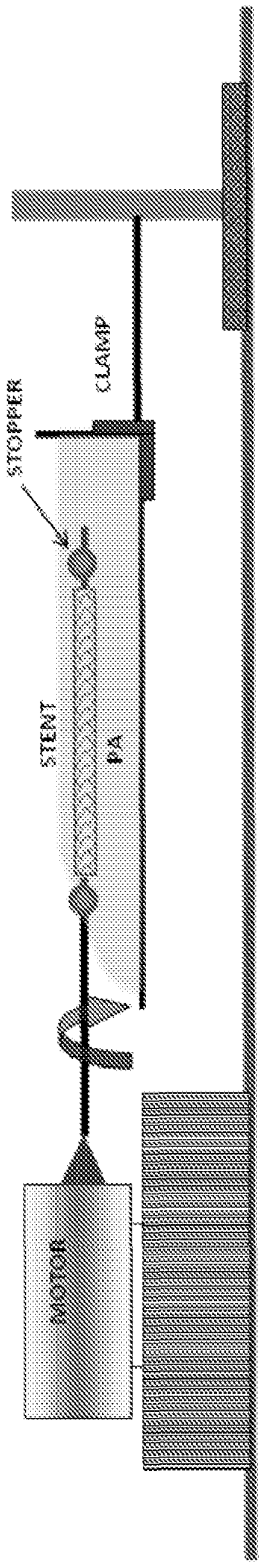


Figure 11

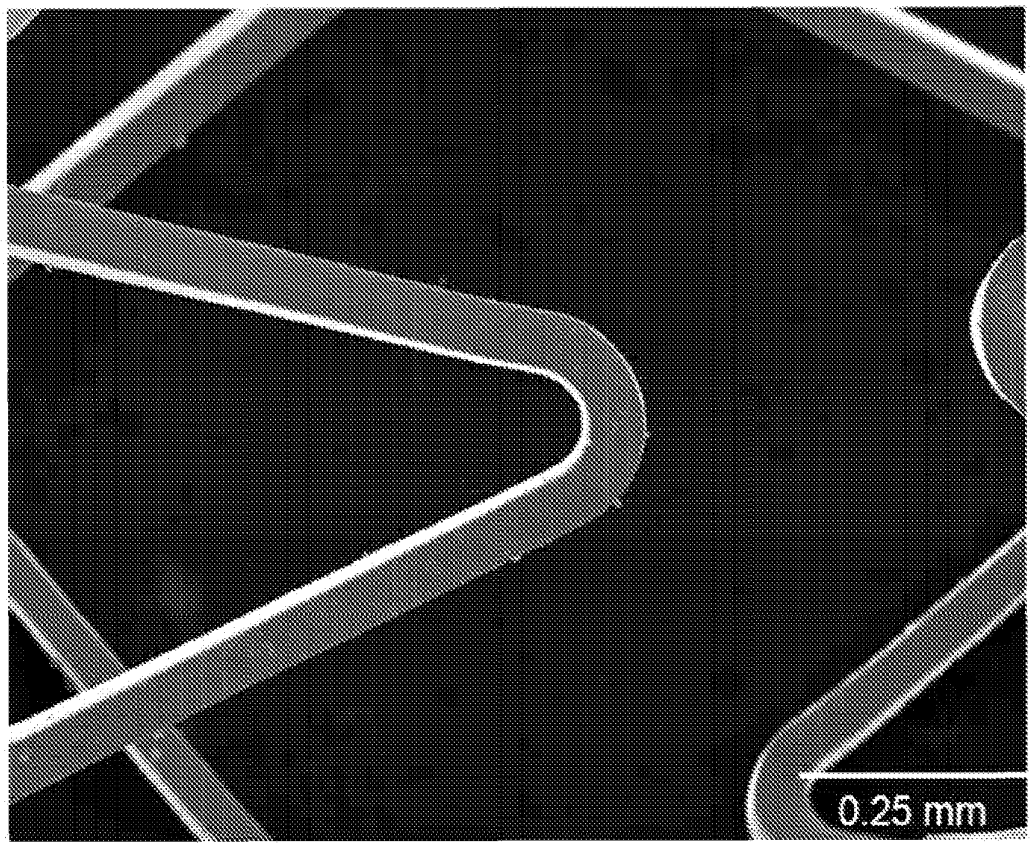


Figure 12

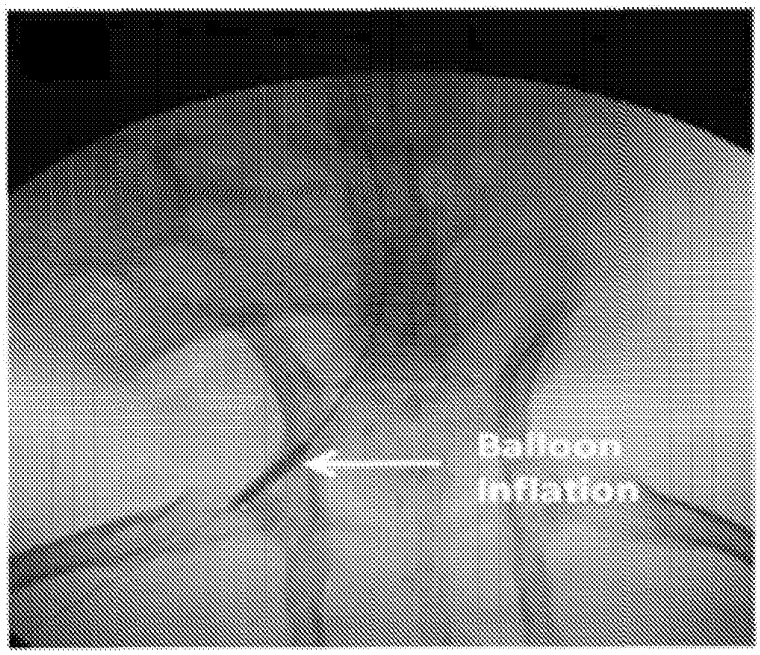


Figure 13A

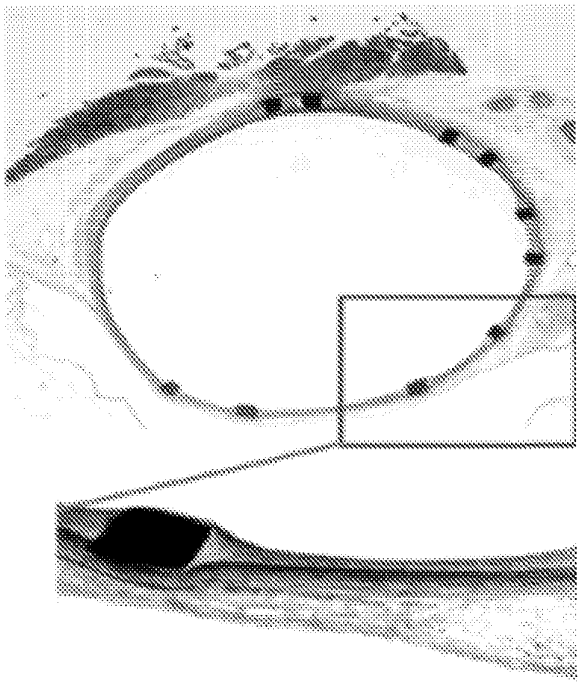


Figure 13B

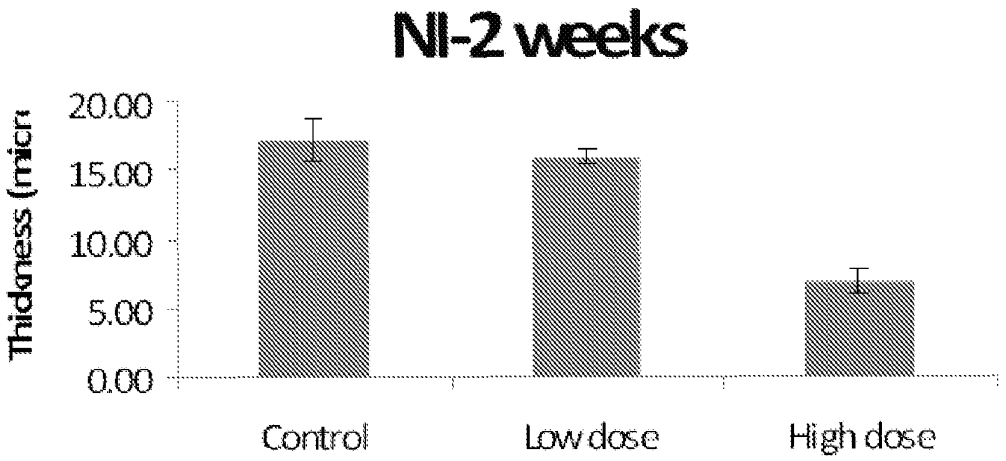


Figure 14

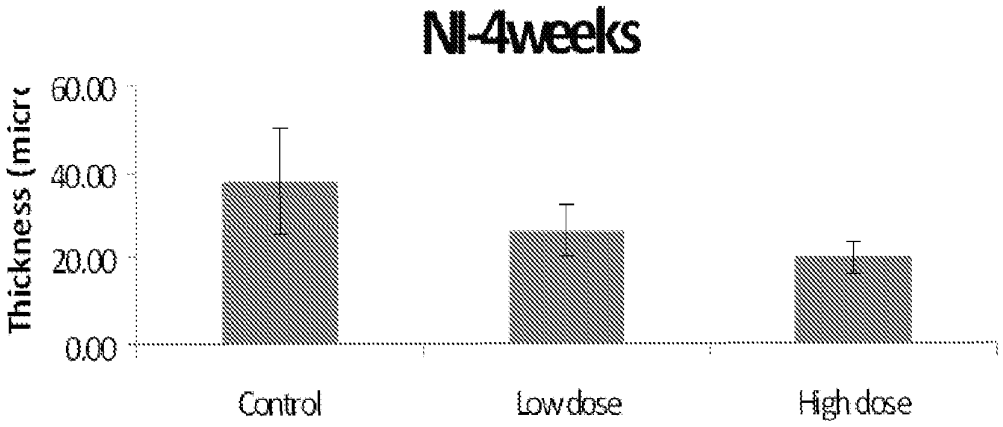


Figure 15

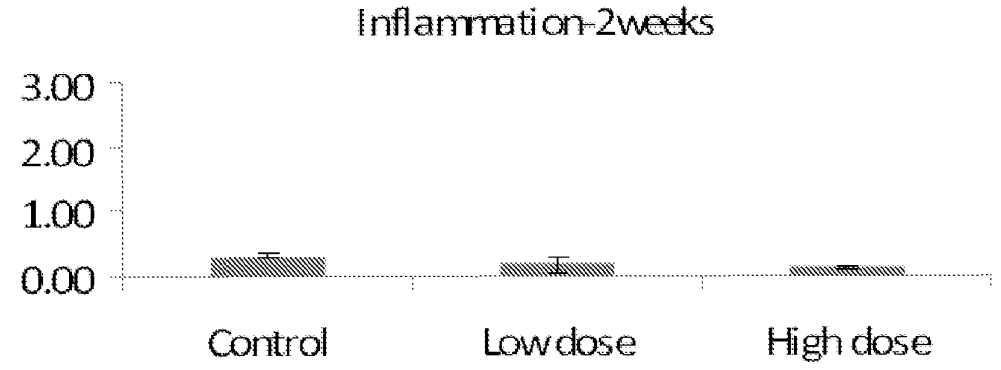


Figure 16

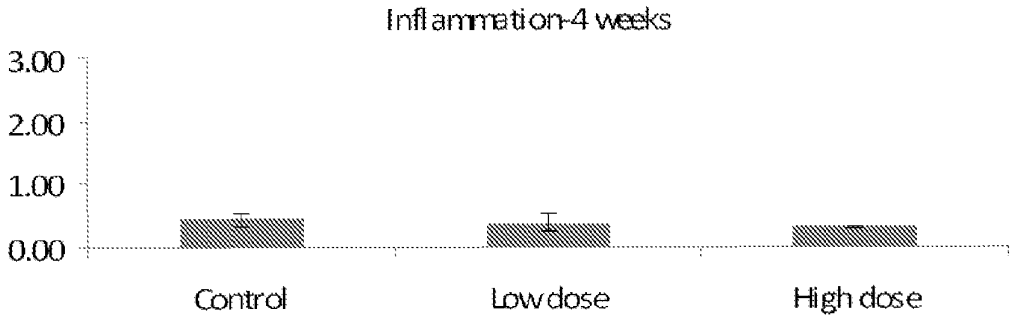


Figure 17

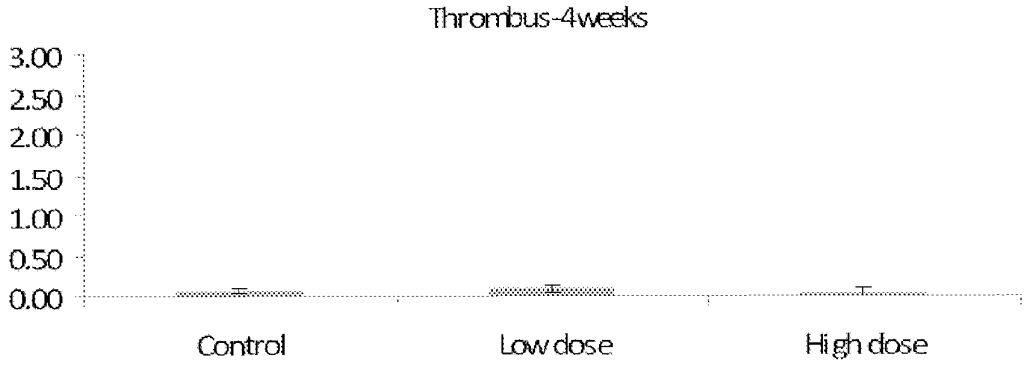


Figure 18

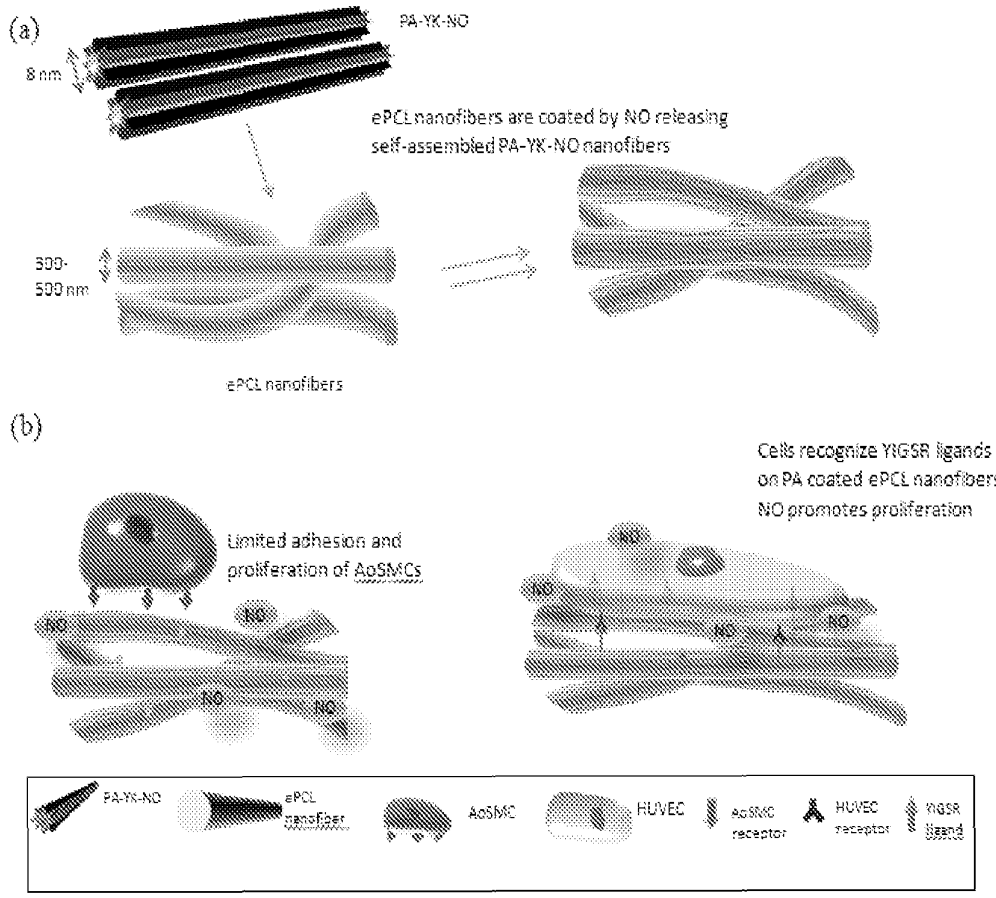


Figure 19

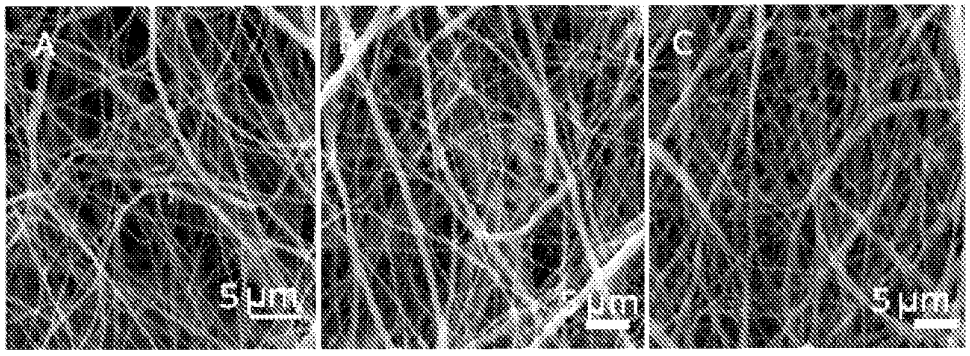


Figure 20

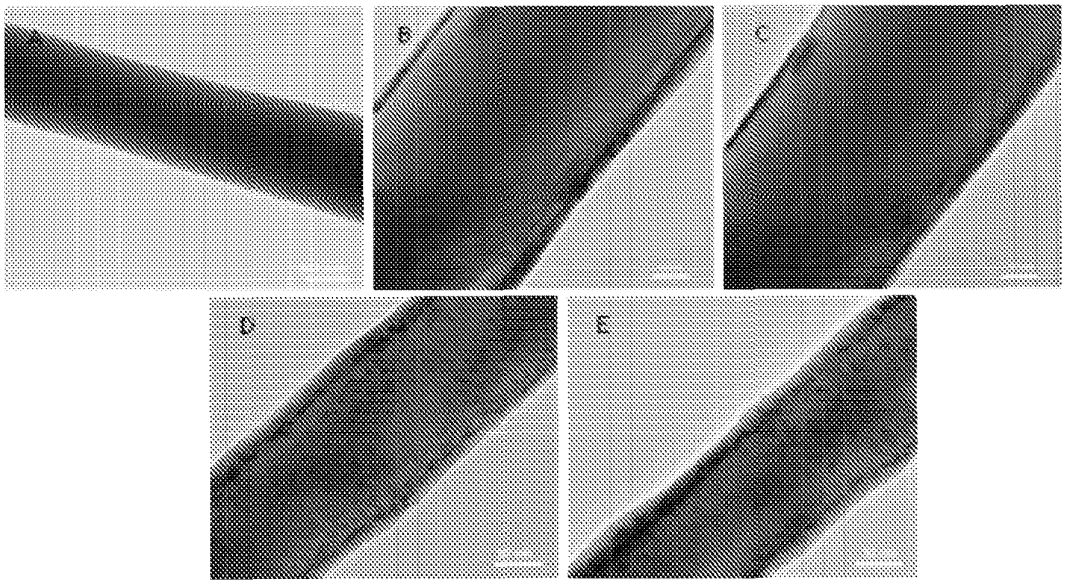


Figure 21

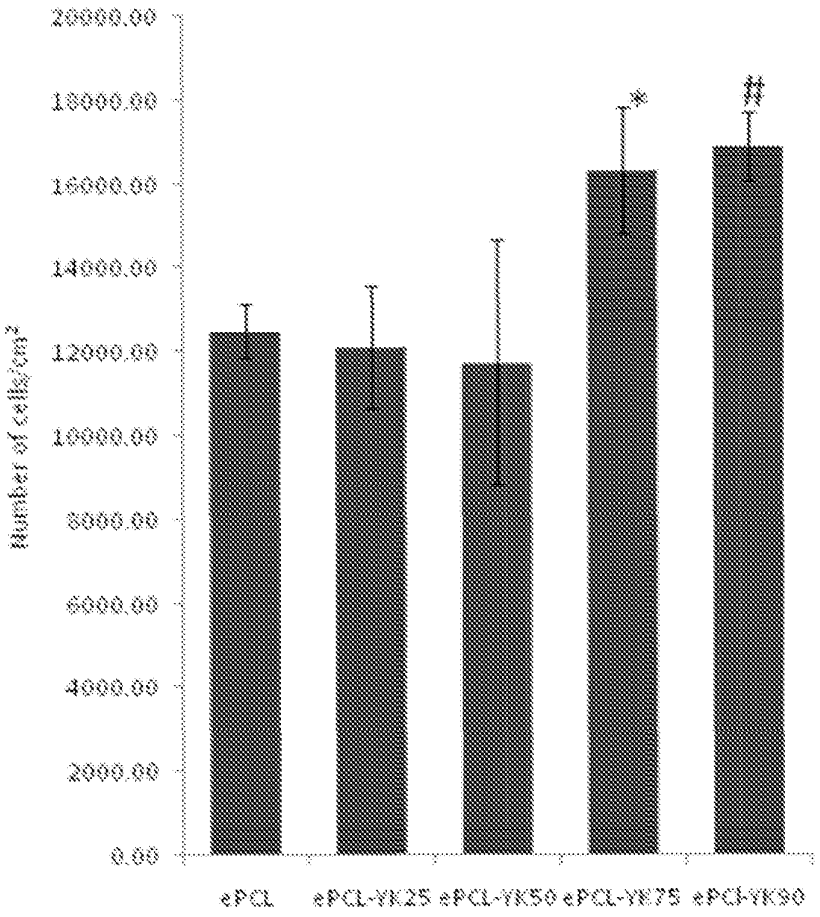

20000.00
18000.00
16000.00
14000.00
12000.00
10000.00
8000.00
6000.00
4000.00
2000.00
0.00
Number of cells/cm²
*
#
ePCL
ePCL-YK25
ePCL-YK50
ePCL-YK75
ePCL-YK90

Figure 22

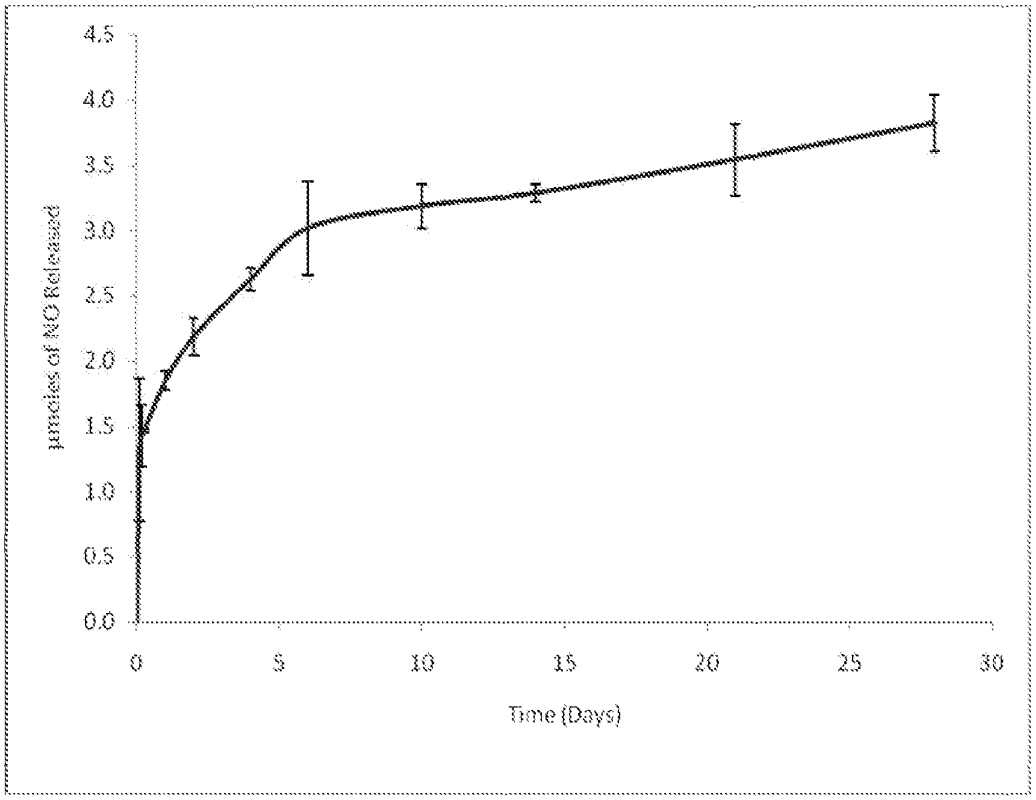


Figure 23

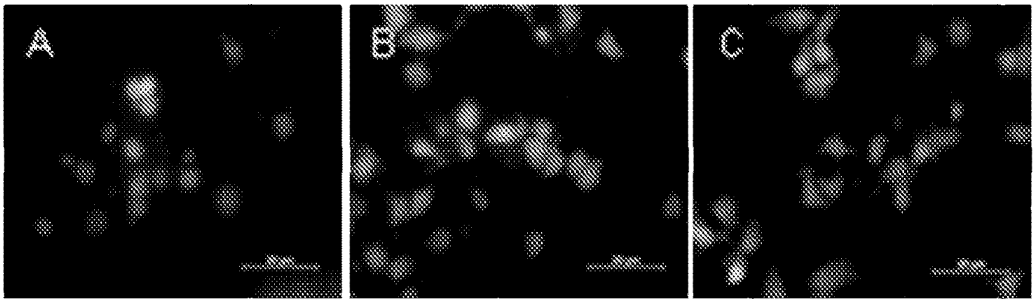


Figure 24

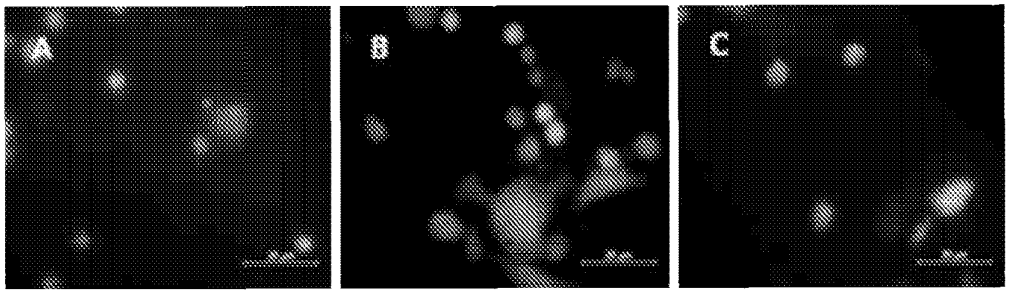


Figure 25

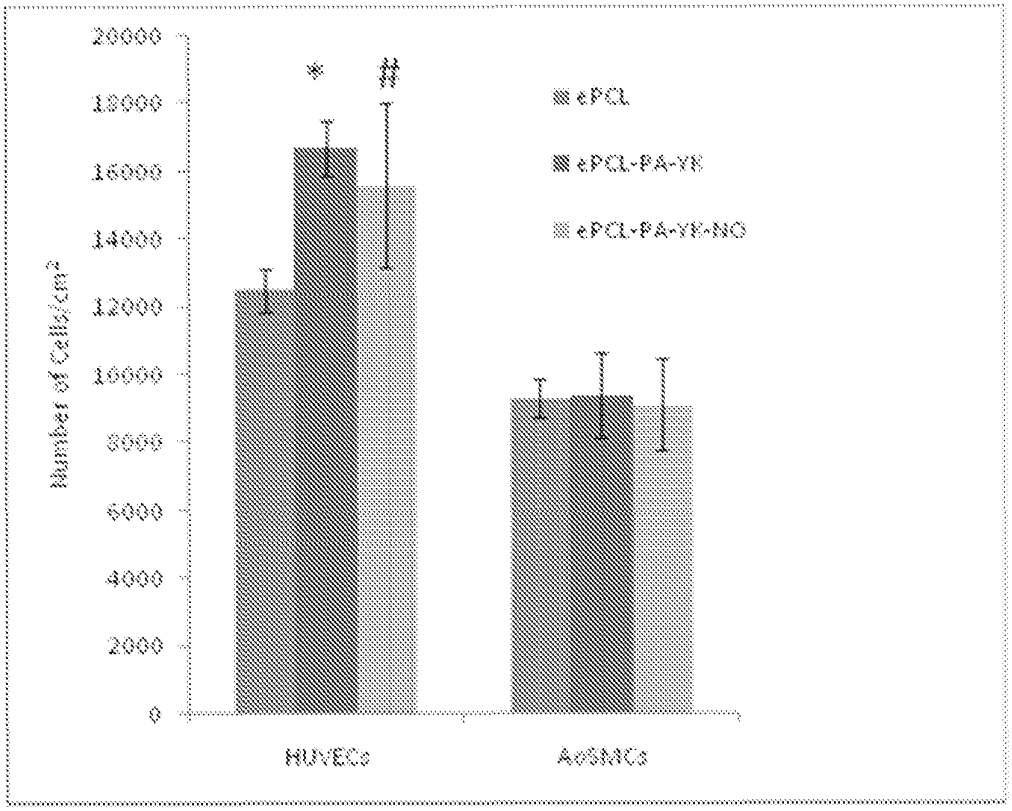


Figure 26

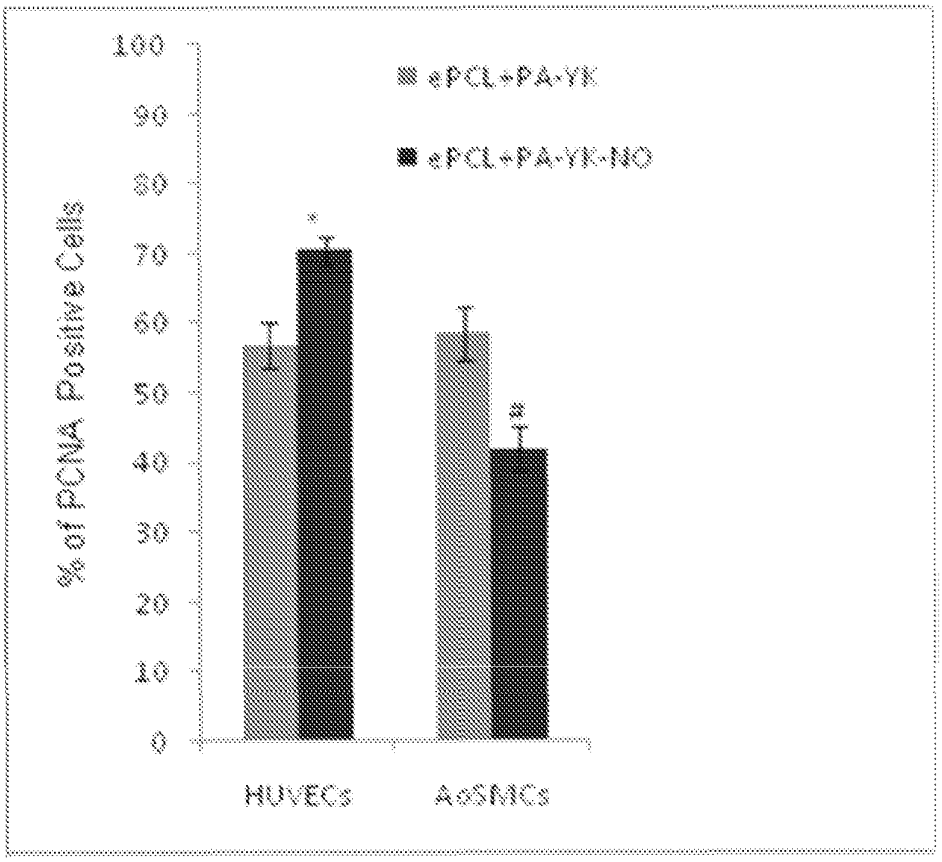

100
90
80
70
60
50
40
30
20
10
0
% of PCNA Positive Cells
ePCL-PA-YK
ePCL-PA-YK-NO
*
#
HUVECs
AoSMCs

Figure 27