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(71) Applicant: THE REGENTS OF THE UNIVERSITY OF CALIFORNIA [US/US]; 1111 Franklin Street, 5th Floor, Oakland, California 94607-5200 (US).

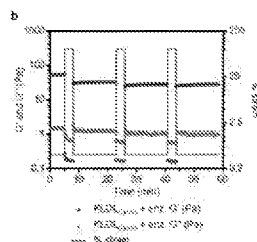
(72) Inventors: GIANNESCHI, Nathan C.; 2145 Sheridan Road, Rm. K148, Evanston, Illinois 60208 (US). CHRISTMAN, Karen L.; 2880 Torrey Pines Scenic Drive, La Jolla, California 92037 (US). CARLINI, Andrea S.; 2145 Sheridan Road, Rm. K148, Evanston, Illinois 60208 (US).

(74) Agent: WARREN, William L. et al.; EVERSLED SUTHERLAND (US) LLP, 999 PEACHTREE STREET, N.E., SUITE 2300, ATLANTA, Georgia 30309 (US).

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(54) Title: CATHETER INJECTABLE CYCLIC PEPTIDE PRO-GELATORS FOR MYOCARDIAL TISSUE ENGINEERING



(57) Abstract: Cyclic peptide pro-gelator compositions, and methods of therapeutic use, which assemble into macromolecular hydrogel when administered through cleavage by endogenous enzymes upregulated at a site of tissue injury, such as a myocardial infarction.

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CATHETER INJECTABLE CYCLIC PEPTIDE PRO-GELATORS FOR MYOCARDIAL TISSUE ENGINEERING

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims the priority benefit of U.S. Provisional Patent
5 Application No. 62/517,213, filed June 9, 2017, the entire contents of which is
incorporated herein by reference.

STATEMENT OF GOVERNMENT INTEREST

[0002] This invention was made with government support under Grant No.
5R01HL117326 awarded by National Institutes of Health (NIH) and Grant No. DGE-
10 1144086 awarded by the National Science Foundation. The government has certain rights
in the invention.

SEQUENCE LISTING

[0003] The instant application contains a Sequence Listing which has been
submitted electronically in ASCII format and is hereby incorporated by reference in its
15 entirety. Said ASCII copy, created on June 11, 2018, is named 24978-0403_SL.txt and is
25,497 bytes in size.

BACKGROUND OF THE INVENTION

[0004] Myocardial infarction (MI) accounts for 46% of all deaths attributed to
cardiovascular disease.¹ Within the first few days post-MI, an inflammatory response
20 causes cardiomyocyte death and degradation of the native extracellular matrix (ECM) by
secreted matrix metalloproteinases (MMPs).² This in turn can lead to aneurysmal thinning
and negative left ventricular (LV) remodeling within days to weeks. Left untreated, heart
failure results as one of the leading causes of death in the western world.

[0005] Injectable hydrogel-based scaffolds have gained attention as a therapeutic
25 approach to prevent negative LV remodeling by utilizing materials to stimulate cardiac
repair. Materials for this purpose have included myocardial ECM,³ alginate,⁴ and
hyaluronic acid hydrogels,⁵ or employing biomaterials as therapeutic delivery scaffolds
such as naturally derived polymeric hydrogels (e.g. collagen,⁶ fibrin,⁷ heparin,⁸ and

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gelatin⁹), synthetic polymeric hydrogels (e.g. PNIPAAm,¹⁰ ureido-pyrimidinone-modified PEG,¹¹ and chitosan¹²) and microparticles (e.g. PLGA¹³ and dextran¹⁴). Despite many successful preclinical studies, wide spread translation and initiation of clinical trials has been slow, with current studies limited to a myocardial ECM hydrogel (clinicaltrials.gov
5 identifier NCT02305602) and alginate (NCT01226563 and NCT01311791). One reason for this lack of translation is that the majority of these hydrogels are not candidates for minimally invasive catheter delivery because of excess material viscosity, their quick gelling nature that can lead to catheter clogging, and concerns regarding hemocompatibility since materials can leak into the blood stream upon injection into a
10 beating heart.¹⁵⁻¹⁷

[0006] One versatile class of materials that has been successfully tested in several preclinical MI models using surgical epicardial injections is self-assembling peptides (SAPs).¹⁸⁻²¹ SAP hydrogels are attractive as they: (1) resemble native ECM, (2) require no additive reagents to induce gelation, (3) are biodegradable, (4) are biocompatible, (5) have
15 pore sizes (~5-200 nm) conducive to promote endothelial cell adhesion and capillary formation, (6) allow rapid cellular migration because of their flexibility, (7) are rehealable, (8) do not suffer from batch-to-batch chemical variability, and (9) are amenable to sequence modification.^{22,23} However, SAPs have not been demonstrated as being amenable to cardiac injection catheter delivery. Other researchers have considered efforts
20 with enzyme-responsive peptides²⁴⁻²⁶ and a study by Nilsson and coworkers utilizing reductively triggered cyclic peptides as progelators²⁷ but the art has not provided cyclic, enzyme triggered, responsive peptide platform that enables minimally invasive delivery of self assembling peptides to the heart. It was previously demonstrated that peptide-polymer based nanoparticles can aggregate into macromolecular scaffolds in diseased tissue
25 through the action of endogenously expressed inflammatory-related enzymes, matrix metalloproteinases (MMPs), providing a viable method for targeted accumulation,²⁸ prolonged tissue retention,²⁹ and therapeutic delivery.³⁰ However, these materials employed a non-biodegradable functionalized polynorbornene backbone and did not possess viscoelastic properties.

30 SUMMARY OF THE INVENTION

[0007] The invention provides a tissue scaffolding composition and method of use for treating tissues in need thereof, e.g., myocardial infarctions, comprising in various

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embodiments a cyclic peptide. In embodiments, the peptide comprises a substrate recognition sequence that when enzymatically cleaved linearizes and self assembles into a hydrogel. In embodiments, the peptide has at least one, preferably three or more, repeating amino acid sequence KFDF (SEQ ID NO:1) or KLDL (SEQ ID NO:5) or other
5 known SAP gelling sequences. In embodiments, the peptide is made cyclic by containing covalent disulfide bonds on cysteine residues or other functional moieties. In embodiments, the substrate recognition sequence is specific for inflammatory-related enzymes, matrix metalloproteinases (MMP)-2/9 and/or elastase. In embodiments, the substrate recognition sequence comprises a MMP-2/9 cleavable amino acid sequence
10 PLGLAG (SEQ ID NO:2). In embodiments, the peptide is labeled for detection or other functional moieties. In alternative embodiments, the composition further comprises a therapeutic agent, a chemotactic agent, an antibiotic, and/or a growth factor.

[0008] The invention provides a method of creating a scaffold for tissue repair in a patient in need thereof comprising administering to the patient a tissue scaffolding
15 composition as described. In embodiments, the scaffold is used for example in negative left ventricular remodeling post-myocardial infarction. In embodiments, the administration is via a catheter, parenteral injection, or implantation.

[0009] In certain embodiments, the present invention provides compositions and methods comprising injecting or implanting in a subject in need an effective amount of the
20 tissue scaffolding composition. The composition can further comprise cells, drugs, proteins, or polysaccharides. The composition can be delivered as a liquid, and can transition to a gel form after delivery and *in situ* enzymatic cleavage. In some instances, the composition is coated on a device such as an implant.

[0010] In some instances, the composition is configured to be delivered to a tissue
25 through a catheter. In some instances, said composition is suitable for direct implantation into a patient.

[0011] In some instances the composition comprises cells, proteins, or therapeutic agents. In some instances, the composition further comprises non-naturally occurring factors that recruit cells into the composition, encourage growth or prevent infection. In
30 some instances, the composition further comprises a therapeutic agent, and as such is configured as a drug delivery vehicle.

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[0012] Enzyme-induced peptide assembly provides a sensitive method for minimally invasive delivery of scaffolding materials to the site of acute MI. Gelling peptides bearing a substrate recognition sequence for the inflammatory-related enzymes, matrix metalloproteinase (MMP)-2/9 and elastase, were cyclized via disulfide bonds to create a water-soluble pro-gelator. When enzymatically cleaved, sterically constrained cyclic peptides linearize and self-assemble via beta-sheets into hydrogels, as shown by TEM, rheometry, and circular dichroism spectroscopy. Furthermore, incorporation of functional ligands and enzyme-responsive amino acids does not disrupt hydrogel assembly. Concentrated peptide solutions in saline were shown to be readily injectable via catheter delivery, representing the first example of a peptide-based gelator for delivery to the MI. Finally, enzyme activation of these materials results in hydrogels that exhibit repeated and rapid healing following excess shear strain. The invention provides compositions and methods for therapeutic hydrogel delivery to acute MI.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] **Figs. 1a-1c. Design of cyclic, enzyme-responsive progelator peptides for activatable gelation.** **Fig. 1a**, cyclic progelator peptides, containing gelling sequence (green greyscales), MMP/elastase enzyme-cleavage recognition sequence (red greyscales), and disulfide bridge (black) resist assembly due to conformational constraint. Rhodamine labeled (pink ellipse greyscales) SAPs were employed for *in vivo* studies as a 5 mol% additive to provide a means for imaging the hydrogels in *ex vivo* microscopy analyses. **Fig. 1b**, Enzymatic cleavage results in linearization into SAPs. **Fig. 1c**, SAPs assemble into viscoelastic hydrogels composed of β -sheets fibrils.

[0014] **Figs. 2a-2c. Sequences and design of SAPs and cyclic, enzyme-responsive progelators.** **Fig. 2a**, Table of SAPs and progelators based on the (KLDL)₃ (SEQ ID NO:3) and (KFDF)₃ (SEQ ID NO:4) amino acid gelling sequence with corresponding diagrammatical representations. Sequences are shown for the base, unmodified SAPs (KLDL (SEQ ID NO:5) and KFDF (SEQ ID NO:1)) and modified SAPs (KLDL_{Linear} and KFDF_{Linear}) primed for cyclization but lacking fluorescent labels, together with the corresponding cyclized versions (progelators, KLDL_{Cyclic} and KFDF_{Cyclic}). Peptide sequences CKLDLKLDLKLDLPLGLAGC (SEQ ID NO:6) and CKFDFKFDFKFDFPLGLAGC ((SEQ ID NO:7) are compared. In addition, rhodamine

labeled modified SAPs (Rho-KL_{DL}_{Linear} and Rho-KF_{DF}_{Linear}) are shown, together with their corresponding labeled cyclized progelators (Rho-KL_{DL}_{Cyclic} and Rho-KF_{DF}_{Cyclic}). “acm” denotes the acetamidomethyl protecting group on Cysteine residues. “Rho” designates the 5(6)-carboxytetramethyl rhodamine label. **Figs. 2b-2c**, Modeling predicts β -sheet re-orientation, rather than self-assembly disruption, following significant SAP functionalization. **Fig. 2b**, Predicted structures and ribbon diagrams (bottom) of KL_{DL} and KL_{DL}_{Linear} SAPs from FibPredictor simulations revealing antiparallel and parallel β -sheet orientations, respectively. **Fig. 2c**, Predicted structures and ribbon diagrams (bottom) of KF_{DF} and KF_{DF}_{Linear} SAPs from FibPredictor simulations revealing antiparallel and parallel β -sheet orientations, respectively (see **Tables 1-2**).

[0015] Figs. 3a-3h. Impact of SAP functionalization on assembly properties. **Fig. 3a**, Experimental circular dichroism (CD) spectra of KL_{DL} and KL_{DL}_{Linear} SAPs, with labeled minimum at 215 nm ($n \rightarrow \pi^*$). **Fig. 3a** discloses SEQ ID NO:7 as "C(KF_{DF})₃PLGLAGC". **Fig. 3b**, Experimental circular dichroism (CD) spectra of KF_{DF} and KF_{DF}_{Linear} SAPs, with labeled minima at 215 nm ($n \rightarrow \pi^*$) and 203 nm ($\pi \rightarrow \pi^*$). **Fig. 3c**, Theoretical CD spectra of KL_{DL} and KL_{DL}_{Linear} SAPs from DichroCalc simulations using FibPredictor models. **Fig. 3d**, Theoretical CD spectra of KF_{DF} and KF_{DF}_{Linear} SAPs from DichroCalc simulations using FibPredictor models. CD spectra minima denoted with dashed lines. **Fig. 3e**, Assembled hydrogel photographs and corresponding TEM images of the KL_{DL} (left) and KL_{DL}_{Linear} (right) SAPs. **Fig. 3f**, Assembled hydrogel photographs and corresponding TEM images of the KF_{DF} (left) and KF_{DF}_{Linear} (right) SAPs. **Fig. 3g**, Viscoelastic measurements of storage moduli, G' (Pa), and damping factor, $\tan \delta$, for KL_{DL} and KL_{DL}_{Linear}. **Fig. 3h**, Viscoelastic measurements of storage moduli, G' (Pa), and damping factor, $\tan \delta$, for KF_{DF} and KF_{DF}_{Linear}. Gel capacity is defined as $\tan \delta < 1$ (dashed line). CD measurements performed at 500 μ M SAP in 10 mM Tris buffer, pH 7.4. (n=3 repeats) SAPs for TEM and rheology prepared at 100 μ M and 15 mg/mL in 1xDPBS (pH 7.4), respectively. Rheological measurements reported for angular frequency of 2.5 rads/s. (n=3 repeats) Values are mean $\pm$ SEM.

[0016] Figs. 4a-4k. Facile synthesis of sterically constrained and labeled cyclic progelators. **Fig. 4a**, Synthetic scheme for unlabeled (route I) and labeled (route II) KL_{DL}_{Cyclic} and KF_{DF}_{Cyclic} progelators. All SAPs were cyclized under dilute conditions (500 μ M) with 5 eq I₂ in a mixture of AcOH/MeOH/H₂O to create a soluble progelator.

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Route I uses N-terminal Fmoc-protected SAPs to improve material separation during chromatographic purification. Fmoc-removal generates the intended unlabeled progelators KLDL_{Cyclic} and KFDF_{Cyclic}. Route II depicts the synthesis of labeled progelators through N-terminal modification with 5(6)-carboxytetramethyl rhodamine prior to cyclization.

Figs. 4b-4c, HPLC (b) monitored at 214nm and corresponding ESI spectra (c) of Fmoc-KLDL_{Linear}, Fmoc-KLDL_{Cyclic}, and KLDL_{Cyclic} (835.79 m/z, 787.76 m/z, and 1070.26 m/z, respectively) verify purity and mass of synthetic modifications to KLDL peptides. **Figs. 4d-4e**, HPLC (d) monitored at 214nm and corresponding ESI spectra (e) of Fmoc-KFDF_{Linear}, Fmoc-KFDF_{Cyclic}, and KFDF_{Cyclic} (781.862 m/z, 856.17 m/z, and 1355.66 m/z, respectively) verify purity and mass of synthetic modifications to KFDF peptides. In both KLDL and KFDF systems, macrocyclization causes a slight decrease in polarity and Fmoc-deprotection increases progelator polarity **Fig. 4f**, KLDL_{Linear} SAP macrocyclization kinetics monitored by LCMS at 214 nm reveal complete cyclization after 120 min. **Fig. 4g**, Absorbance spectra of unlabeled and labeled KLDL progelators ($\lambda_{\text{max}} = 565$ nm for rhodamine signal). **Fig. 4h**, KFDF_{Linear} SAP macrocyclization kinetics monitored by LCMS at 214 nm reveal complete cyclization after 120 min. **Fig. 4i**, Absorbance spectra of unlabeled and labeled KFDF progelators ($\lambda_{\text{max}} = 565$ nm for rhodamine signal). **Fig. 4j**, KLDL_{Linear} precursor assembles as a hydrogel (left) and the resulting KLDL_{Cyclic} progelator exists as a soluble solution (right). **Fig. 4k**, KLDL_{Linear} precursor assembles as a hydrogel (left) and the resulting KLDL_{Cyclic} progelator exists as a soluble solution (right).

[0017] Figs. 5a-5f. Enzyme responsiveness of Rho-KLDL_{Cyclic} and Rho-KFDF_{Cyclic} progelators. **Figs. 5a-5c**, Analysis of Rho-KLDL_{Cyclic} responsiveness to MMP-9, elastase, and thermolysin. (**Fig. 5a**) Peptide sequence and indicated enzymatic cuts sites. **Fig. 5a** discloses SEQ ID NO:6. (**Fig. 5b**), Progelator incubated with active enzymes (left insets) shows material aggregation and settling from solution, and progelator incubated with denatured enzymes (right insets) show fully dispersed peptide solutions. Corresponding TEM images (bottom) of active enzyme cleavage products show fiber formation. (**Fig. 5c**), CD spectra of cleavage kinetics with thermolysin. Disappearance of signal at 204 nm (black arrow), corresponds to ring-opening. **d-f**, Analysis of Rho-KFDF_{Cyclic} to responsiveness MMP-9, elastase, and thermolysin. (**Fig. 5d**) Peptide sequence and indicated enzymatic cuts sites. **Fig. 5d** discloses SEQ ID NO:7. (**Fig. 5e**), Progelator incubated with active enzymes (left insets) shows material aggregation and

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settling from solution, and progelator incubated with denatured enzymes (right insets) show fully dispersed peptide solutions. Corresponding TEM images (bottom) of active enzyme cleavage products show fiber formation. **(Fig. 5f)** CD spectra of cleavage kinetics with thermolysin. Disappearance of signal at 204 nm (black arrow), corresponds to ring-opening. CD of 500 μ M progelator in 10 mM Tris buffer, pH 7.4. Enzyme cleavages performed at 1 mM progelator with 1:1000, 1:250, and 1:4500 enzyme/substrate molar ratio in 1x cleavage buffers (see Methods). Samples diluted to 100 μ M for TEM.

[0018] Figs. 6a-6g. Bulk Scale Gelation of Unlabeled Cyclic Progelators. Fig. 6a, Time course rheological analysis of progelators before and after addition of thermolysin ($t=40$ min). Divergence of G' from G'' ($G'>G''$) indicates formation of crosslinked hydrogel. **Figs. 6b-6c,** Step-strain oscillation plots of resulting **(Fig. 6b)** linearized KLDEL and **(Fig. 6c)** linearized KFDF SAP hydrogels at 9 hr post-enzyme-activation reveal repeat healing (3 min at 100 % strain, 15 min at 0.1% strain, $n=3$ cycles). Angular frequency 2.5 rad/s, 0.1% strain. **Figs. 6d-6e,** Images of **(Fig. 6d)** KLDEL_{Cyclic} and **(Fig. 6e)** KFDF_{Cyclic} progelators in the absence (-) and presence (+) of thermolysin with corresponding TEM. Enzyme cleavages at 10 mM in 1xDPBS with 1:4500 enzyme/substrate molar ratio. TEM of samples diluted to 100 μ M. **Fig. 6f,** CD of KFDF_{Cyclic} before and after subsequent additions of thermolysin at 0.5, 1.2, 25, and 73 hr shows gradual disappearance of minimum around 204 nm from π - π^* interactions between stacked cyclic peptides. 500 μ M peptide in 10 mM Tris, pH 7.4. **Fig. 6g,** TCEP (1.2 eq) induced reduction of progelator disulfide bond shows ideal assembly of linearized progelators. Two minima at 204 nm ($\pi \rightarrow \pi^*$) and 215 nm ($n \rightarrow \pi^*$) reveal weak aromatic π - π^* interactions and β -sheets assembly, respectively. 500 μ M peptide in H₂O, pH 7.4.

[0019] Figs. 7a-7d. In vitro catheter injection of low viscosity progelators. Fig. 7a, Complex viscosity of KLDEL_{Cyclic} and KFDF_{Cyclic} progelators reveal >20x decrease in comparison to corresponding unmodified KLDEL and KFDF SAPs. **Fig. 7b,** Schematic illustrating catheter applicability of KLDEL and KFDF SAPs vs that of enzyme-responsive KLDEL_{Cyclic} and KFDF_{Cyclic} progelators. Injection of SAP hydrogels causes clogging. **Fig. 7c,** Experimental setup of progelator (2 mol% labeled) catheter injection test. Peptide was (1) loaded in a 1mL syringe, (2) mounted on a syringe pump, and (3) flowed at 0.6 mL/min through the inner nitinol tubing (27G) of a MyoStar transendocardial injection catheter. The catheter was (4) submerged in a circulating water bath heated to 37 $^{\circ}$ C. The

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collection tube (5) before (top) and after (bottom) injection show successful injection. **Fig. 7d**, Image of KLDL_{Cyclic} and KFDF_{Cyclic} progelators (2 mol% labeled) after catheter injection into an empty vial (left) or a vial containing thermolysin (right), demonstrating that injection does not disrupt enzyme-responsiveness. Progulator formulated as 10 mM in 1xDPBS (pH 7.4) and treated with 1:4500 enzyme/substrate molar ratio.

[0020] Figs. 8a-8g. Hemocompatibility in human blood and *in vivo* analysis in a rat ischemia reperfusion model. Figs. 8a-8d, Hemocompatibility analysis of progulator in human blood. **Fig. 8a**, Activated clotting time (ACT) in whole human blood. Collagen (pos) and blood without calcium (neg) controls. Samples without calcium exceed instrument maximum time range (>1500 sec). (n=6 per group) **Fig. 8b**, Whole blood hemostasis kinetics monitored at 5, 15, 30, and 45 min in recalcified human blood. Collagen (pos), glass coverslip (pos), and blood without calcium (neg) controls. (n=4 per group) **Fig. 8c**, Hemolysis of isolated RBCs after 1 hr incubation. 1% Triton X-100 (pos) and vehicle only (neg) normalization controls. (n=4 per group) **Fig. 8d**, Pro-thrombotic profile in platelet poor plasma (PPP) plotted with respect to time. Onset of coagulation is accompanied by an increase in absorbance. Collagen (pos) and no calcium (neg) controls. (n=6 per group) Vehicle in all samples contain biological fluid without progulator. Progulator concentrations are 1:20000, 1:10000, 1:5000, 1:1000, 1:500, 1:100, and 1:10 blood volume dilution of injected dosage. ns ($p > 0.05$), *** ($p \leq 0.001$), **** ($p \leq 0.0001$). Ordinary one-way ANOVA for comparison with vehicle standard for **Fig. 8a** and **Fig. 8c**. Two-way ANOVA at each timepoint for comparison with vehicle standard for **Fig. 8b**. Values are mean $\pm$ SEM. **e**, *In vivo* study timeline. Female Sprague-Dawley rats that received ischemic reperfusion surgery (35 min occlusion) were provided a single 75 μ L intramyocardial injected of KFDF_{Cyclic} progulator (5 mol% Rho-KFDF_{Cyclic}) at 7 days post-MI, to simulate a local injection. Animals were euthanized at 24 hr post-injection, and hearts were collected. Progulator formulated at 10 mM peptide in 1xDPBS (pH 7.4) (n=5 animals). **Fig. 8f**, H&E stained representative heart section. Selected regions at 10x magnification (right) illustrate hydrogel assembly in the infarct. Arrows point to peptide material. **Fig. 8g**, Fluorescence images of the neighboring section, stained for nuclei (blue) and α -actinin (green greyscales), with rhodamine-labeled peptide gels in red. LV is left ventricle. Scale bars of whole heart and zoomed in 10x magnification, 1 mm and 200 μ m, respectively.

[0021] **Figs. 9a-9f. Chemical structures of KLDL peptides synthesized. Figs. 9a-9f, KLDL (Fig. 9a), KLDL_{Control} (Fig. 9b), KLDL_{Linear} (Fig. 9c), Rho-KLDL_{Linear} (Fig. 9d), KLDL_{Cyclic} (Fig. 9e), and Rho-KLDL_{Cyclic} (Fig. 9f).**

[0022] **Figs. 10a-10f. Chemical structures of KFDF peptides synthesized. Figs. 10a-10f, KFDF (Fig. 10a), KFDF_{Control} (Fig. 10b), KFDF_{Linear} (Fig. 10c), Rho-KFDF_{Linear} (Fig. 10d), KFDF_{Cyclic} (Fig. 10e), and Rho-KFDF_{Cyclic} (Fig. 10f).**

[0023] **Figs. 11a-11m. ESI mass spectra and sequences of peptides synthesized. Figs. 11a-11l, Spectra with accompanied m/z species identifications (shown in red) for unmodified SAPs, functionalized SAPs, and progelators in study. (Fig. 11a) KLDL, (Fig. 11b) KFDF, (Fig. 11c) KLDL_{Control}, (Fig. 11d) KFDF_{Control}, (Fig. 11e) KLDL_{Linear}, (Fig. 11f) KFDF_{Linear}, (Fig. 11g) Rho-KLDL_{Linear}, (Fig. 11h) Rho-KFDF_{Linear}, (Fig. 11i) KLDL_{Cyclic}, (Fig. 11j) KFDF_{Cyclic}, (Fig. 11k) Rho-KLDL_{Cyclic}, and (Fig. 11l) Rho-KFDF_{Cyclic}. Fig. 11m, Corresponding sequences (Fig. 11m discloses SEQ ID NOS:3, 8-11, 10-11, 6-7 and 6-7, respectively, in order of appearance) with expected molecular weight (MW) and observed m/z values. * indicates a disulfide bond between neighboring cysteines.**

[0024] **Figs. 12a-12d. Morphology and rheological properties of KLDL and KFDF. Unmodified linear SAP compared with respect to molar concentration (10 mM) present as porous networks hydrogel networks with rehealable properties. Stiffer gels obtained when Phe substituted for Leu in the KFDF SAP. Figs. 12a-12b, SEM of (Fig. 12a) KLDL and (Fig. 12b) KFDF SAPs. Fig. 12c, Storage modulus (G') for KLDL and KFDF SAPs in H₂O or 1xDPBS show increasing stiffness with increasing saline content and peptide hydrophobicity. Angular frequency 2.5 rad/s, 0.1% strain. Figs. 12d-12e, Step-strain oscillation analyses of (Fig. 12d) KLDL and (Fig. 12e) KFDF SAPs show rapid and repeat healing capacity of hydrogels ($G' > G''$) following complete network disruption ($G'' > G'$) at high strains. Viscoelastic storage (G') and loss (G'') moduli are plotted as a function of time with step cycles at 100 % destructive strain (t=3 min) followed by 0.1% recovery strain (t=15 min) to monitor healing. Hydrogels prepared at 10 mM in 1x DPBS. Angular frequency 2.5 rad/s.**

[0025] **Figs. 13a-13f. Strain sweeps of SAPs.** **Figs. 13a-13e**, Storage (G') and loss (G'') moduli were measured as a function of strain (0.5 – 50%) to identify the linear viscoelastic region (LVR) and appropriate rheological measurement conditions for all SAPs. Corresponding plots of (**Fig. 13a**) KLDL, (**Fig. 13b**) KFDF, (**Fig. 13c**) KFDF_{Control},
 5 (**Fig. 13d**) KFDF_{Linear}, (**Fig. 13e**) KFDF_{Cyclic} + thermolysin, and (**Fig. 13f**) KLDL_{Cyclic} + thermolysin. A strain of 0.5% lies within the LVR for these strain sweeps and was chosen for all rheological measurements in this study. SAPs were prepared at 15 mg/mL in 1x DPBS. Angular frequency 2.5 rad/s.

[0026] **Figs. 14a-14d. MALDI of labeled progelators before and after cyclization.** **Figs. 14a-14b**, Rho-KLDL_{Linear} (**Fig. 14a**) and resulting cyclization product (**Fig. 14b**) Rho-KLDL_{Cyclic}. Masses are 2694.00 m/z $[M+H]^+1+$ and 2549.46 m/z $[M+H]^+1+$, respectively. **Figs. 14c-14d**, Rho-KFDF_{Linear} (**Fig. 14c**) and resulting cyclization product (**Fig. 14d**) Rho-KFDF_{Cyclic}. Masses are 2989.69 m/z $[M+H]^+1+$ and 2754.18 m/z $[M+H]^+1+$, respectively.

15 [0027] **Figs. 15a-15f. HRMS unlabeled and labeled progelators.** Analysis conducted to identify whether final progelator peptides were in closed cyclic or open linear conformations. **Figs. 15a-15b**, HRMS spectra for (**Fig. 15a**) KLDL_{Cyclic} and (**Fig. 15b**) Rho-KLDL_{Cyclic}. **Fig. 15c**, Corresponding summary of theoretical m/z, measured m/z, measurement error, and elemental compositions for opened and closed conformations.
 20 **Figs. 15d-15e**, HRMS spectra for (**Fig. 15d**) KFDF_{Cyclic} and (**Fig. 15e**) Rho-KFDF_{Cyclic}. **Fig. 15f**, Corresponding summary of theoretical m/z, measured m/z, measurement error, and elemental compositions for opened and closed conformations. All measurements reveal closed conformation in contrast to open linear peptide.

[0028] **Figs. 16a-16d. Tandem-MS of Rho-KLDL_{Cyclic} and Rho-KFDF_{Cyclic} progelators.** Fragmentation of the peptide sequence enables identification of the peptide sequence, notably those that contain the less labile covalent disulfide bond. **Fig. 16a**, MS¹ spectrum for Rho-KLDL_{Cyclic} with peak identities labeled in green. **Fig. 16b**, Corresponding MS² fragmentation spectrum filtered for the 1276 m/z ion. Peak identities, as determined by DisConnect software, are displayed in **Table 3**. **Fig. 16c**, MS¹ spectrum
 25 for Rho-KLDL_{Cyclic} with peak identities labeled in green. **Fig. 16d**, Corresponding MS² fragmentation spectrum filtered for the 1378 m/z ion. Peak identities, as determined by
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DisConnect software, are displayed in **Table 4**. Note that only normal fragmentation peaks (no H₂O, NH₃, or CO losses) are identified.

[0029] Figs. 17a-17d. SLS profiles for unlabeled progelator in different buffers. Solutions of KFDF_{Cyclic} were formulated in solvents commonly used for formulating SAPs to identify optimal solvents that minimize the occurrence of large assemblies in solution. **Fig. 17a**, KFDF_{Cyclic} was pre-dissolved at 10 mg/mL in H₂O at pH 9 and added to solvent in the DLS cuvette such that final concentrations were 1 mg/mL in H₂O, 1x DPBS, or 295 mM sucrose (pH 7.4). Scattering was highest for sucrose solutions and equivalent in H₂O and 1x DPBS after 2 hr incubation indicating smaller assemblies in the later solvent. Intensities were corrected for background solvent scattering. Scattering intensities were normalized to individual solvents. **Figs. 17b-17d**, TEM analysis in H₂O (**Fig. 17b**), 1x DPBS (**Fig. 17c**), and 295 mM sucrose (**Fig. 17d**), diluted to 100 μ M. Large structures were absent in H₂O and 1x DPBS with some small clusters of fibrils in sucrose.

[0030] Figs. 18a-18e. Effect of cyclic peptide progelator formulation on aggregation. Solutions of KFDF_{Cyclic} and KFDF_{Linear} (data not shown) were optimally formulated in 1x DPBS using a pH switch from basic (pH 9.0) to neutral (pH 7.4) conditions prior to addition of saline to limit aggregation. These formulations in 1x DPBS are stable over prolonged incubation periods (up to 3 days) under dilute (100 μ M) and concentrated (10 mM) conditions. a-c, TEM (100 μ M) micrographs of progelator formulated in 1x DPBS after solvent switch from acidic to neutral pH (**Fig. 18a**), no pH switch (**Fig. 18b**), or switch from basic to neutral pH (**Fig. 18c**). d-e, DLS (1 mg/mL) (**Fig. 18d**) and the corresponding normalized autocorrelation functions (**Fig. 18e**) depict the influence of different formulation conditions on aggregation.

[0031] Fig. 19. Circular dichroism (CD) of KFDF_{Cyclic} progelator. a, CD spectrum of KFDF_{Cyclic} progelator (500 μ M, pH 7.4) formulated with basic to neutral pH solvent switch. (n=3 repeats). The magnitude of the high energy minimum at 204 nm is increased significantly following cyclization of KFDF_{Linear} (**Fig. 2b** in main text), which is attributed to aromatic π - π^* effects achieved through vertically stacked macrocycles.

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[0032] **Figs. 20a-20d. Representative enzyme cleavage of unlabeled cyclic progelator.** KFDF_{Cyclic} and KLDL_{Cyclic} (data not shown) were treated with MI-associated enzymes, MMP-9 and elastase to confirm enzyme responsiveness. **Fig. 20a**, Sequences of the KFDF_{Cyclic} progelator cleavage reactions and KFDF_{Linear} analogue for comparison. **Fig. 20a** discloses SEQ ID NOS 7, 12-13, 7, 14 and 7, respectively, in order of appearance. **Fig. 20b**, LCMS spectrum of KFDF_{Linear} analogue ($R_t = 12.5$ min) as a positive control. **Fig. 20c**, LCMS spectra of KFDF_{Cyclic} progelator treated with denatured (top) and active (bottom) MMP-9 catalytic domain for 5 hr. **Fig. 20d**, LCMS spectra of KFDF_{Cyclic} progelator treated with denatured (top) and active (bottom) porcine elastase for 5 hr. Calculated percent cleavages are reported in the spectra. Cleavage products are indicated with red asterixis. Due to the large number of cis/trans configurational isomers and amphiphilic self-assembling nature of our cyclic peptides, our pure KFDF_{Cyclic} progelator eluted within an R_t range between 9.7-12.5 min. Cleaved peptide peaks ($R_t \sim 12.4$ min) have better resolution likely due to increased molecular flexibility. Cleavage reactions are described in the Methods section of the main text.

[0033] **Figs. 21a-21b. Voltage plots for thermolysin cleavage of labeled progelators by CD.** Corresponding data for **Fig. 5c,5f** CD spectra of time-course analysis of thermolysin cleavage. **Fig. 21a**, High tension (HT) voltage plot of thermolysin cleavage of Rho-KLDL_{Cyclic} over time. **Fig. 21b**, High tension (HT) voltage plot of thermolysin cleavage of Rho-KFDF_{Cyclic} over time. Detector saturation at wavelengths where HT voltage > 800 mV. Peptide concentration 125 μ M. [substrate:enzyme] = 4500:1.

[0034] **Figs. 22a-22e. Simulated control SAPs, KLDL_{Control} and KFDF_{Control}, from progelator cleavage.** KLDL_{Control} and KFDF_{Control} SAPs were used to represent the enzymatically cleaved progelators containing flanking substrate residues on the geling sequences. **Fig. 22a**, Sequences, chemical structures, and corresponding diagrammatical representations of peptides, with green denoting gelator sequence and red denoting cleaved substrate recognition sequences. **Fig. 22a** discloses SEQ ID NOS:8-9, respectively, in order of appearance. **Fig. 22b**, Predicted structures and ribbon diagrams (bottom) of KLDL_{Control} and KFDF_{Control} SAPs from FibPredictor simulations revealing parallel β -sheet orientations (see Supplementary Tables 1-2). **Fig. 22c**, Experimental circular dichroism (CD) spectra of KLDL_{Control} and KFDF_{Control} SAPs, with labeled

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minima at 215 nm ($n \rightarrow \pi^*$) and 203 nm ($\pi \rightarrow \pi^*$). **Fig. 22d**, Assembled hydrogel photographs and corresponding TEM images of KLDL_{Control} (left) and KFDF_{Control} (right) SAPs. **Fig. 22e**, Viscoelastic measurements of storage moduli, G' (Pa), and damping factor, $\tan\delta$, for KLDL_{Control} and KFDF_{Control}. Gel capacity is defined as $\tan\delta < 1$ (dashed line). CD measurements performed at 500 μ M SAP in 10 mM Tris buffer, pH 7.4. (n=3 repeats) SAPs for TEM and rheology prepared at 100 μ M and 15 mg/mL in 1xDPBS (pH 7.4), respectively. Rheological measurements reported for angular frequency of 2.5 rad/s. (n=3 repeats) Values are mean $\pm$ SEM.

[0035] Figs. 23a-23b. TCEP reduction of progelator. TCEP reduction indicates ideal assemblies from when cleavage of cyclic progelators is complete. TCEP reduction is shown to be reversible through TCEP inactivation at lower pH **Fig. 23a**, Progelator treated with 1.2, 2.4, and 3.6 eq of TCEP. Gradual decrease in pH (pH 7.4 to 4.5) occurs from successive TCEP additions. Neutralization to pH 7.4 with NaOH revives TCEP reductive potential. **Fig. 23b**, Corresponding high tension (HT) voltage plots. Concentration 500 μ M progelator. (n=3 repeats).

[0036] Fig. 24. SLS of cyclic progelator before and after enzyme addition. This scattering profile demonstrates that progelator in solution is stable to flocculation for hours until addition of thermolysin induces a steep increase in scattering intensity as self-assembly occurs. Complete disruption of macromolecular structures via sonication at 48 hr post-activation results in a return to nominal scattering intensity like that of the cyclic progelator. Reassembly is observed by a gradual increase in scattering intensity of this dilute solution. Final peptide concentration is 1 mg/mL in 1xDPBS at 37 °C. Hydrogel disruption was achieved with 10 min sonication.

[0037] Fig. 25. Hemostasis kinetics as a response of dosing. Supplementary data corresponding to **Fig. 8b** in main text. Extent of clotting measured as absorbance at 405 nm as a function of dosing at 5, 15, 30, and 45 min in whole human blood. ns ($p > 0.05$). (n=4 per group). Two-way ANOVA at each timepoint for comparison with vehicle standard. Values are mean $\pm$ SEM.

[0038] Figs. 26a-26. Pro-thrombotic assay statistics. Supplementary data corresponding to **Fig. 8d** in main text. **Fig. 26a**, Coagulation rate measured as the maximal

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first derivative, or slope, of coagulation. **Fig. 26b**, Time at which coagulation is at half maximal completion. **Fig. 26c**, Extent of coagulation measured as the plateau absorbance. (n=6 group). Progelator concentrations are 1:20000, 1:10000, 1:5000, 1:1000, 1:500, 1:100, and 1:10 volume dilution in PPP. ns ($p > 0.05$), *** ($p \leq 0.001$), **** ($p \leq 0.0001$).

5 Ordinary one-way ANOVA for comparison with vehicle standard. Values are mean $\pm$ SEM.

[0039] **Figs. 27a-27b. Heart slices visualized through IHC staining. Fig. 27a**, Merged (left) and red channel-only (right) fluorescence images of 18 slices from 5 rat hearts that received intramyocardial injections of KFDF_{Cyclic} progelator at 7 days post-MI and harvested 24 hr later. Images correlate with Fig. 6. Tissue is stained for myocardium with anti- α -actinin antibody (green) and nuclei with Hoechst (blue), and peptides are visualized through the rhodamine tag (red). Peptide is localized within the MI region (+blue-green). **Fig. 27b**, Representative slice of heart receiving saline injection at 7 days post-MI and harvested 24 hr later. Scale bar = 2 mm.

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15 DETAILED DESCRIPTION OF THE INVENTION

[0040] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

20 DEFINITIONS

[0041] To facilitate understanding of the invention, a number of terms and abbreviations as used herein are defined below as follows:

[0042] When introducing elements of the present invention or the preferred embodiment(s) thereof, the articles “a”, “an”, “the” and “said” are intended to mean that there are one or more of the elements. The terms “comprising”, “including” and “having” are intended to be inclusive and mean that there may be additional elements other than the listed elements.

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[0043] The term “and/or” when used in a list of two or more items, means that any one of the listed items can be employed by itself or in combination with any one or more of the listed items. For example, the expression “A and/or B” is intended to mean either or

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both of A and B, i.e. A alone, B alone or A and B in combination. The expression “A, B and/or C” is intended to mean A alone, B alone, C alone, A and B in combination, A and C in combination, B and C in combination or A, B, and C in combination.

[0044] It is understood that aspects and embodiments of the invention described
5 herein include “consisting” and/or “consisting essentially of” aspects and embodiments.

[0045] It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible sub-ranges as well as individual numerical
10 values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed sub-ranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range. Values or ranges may be also be expressed herein as “about,” from “about” one
15 particular value, and/or to “about” another particular value. When such values or ranges are expressed, other embodiments disclosed include the specific value recited, 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 there are
20 a number of values disclosed therein, and that each value is also herein disclosed as “about” that particular value in addition to the value itself. In embodiments, “about” can be used to mean, for example, within 10% of the recited value, within 5% of the recited value, or within 2% of the recited value.

[0046] As used herein, “patient” or “subject” means a human or animal subject to
25 be treated.

[0047] As used herein the term “pharmaceutical composition” refers to a pharmaceutical acceptable compositions, wherein the composition comprises the peptides of the present invention, and in some embodiments further comprises a pharmaceutically acceptable carrier. In some embodiments, the pharmaceutical composition may be a
30 combination of pharmaceutically active agents, including cells.

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[0048] As used herein the term “pharmaceutically acceptable” means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopoeia, other generally recognized pharmacopoeia in addition to other formulations that are safe for use in animals, and more particularly in humans and/or non-human mammals.

[0049] As used herein the term “pharmaceutically acceptable carrier” refers to an excipient, diluent, preservative, solubilizer, emulsifier, adjuvant, and/or vehicle with which demethylation compound(s), is administered. Such carriers may be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents. Antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; and agents for the adjustment of tonicity such as sodium chloride or dextrose may also be a carrier. Methods for producing compositions in combination with carriers are known to those of skill in the art. In some embodiments, the language “pharmaceutically acceptable carrier” is intended to include any and all solvents, dispersion media, coatings, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art.

[0050] As used herein, “therapeutically effective” refers to an amount of a peptide of the present invention that is sufficient to treat or ameliorate, or in some manner reduce the symptoms associated with ischemic or inflamed tissue, such as but not limited to a myocardial infarction by providing a scaffolding for tissue remodeling and/or cellular growth. When used with reference to a method, the method is sufficiently effective to treat or ameliorate, or in some manner reduce the symptoms associated with ischemia. For example, an effective amount in reference to myocardial infarction is that amount which is sufficient to palliate, ameliorate, stabilize, reverse or slow progression of the disease, or otherwise reduce pathological consequences of the disease. In any case, an effective amount may be given in single or divided doses.

[0051] As used herein, the term “treatment” including co-administration with other pharmaceutically active agents (e.g., cells) embraces at least an amelioration of the symptoms associated with ischemic conditions in the patient, where amelioration is used

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in a broad sense to refer to at least a reduction in the magnitude of a parameter, e.g. a symptom associated with the disease or condition being treated. As such, "treatment" also includes situations where the disease, disorder, or pathological condition, or at least symptoms associated therewith, are completely inhibited (e.g. prevented from happening) or stopped (e.g. terminated) such that the patient no longer suffers from the condition, or at least the symptoms that characterize the condition, due at least in part to the administration of the present compositions.

[0052] The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry and immunology, which are within the skill of the art. Such techniques are explained fully in the literature, such as, Molecular Cloning: A Laboratory Manual, 2nd ed. (Sambrook et al., 1989); Oligonucleotide Synthesis (M. J. Gait, ed., 1984); Animal Cell Culture (R. I. Freshney, ed., 1987); Methods in Enzymology (Academic Press, Inc.); Current Protocols in Molecular Biology (F. M. Ausubel et al., eds., 1987, and periodic updates); PCR: The Polymerase Chain Reaction (Mullis et al., eds., 1994); Remington, The Science and Practice of Pharmacy, 20th ed., (Lippincott, Williams & Wilkins 2003), and Remington, The Science and Practice of Pharmacy, 22th ed., (Pharmaceutical Press and Philadelphia College of Pharmacy at University of the Sciences 2012).

[0053] The invention provides a tissue scaffolding composition and method of use for treating tissues in need thereof, e.g., ischemic tissue in myocardial infarctions, comprising in various embodiments a cyclic peptide. In embodiments, the cyclic peptide comprises a substrate recognition sequence that when enzymatically cleaved linearizes and self assembles the peptide with like-kind peptides, e.g., via beta-sheets, into a hydrogel.

[0054] In embodiments, the peptide has at least one, preferably three or more, repeating amino acid sequence KFDF (SEQ ID NO:1) or KLDL (SEQ ID NO:5) or other known self-assembling peptide (SAP) gelling sequences. Another exemplary peptide gelling sequence that forms a hydrogel via self-assembly is QQFEWEFEQQ (SEQ ID NO:108). The invention provides cyclic peptides that can self-assemble into a hydrogel when enzymatically cleaved, such as via beta-sheets, (EAK16, RADA16-I, RADA16-II, and Rp11) alpha-helices (hSAFaaa and other two-component systems based on coiled-coil heptad sequence repeats, abcdefg) or beta-hairpins (MAX1 and MAX8), that further

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assemble viscoelastic hydrogels. Well-known SAPs are described in the literature, and incorporated by reference herein, for example: Sun et al., "Self-Assembled Peptide Nanomaterials for Biomedical Applications: Promises and Pitfalls," International Journal of Nanomedicine, 2016, 12:73-86; Habibi et al., "Self-Assembled Peptide-Based Nanostructures: Smart Nanomaterials Toward Targeted Drug Delivery," Nanotoday, 2016, 11(1):41-60; Eskandari et al., "Recent Advances in Self-Assembled Peptides: Implications for Targeted Drug Delivery and Vaccine Engineering," Advanced Drug Delivery Reviews, 2017, 110-111:169-187; and Banwell et al., "Rational Design and Application of Responsive α -Helical Peptide Hydrogels," Nature Materials, 2009, 8:596-600.

10 **[0055]** In embodiments, the peptide is cyclized by disulfide bonds on cysteine residues, but other functional moieties and chemistries can be used to cyclize the peptides. In embodiments, the substrate recognition sequence is specific for inflammatory-related enzymes, matrix metalloproteinases (MMP)-2/9 and/or elastase. In embodiments, the peptide sequence comprises a MMP-2/9 cleavable sequence PLGLAG (SEQ ID NO:2).
15 Cleavage *in situ* allows assembly of the gelling sequence into a hydrogel. In embodiments, the peptide comprises or consists essentially of the amino acid sequence CKFDFKFDKFDKFDPLGLAGC (SEQ ID NO:7). In embodiments, the peptide is labeled for detection. In alternative embodiments, the composition further comprises a therapeutic agent (including small molecules, biologics and cells), a chemotactic agent, an antibiotic, and/or a growth factor.
20

[0056] The invention provides a method of creating a scaffold for tissue repair in a patient in need thereof comprising administering to the patient a tissue scaffolding composition as described. In embodiments, the scaffold is used in treating inflamed ischemic or damaged tissue, for example in negative left ventricular remodeling post-myocardial infarction. In embodiments, the administration is via a catheter, parenteral injection, or implantation.
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[0057] In certain embodiments, the present invention provides compositions and methods comprising injecting or implanting in a subject in need an effective amount of the tissue scaffolding composition. The composition can further comprise cells, drugs, proteins, or polysaccharides. The composition can be delivered as a liquid, and can transition to a gel form after delivery and *in situ* enzymatic cleavage. In some instances, the composition is coated on a device such as an implant.
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[0058] In some instances the composition comprises cells, proteins, or therapeutic agents. In some instances, the composition further comprises non-naturally occurring factors that recruit cells into the composition, encourage growth or prevent infection. In some instances, the composition further comprises a therapeutic agent, and as such is configured as a drug delivery vehicle.

[0059] In some instances, the composition herein can recruit endogenous cells within the recipient and can coordinate the function of the newly recruited or added cells, such as stem cells, allowing for cell proliferation or migration within the composition. A composition herein can further comprise one or more additional components, for example without limitation: an exogenous cell, a peptide, polypeptide, or protein, a vector expressing a DNA of a bioactive molecule, and other therapeutic agents such as drugs, cellular growth factors, nutrients, antibiotics or other bioactive molecules.

[0060] The composition of the invention can further comprise cells, drugs, proteins, or other biological material such as, but not limited to, erythropoietin (EPO), stem cell factor (SCF), vascular endothelial growth factor (VEGF), transforming growth factor (TGF), fibroblast growth factor (FGF), epidermal growth factor (EGF), cartilage growth factor (CGF), nerve growth factor (NGF), keratinocyte growth factor (KGF), skeletal growth factor (SGF), osteoblast-derived growth factor (BDGF), hepatocyte growth factor (HGF), insulin-like growth factor (IGF), cytokine growth factor (CGF), stem cell factor (SCF), platelet-derived growth factor (PDGF), endothelial cell growth supplement (EGGS), colony stimulating factor (CSF), growth differentiation factor (GDF), integrin modulating factor (IMF), calmodulin (CaM), thymidine kinase (TK), tumor necrosis factor (TNF), growth hormone (GH), bone morphogenic proteins (BMP), tissue inhibitor matrix metalloproteinase (TIMP), interferon, interleukins, cytokines, integrin, collagen, fibrillins, fibronectin, laminin, glycosaminoglycans, hemonection, thrombospondin, heparan sulfate, dermatan, chondroitin sulfate (CS), hyaluronic acid (HA), vitronectin, proteoglycans, transferrin, cytactin, tenascin, and lymphokines.

[0061] In some instances, the composition can comprise a biological group that can act as an adhesive or anchor where the composition is delivered. In one instance, a composition can be a bioadhesive, for example, for wound repair. In some instances, a composition herein can be configured as a cell adherent. For example, the composition herein can be coating or mixed with on a medical device or a biologic that does or does

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not comprises cells. For example, the composition herein can be a coating for a synthetic polymer graft. In some instances, the composition includes an anti-bacterial or anti-bacterial agents. Methods herein can comprise delivering the composition as a wound repair device.

5 **[0062]** In embodiments, the invention utilizes a cyclic peptide pro-gelator which is soluble and nonviscous in buffered saline and self-assembles into a macromolecular hydrogel at the site of tissue injury, such as myocardial infarction (MI). Upon linearization through peptide cleavage by enzymes upregulated at the site of MI, rapid self-assembly creates a macromolecular network (millimeter scale) which traps itself into the tissue it is
10 activated and enhances tissue retention over that of unimers or nano-scale materials. These biomaterials act to viscoelastically mimick and supplement the supportive native extracellular matrix (ECM) that MMP-2/9 degrade following myocardial infarction. Supplementation of the degraded ECM with a synthetic biopolymer pro-gelator enables targeted accumulation of a viscoelastic network and minimally invasive delivery to heart
15 tissue post-MI.

[0063] Low viscosity makes this material not only injectable, but amenable to tranendocardial catheterization and catheter-based coronary infusion, a characteristic which has only been demonstrated with three other materials: decellularized myocardial ECM hydrogel, aliginate hydrogel, and oxime cross-linked hydrogel. This allows for
20 minimally invasive and local delivery to the heart tissue. In contrast, known promising hydrogel-based biomaterials require invasive delivery as a patch or direct epicardial injection into compromised tissue, a quality that is not feasible for clinical application.

[0064] As coronary infusion of the prior art is known to be limited by inefficient spreading, materials which are amenable to catheterization may still not reach their
25 intended target (infarct). Additionally, the risk of embolization is a current concern due to the propensity for injected materials to leak into the LV chamber with transendocardial catheterization. The material of the invention is inert in the cyclic confirmation and only gels when linearized, thus making these peptides selective to areas with excess inflammation where protease activity is upregulated, in particular, that of MMP-2/9.
30 Furthermore, the material resists gelation in serum and healthy myocardial tissue. This prevents off-target accumulation and reduces the risk of potential gelling in the blood stream.

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[0065] In contrast to covalently crosslinked hydrogels in preclinical studies, these materials exhibit repeat healing properties (useful in an organ constantly in motion) and rheological stiffnesses similar to that found in native ECM.

[0066] As the ECM is an important hydrogel network in tissue, providing structural support and a niche for biochemical signaling and cellular infiltration, biomaterial hydrogels are being used as ECM mimicks in the wake of enzymatic degradation post-MI. Currently, there are no clinically approved hydrogel based materials for treating MI, primarily due to delivery and formulation limitations stated above. However, clinical trials using aliginate (phase II) and decellularized myocardial ECM hydrogel (phase I) delivered via catheter-based intracoronary infusion and transendocardial catheter delivery, respectively, are currently underway.

[0067] There are currently no known stimuli responsive or catheter deliverable peptide-based formulations reported in pre-clinical studies. Pre-clinical studies with RAD16-II peptide nanofibers have only utilized direct epicardial injection either as formulations with scaffold itself or in conjunction with stem cells and growth factors (VEGF). Thus only invasive delivery techniques are being explored with peptide-based systems, potentially due to excess viscosity of the scaffold formulations.

[0068] In embodiments, these peptides are primarily composed of an amphipathic peptide sequence characterized by three repeats of Lys-Phe-Asp-Phe (SEQ ID NO:1), (KFDF)₃ (SEQ ID NO:4), or more, which self assemble by stacking as beta-sheets through electrostatic and hydrophobic interactions. The macromolecular hydrogel it forms is porous, elastic, and rehealable upon disruption, similar to native ECM. Modification of this gelator sequence with a matrix metalloproteinase (MMP)-2/9 cleavable sequence (e.g., PLGLAG (SEQ ID NO:2)) enables infarct-specific activation. Macrocyclization through covalent disulfide bonds on N- and C-terminal cysteine residues sterically constrains the peptide, preventing beta-sheet assembly as a hydrogel, thus affording a pro-gelator.

[0069] Further, in embodiments, this material is highly modular, in that small molecules (e.g. dyes, contrast agents, and drugs) can easily be incorporated on the N-terminal amine and used in mixed formulations of tagged and untagged peptide (*in vitro* enzyme cleavage using 100% rhodamine labeled peptide, and *in vivo* studies using 5% rhodamine labeling). Together, these modifications provide a novel way of delivering

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biosynthetic tissue scaffolds to diseased tissue in a minimally invasive (catheterization) and targeted (enzyme-responsive) manner.

EXAMPLES

[0070] In embodiments, the invention provides compositions and methods of use comprising a cyclic peptide progelator that flows freely in solution and resists assembly into hydrogel until acted upon by an endogenous signal (**Figs. 1a-1c**), namely MMP-2/9^{2,31} and elastase,^{32,33} which are expressed at the site of MI during the acute inflammatory (days) and fibrotic (weeks) phases. These enzymes play key roles in the healing process through degradation of extracellular matrix (ECM) and fibrinogen.²

10 [0071] SAP sequences were prepared as water soluble, dispersed cyclic progelators (**Fig. 1a**) that contain a substrate recognition sequence for MMP-2/9 and elastase (red). Two different cyclic progelators were prepared; one without a fluorescent label and one with a rhodamine label (pink ellipse) located adjacent to the substrate sequence. The data shows that enzymatic cleavage of these sterically constrained cyclic

15 progelators results in linearization to generate SAPs (**Fig. 1b**) which subsequently assemble into rehealable viscoelastic hydrogels (**Fig 1c**). Their low viscosity, ability to gel at the site of MI, and hemocompatible nature of the concentrated progelator are demonstrated. In addition, the data shows that the progelators are amenable to minimally invasive catheter injection using an *in vitro* model system. This enables the delivery of

20 SAPs to the heart via catheter.

[0072] **Design of stimuli-responsive SAPs for activatable gelation *in vivo*.** SAPs undergo spontaneous assembly through electrostatic and amphiphilic interactions into ordered nanostructures.^{34,35} In many instances, variation of sequence and charge distribution in SAPs can influence properties such as secondary structure,³⁶ fiber diameter,³⁷ and bulk viscoelasticity³⁸. The present invention began with a gellable core based on a SAP consisting of the repeat sequence (KLDL)₃ (SEQ ID NO:3) (referred to in this study as KLDL), which has been studied as a non-immunogenic, non-hemolytic, and antimicrobial scaffold for tissue engineering applications.³⁹⁻⁴¹ This peptide self-assembles

25 spontaneously into β -sheets through cationic and anionic crosslinking of the Lys and Asp residues and hydrophobic interactions along the Leu residues. As a proof-of-concept for

30 sequence control, an analogue of KLDL was chosen to alter secondary structural assembly

and achieve modified gel properties. For this purpose, (KFDF)₃ (SEQ ID NO:4) (referred to as KFDF) was designed, in which the incorporation of aromatic Phe residues introduce π - π interactions. From the KLDL and KFDF SAPs, a small library of functionalized SAPs and progelators was engineered for studying assembly within a biological relevant environment (**Figs. 2a-2c** and **Figs. 9a-11**). We first evaluated whether the addition of non-gelling amino acids from the MMP-2/9 recognition sequence (PLGI $\overline{\text{L}}$ AG) (SEQ ID NO:2) and cysteine residues used for macrocyclization into the SAP sequences would interfere with their ability to self-assemble (**Fig. 2a**). Predictive computational modeling with open-access FibPredictor software⁴² provided initial evidence that functionalized KLDL_{Linear} and KFDF_{Linear} SAPs would experience a switch in self-assembly orientation from antiparallel to parallel β -sheets. Regardless, such a design would retain the capacity to form fibrillar bilayers (**Figs. 2b-c** and **Tables 1-2**). Notably in KFDF_{Linear}, Phe residues on each strand are sandwiched with analogous residues on neighboring strands, which facilitate π - π stacking and stronger hydrophobic interactions than those of Leu-containing peptides. Indeed, with the design validated *in silico*, synthetic preparation of the KFDF SAP resulted in materials with increased mechanical strength without sacrificing re-healing capacity or fibrous morphology, inherent to the known KLDL system⁴³ (**Figs. 12a-12d**).

[0073] In practice, modified SAPs capable of going on to form the cyclic progelators, namely KLDL_{Linear} and KFDF_{Linear}, retained the capacity to self-assemble (both experimentally and predicted) despite modest changes in secondary structure and viscoelastic properties (**Figs. 3a-3h**). Experimental CD demonstrated that KLDL_{Linear} and KFDF_{Linear} retained β -sheet conformations ($n \rightarrow \pi^*$ transition at 215 nm). However, the positive peak at ~200 nm ($\pi \rightarrow \pi^*$ transition) found in KLDL and KFDF is blue-shifted to ~195 nm, indicative of a parallel to antiparallel orientation switch (**Fig. 3a-3b**).⁴⁴ Additionally, KFDF_{Linear} reveals a new high energy minimum at 203 nm corresponding to π - π^* effects resulting from aromatic π - π interactions in the hydrophobic interior of the bilayer structure (**Fig. 3b**).²⁷ This transition is predicted in theoretical spectra from DichroCalc⁴⁵ for KFDF_{Linear} (202 nm) and not for KLDL_{Linear}, which contains no aromatic residues. (**Fig. 3c-3d**). Regardless of altered secondary structure, fiber morphology and bulk viscoelastic properties exhibited minimal changes to that of unmodified KLDL and KFDF (**Fig 3e-3h**). Slight drops in storage moduli for KLDL_{Linear} and KFDF_{Linear} are

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attributed to the decreasing molar concentration of amino acids (hydrogels prepared with respect to weight at 15 mg/mL) contributing to β -sheet formation. Regardless, no significant disruptive effects to gelling capacity, as defined by $\tan\delta < 1$ (**Fig. 3g-3h**) or strain tolerance (**Figs. 13a-13f**) were observed through the introduction of over 66% more amino acid residues to the SAP sequence. This exemplifies the robustness of these linear SAPs to sequence modification and potential capacity to tolerate functional moieties such as fluorescent tags or small molecule drugs.

[0074] Cyclization and progelator formulation. SAP steric constraint provides a simple and versatile engineering approach for preventing network assembly in a biological environment (**Figs. 4a-4k**). Functionalized SAP analogues containing an Fmoc protecting group or rhodamine dye on the N-terminus were cyclized through oxidation of terminal cysteine residues to generate unlabeled and labeled progelators (**Fig. 4a**). Fmoc was temporarily used for unlabeled progelators to improve peak separation during purification and to monitor cyclization by HPLC and ESI (**Fig. 4b-4e**). Dilute solution phase macrocyclization was complete within 125 min by LCMS (**Fig. 4f,4h**). Combined, MALDI, HRMS and Tandem-MS confirm synthesis of the cyclic products (**Figs. 14a-16d and Tables 3-4**). UV spectra confirm the presence of rhodamine absorbance in Rho-KLDL_{Cyclic} and Rho-KFDF_{Cyclic} progelators (**Fig. 4g,4i**). Nonviscous progelators (**Fig. 4j-4k**) were dialyzed into milliQ H₂O, sterile filtered (0.2 μ m PES), and lyophilized for storage prior to use *in vitro* and *in vivo*. To ensure synthetic reproducibility and appropriate biological analysis in this study, our cyclic progelators were structurally characterized and formulated by first dissolving in aqueous solution under basic conditions, followed by neutralization to give final solutions in 1xDPBS, pH 7.4 (10 mM peptide). This formulation was determined by searching for conditions that minimized aggregation in different solvents and pH values as determined by light scattering and TEM (**Figs. 17a-18**). Conformational rigidification via macrocyclization is suspected to have induced limited oligomerization via intermolecular stacking of cyclic constructs into transient nanotubes (**Fig. 19**), as has been reported in the literature.⁴⁶⁻⁴⁸ Despite these observed secondary structural characteristics, large structures were absent by TEM (**Figs. 18a-18e**). Thus, our formulated cyclic progelators persist as free-flowing solutions for easy injection *in vivo*.

[0075] **Cyclic progelators are responsive to inflammation associated enzymes.** Labeled cyclic peptide progelators were tested for their responsiveness to inflammatory-related proteases overexpressed post-MI (**Figs. 5a-5f**). The initial inflammatory response is marked by the recruitment of abundant neutrophils, which contribute to the release of proteases such as matrix metalloproteinases (MMPs) and elastase in the first days post-MI.^{31,49} The fibrotic phase (weeks post-MI) is notable for residually high MMP concentrations^{2,31} and elevated sources of elastase from neutrophil extracellular traps (NETs), remaining weeks after neutrophil apoptosis.^{32,33} Rho-KLDL_{Cyclic} and Rho-KFDF_{Cyclic} were incubated with MMP-9 catalytic domain and porcine elastase to cleave at preferential cut sites (**Figs. 5a,5d**). Enzymatic cleavage by active MMP-9 and elastase (**Figs. 20a-20d**) induced visible aggregation in the reaction vial and entangled fibrous meshes by TEM, whereas denatured enzymes elicited no response (**Figs. 5b,5e**). At the site of MI, the presence of very high MMP and elastase concentrations is expected to induce rapid cleavage, forcing the peptide progelator to assemble and solidify at the target site. However, other inflammation-associated enzymes or constitutively expressed extracellular proteases could play a role in nonspecific degradation. Thus, thermolysin was used as a robust nonspecific enzyme to assess general stability of our material to excess proteolysis and subsequent hydrogel disassembly *in vivo*.⁵⁰ Despite the promiscuous activity of thermolysin, incubation with Rho-KLDL_{Cyclic} and Rho-KFDF_{Cyclic} induced fiber formation by TEM (**Figs. 5b,5e**). CD revealed structural rearrangements during this linearization process (**Figs. 5c,5f** and **Figs. 21a-21b**). The similar responsiveness of both unlabeled progelators to inflammatory enzymes and resistance to dissolution by thermolysin demonstrate the versatility of our conformational control strategy.

[0076] **Progelators assemble as viscoelastic and rehealable hydrogels despite robust proteolysis.** The concept behind our cyclic peptide progelators is that upregulated enzymes will initiate assembly and ultimately viscoelastic gel formation at the site of MI. However, this location contains a soup of robust proteases involved in the degradation of peptides and proteins. Thus, excess proteolysis *in vivo* may prevent gelation or retention of hydrogels at the site of MI. To simulate this environment, we treated our material with a large quantity of the robust and non-specific enzyme, thermolysin. Hydrogel assembly kinetics and resulting mechanical

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properties were then measured rheologically (**Figs. 6a-6g**). Initially, KL_{DL}_{Cyclic} and KF_{DF}_{Cyclic} progelators formulated as non-viscous liquids displayed overlapping G' and G'' , indicative of free-flowing solutions lacking significant crosslinks or chemical interactions (**Fig. 6a**). Upon incubation with thermolysin, a gradual increase in G' and divergence from G'' shows the formation of viscoelastic hydrogels. A steady state was reached at 200 min and continuous measurement of both shear moduli revealed no change for up to 3 days in the presence of thermolysin. The tightly entangled macromolecular scaffolds are suspected to confer some degree of resistance to further proteolysis. Furthermore, the resulting gels displayed rapid healing capacity when subjected to repeat cycles of excess strain (100%) (**Fig. 6b-6c**). Resulting bulk scale hydrogels and dilute TEM images of fiber assemblies from the rheometer are depicted in **Fig. 6d-6e**. Synthetic product analogue SAPs in which the gelator sequence contained flanking substrate residues (KL_{DL}_{Control} and KF_{DF}_{Control}) were used for simple comparison with enzyme-induced gelation (**Figs. 22a-22e**). Indeed, secondary assembly characteristics (CD), fibril morphology (TEM), and viscoelastic properties (rheology) of KF_{DF}_{Control} match closely to that of enzymatically cleaved KF_{DF}_{Cyclic} in **Figs. 6a-6g**. However, KL_{DL}_{Control} SAP produced a viscoelastically stronger gel than that demonstrated by KL_{DL}_{Cyclic} following enzyme directed linearization.

[0077] To understand the initial stages of self-assembly when progelators first encounter their target protease *in vivo*, slow step-wise cleavage of KF_{DF}_{Cyclic} progelator was achieved through serial additions of dilute thermolysin (**Fig. 6f**). CD reveals that linearization by enzyme cleavage causes an increase in peptide flexibility, shown by a steep decrease in the signal at 204nm, and unchanged β -sheet signal from lateral alignment at 215 nm. Tris(2-carboxyethyl)phosphine (TCEP) was used as a positive control to represent ideal assemblies of linearized peptides through complete reduction of the disulfide bond (**Fig. 6f and Figs. 23a-23b**). By TEM, reduced peptides assemble as tangled fibrils, similarly to enzymatically cleaved peptides (**Fig. 6g**). Thus, the enzyme-induced gels observed in **Figs. 6a-6g** are likely forming through the same self-assembly mechanisms predicted with KF_{DF}_{Control}. The rheological and spectroscopic results presented here indicate that our system has to potential to tolerate excess proteolytic degradation *in vivo*.

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[0078] **Catheter compatibility of cyclic peptide progelators.** Minimally invasive injection of hydrogels into the heart is typically performed through transendocardial injections. Unlike direct epicardial injections performed in small animal models, transendocardial delivery has certain constraints that are not amenable to most hydrogel systems, namely materials should be able to reside within the catheter during an up to hour long procedure, yet still be injectable over multiple injections during this time, and finally only form a solid gel once it has entered the tissue.^{17,51} Rapid gelation and/or high viscosity prohibit many injectable hydrogels from being considered for this minimally invasive delivery route. KLDDL_{Cyclic} and KFDF_{Cyclic} progelators were amenable to cardiac catheter injection (**Figs. 7a-7d**). As shown in **Fig. 7a**, both unmodified SAPs and cyclic progelators exhibit shear-thinning behavior, but the viscosities for the latter are over 20x lower for both KLDDL_{Cyclic} and KFDF_{Cyclic}. Thus, conformational constraint of β -sheet forming SAPs was sufficient to disrupt favorable unimer alignment and weakened structural interactions. We reasoned that SAP cyclization would be sufficient to prevent clogging during catheter injection (**Fig. 7b**).

[0079] To mimic injection of the cyclic progelators through a cardiac injection catheter *in vivo*, both (2 mol% labeled for visualization) were flowed through the inner nitinol tubing of a 27G (0.21 mm inner diameter) MyoStar transendocardial injection catheter at 37 °C using a syringe pump (**Fig. 7c**). No excess resistance was detected by manual operation following incubation of cyclic progelator within the catheter loop for 60 min for either peptide. In contrast, unmodified KLDDL and KFDF SAPs caused immediate catheter clogging. Excess resistance from high viscosity and tendency to rapidly self-assemble during shearing injection could explain why there are no previous reports of SAPs for cardiac catheter delivery. To ensure that the shear forces the cyclic progelator are exposed to during catheter injection do not inhibit enzyme activation and prevent gelation *in vivo*, the cyclic peptides were injected through the catheter into a vial containing thermolysin and incubated for 3 hr (37 °C), resulting in hydrogel formation (**Fig. 7e**).

[0080] **Hemocompatibility and intramyocardial injections in a rat myocardial infarction model.** For proof-of-concept analyses in biological systems, we examined the KFDF_{Cyclic} progelator in hemocompatibility studies and assessed *in vivo* gelation (**Figs.**

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8a-8g). Biomaterial leakage is a concern with transendocardial injection since injections are performed into a beating heart.⁵² Therefore, we assessed hemocompatibility of the cyclic progelator to ensure that modification and cyclization of the SAPs would not generate a thrombogenic response. Whole human blood clotting times, hemostasis kinetics, red blood cell (RBC) hemolysis, and pro-thrombotic profiles were evaluated in the presence of increasing cyclic peptide progelators concentrations in blood. Activated clotting times (ACT) were used as a standard method⁵³ that encompasses intrinsic and common coagulation pathways to assess thrombogenicity. Furthermore, as this method is influenced by increased sample viscosity due to clot formation, potential peptide cleavage by blood proteases and resulting self-assembly would decrease clotting times. No adverse effects were observed for up to 1:10 peptide:blood concentrations (**Fig. 8a** and **Table 5**). In contrast, collagen showed a significant clot time reduction, and chelation of calcium prevented clotting altogether. Whole blood clotting kinetics were also measured in the absence of an activator to monitor the influence of progelator on hemostasis. Similarly, no statistical difference in clotting was observed between the vehicle and the highest progelator concentration (1:10) at any timepoint (**Fig. 8b** and **Fig. 25**). In contrast, collagen and glass controls increased the clot rate.

[0081] Red blood cell (RBC) hemolysis in the presence of progelator was used to assess acute toxicity (**Fig. 8c** and **Table 6**). Doses up to 1:10 revealed <5% hemolysis, which is below the limit for consideration as a nonhemolytic biomaterial.⁵⁴ Finally, a pro-thrombotic assay using platelet poor plasma (PPP) was used to monitor non-platelet and phospholipid-specific effects on blood (**Fig. 8d**), such as potential to inhibit thrombin activity, prevent crosslinking of fibrinogen, or sequestration of calcium for decreased platelet activation. This assay has the advantage of monitoring the intrinsic pathway only, which is useful for studying surface contact activation in blood-biomaterial interactions. The onset of coagulation is indicated by a steep rise in the absorbance vs time curve. A plateau in absorbance was reached within a few minutes and maintained over time, indicating a fully formed, stable clot. No changes to coagulation rate (slope), half-maximal coagulation time, and extent of coagulation (**Figs. 26a-26c**) were observed in samples incubated with progelator up to doses of 1:100 peptide/blood.

[0082] With hemocompatibility verified, we applied the progelator in an initial proof-of-concept study *in vivo*. Enzyme-responsive progelator, KFDF_{Cyclic}, doped with a

small amount of Rho-KFDF_{Cyclic} (5 mol%) for imaging, was injected into the infarct region 7 days post-MI in a rat ischemia-reperfusion model (**Fig. 8e**). Assessment of hearts at 24 hr post-injection confirmed that progelator effectively gelled at the heart (**Fig. 8e**, **Figs. 27a-27b**). Histological and fluorescence analysis of heart sections revealed hydrogel assembly occurred at the site of MI in all rats (**Figs. 8f-8g**). This experiment demonstrates the activation and subsequent gelation of MMP responsive progelators to inflamed biological tissue.

[0083] The number of candidate biomaterials amenable to minimally invasive cardiac catheter delivery to the heart post-MI is limited. Consequently, translation of promising scaffolds from preclinical to clinical trials has been rare. Provided herein is a platform modification strategy to enable one class of hydrogels, SAPs, to be injectable via a cardiac injection catheter by conformationally constraining linear SAPs into macrocyclic progelators which resist self-assembly (**Figs. 1a-1c**). These progelators were further modified with a substrate recognition sequence for endogenously expressed MI-associated proteases. The invention provides peptide sequences based on a known SAP, and then utilizing *in silico* modeling to provide preliminary insight into any modifications that may cause disruption of gelation (**Figs. 2a-2c**). These modified sequences were then prepared synthetically for testing. Subsequent experimental analysis of self-assembly characteristics and mechanical properties agreed well with these predictions, namely that sequences modifications did not interfere with self-assembly as β -sheets into fibrils and viscoelastic hydrogels (**Figs. 3a-3h**). It is hypothesized that the platform for modifications could be well-tolerated by other known SAP sequences used for biomedical applications.^{55,56}

[0084] The versatility of the platform is demonstrated through the functionalization of two different SAPs sequences, which exhibit disparate self-assembly mechanisms, and yet form progelators with identical responsiveness. Cyclic progelators synthesized in **Figs. 4a-4k** lacked the capacity to gel when sterically constrained. However, they exhibited responsiveness to enzymes that are inherently active in the MI during both the acute inflammatory phase and fibrotic phase (**Figs. 5a-5f**). Despite the different cut sites recognized by MMP-9 catalytic domain, elastase, and thermolysin, similar secondary structure and nanoscale morphology changes were observed. Bulk cleavage of these sterically constrained materials resulted in healable viscoelastic hydrogels that were stable against excess proteolysis (**Figs. 6a-6g**).

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[0085] The development of smart structurally dynamic and responsive materials for tissue engineering post-MI is a relatively untapped field. With many scaffolds, the common issue remains that chemical modification (e.g. crosslinks, binding moieties, targeting motifs, or therapeutics drugs) can negatively impact material properties such as sample viscosity and gelation kinetics, which would preclude their use in catheters. With the present design, mechanical differences (e.g. stiffness or healing kinetics) in the resulting hydrogel would not affect the initial progelator formulation. Importantly, we provide the first demonstration of low viscosity peptide-based progelators for use in a cardiac injection catheter with little resistance to flow (**Figs. 7a-7d**). The results of these experiments demonstrate that the cyclic progelator acts as a Newtonian fluid for smooth catheter-based injection and still retains the capacity to solidify when acted upon by endogenously expressed inflammatory enzymes in the heart, suggesting this could provide a useful strategy for cardiac catheter delivery *in vivo*.

[0086] With the potential for tissue leakage into the bloodstream, thrombogenicity of transendocardially injected biomaterials is a concern. Synthetic modifications to the KFDF SAP were shown to be hemocompatible and non-thrombogenic through a comprehensive assessment of whole blood clotting times, hemostatic kinetics, RBC hemolysis, and pro-thrombotic assays (**Figs. 8a-8g**). A dosing analysis shows no statistically significant difference in hemostasis due to increasing progelator concentration in whole blood. The extent of coagulation in platelet poor plasma was only altered at the highest peptide dose which indicates that local high concentrations of peptide have a minor anti-coagulative effect on the intrinsic coagulation (contact-based) pathway; furthermore, the impact that the peptide does have on coagulation was shown to be independent of platelet-dependent thrombus formation, a key player in the pathogenesis of acute MI.⁵⁷ This conclusion is supported by the lack of change in pro-thrombotic profiles when incubated with collagen, which increases clotting rates only through the activation of platelet aggregation.⁵⁸ Given a “standard” clinical concentration of 1:10,000,⁵⁹ which assumes that no material was injected into the heart wall and instead escaped into the bloodstream, no adverse effects were observed on coagulation at this clinically relevant dose. These results indicate that this platform for generating an inert cyclic adduct of known SAPs should not result in unwanted blood interactions during delivery.

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[0087] The present platform enables facile delivery of a free-flowing progelator, and on-demand gelation at the site of inflammation (**Fig. 8**). *In vivo* analysis of KFDF_{Cyclic} progelator revealed successful gelation of activated hydrogel scaffolds in a rat MI model. As such, the invention demonstrated a simplistic strategy that uses the naturally complex
5 MI microenvironment for delivery and smart assembly in tissue. This proof-of-concept test paves the way for exploring the amenability of various other SAP systems (e.g. RAD16-II) with the progelator system for cardiac catheterization *in vivo*. In addition, there exists the potential to combine this approach with various therapeutics. In this study, appendage of a rhodamine dye to cyclic progelators did not disrupt gelation. The invention
10 envisions a modular system to deliver and sustain any number of covalently bound therapeutics (e.g. small molecule drugs or short peptides). For larger therapeutics (e.g. stem cells and growth factors), simple formulation as a nonviscous solution for delivery, and subsequent encapsulation within the locally activated networks, might provide a means for targeted drug delivery without the need for complex synthetic manipulation.
15 Ultimately, the simple strategy for structural control of self-assembling peptides can be employed for minimally invasive delivery of therapeutic peptides to other forms of injury or disease where MMPs are upregulated, including osteoarthritis,⁶⁰ cartilage tissue repair,⁴¹ nerve damage,^{61,62} and acute brain injury⁶³.

[0088] **General information.** Amino acids used in Fmoc SPPS were purchased
20 from AAPPTec and NovaBiochem. All other synthetic materials were obtained from Sigma-Aldrich and used without further purification unless otherwise noted. MMP-9 (recombinant human catalytic domain) (BML-SEro360) was acquired from Enzo Life Sciences, as a 1.0 mg/mL solution at 40 U/ μ L in 50mM TRIS, pH 7.5, 1mM CaCl₂, 300mM NaCl, 5 μ M ZnCl₂, 0.1% Brij-35, and 15% glycerol. Porcine pancreatic elastase
25 (3246821000U) was acquired from EMD Millipore, as a lyophilized powder (22 U/mg). Thermolysin (V400A) was acquired from Promega, as a lyophilized powder. Unspun whole human blood (citrate, non-heparinized) was obtained from Biological Specialty Corporation (LS23 95099) and stored at 1-6 °C.

[0089] **Peptide synthesis.** Peptides were synthesized in an AAPPTec Focus XC
30 peptide synthesizer. Peptides with C terminal amides were synthesized on rink amide MBHA resin and peptides with C-terminal carboxylic acids were synthesized on Wang-OH resin using double coupling conditions for the first amino acid. HBTU (*N,N,N',N'*-

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tetramethyl-O-(1H-benzotriazol-1-yl) uronium hexafluorophosphate) was used as the general coupling agent. When included, rhodamine was incorporated during the synthesis at the N-terminus of the sequence as 5(6)-carboxytetramethyl rhodamine using HATU (1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate) as the coupling agent. 5(6)-carboxytetramethyl rhodamine was synthesized as previously described.⁶⁴ General peptide cleavage and deprotection was performed in 95:2.5:2.5 (%v/v) trifluoroacetic acid (TFA), triisopropyl silane (TIPS), and H₂O, respectively, for 2 hr. Cleaved peptides were precipitated in cold anhydrous ether (3x) to yield solid crude. Semi-protected peptides containing Cys(acm) were purified prior to cyclization. To synthesize cyclic progelators, iodine was used to simultaneously deprotect Cys(acm) and initiate disulfide bond formation under dilute conditions to favor intramolecular macrocyclization.⁶⁵ To a solution of semi-protected peptides (500 µM) in a mixture of acetic acid/methanol/H₂O (1:16:4) was slowly added 0.1 M methanolic iodide until the yellow color persisted (~4-5 eq). The reaction was vigorously stirred at room temperature for 2 h and reaction completion was confirmed by LCMS. After 2 hr reaction, Amberlite IRA-400 Resin (chloride form) was stirred in the solution for 1 hr to quench excess iodine and absorb reacted iodide ions. Filtrate was placed on a rotary evaporator to remove acetic acid and methanol. The remaining solution was diluted with H₂O and lyophilized to a white powder. By HPLC no dimerization was observed. For cyclization kinetics analysis (**Figs. 2c-2e**), the N-terminal Fmoc was temporarily left on unlabeled peptides for improved peak separation during purification.

[0090] Peptide purification and analysis. Analytical scale RP-HPLC analysis of peptides was performed on a Jupiter Proteo90A Phenomenex column (150 x 4.60 mm) using a Hitachi-Elite LaChrom L2130 pump with a UV-Vis detector (Hitachi-Elite LaChrom L-2420) monitoring at 214 nm, 256 nm, 290 nm, and 565 nm. Gradients performed over 30 min. LCMS was performed with a Waters AQUITY UPLC System using a C-18 column over a 4 or 10 min gradient. Peptides were purified with a Jupiter Proteo90A Phenomenex column (2050 x 25.0 mm) on an Armen Glider CPC preparatory phase HPLC over a 43 min gradient to yield 90-95% purity. For all RP-HPLC assays and purifications, gradient solvent systems utilized Buffer A (H₂O with 0.1% TFA) and Buffer B (acetonitrile with 0.1% TFA). For all LCMS assays, gradient solvent systems used Buffer A (H₂O) and Buffer B (acetonitrile with 0.1% formic acid). Crude peptide was

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prepared for purification in 5:25:70 acetic acid/Buffer A/Buffer B via initial dissolution in acetic acid with sonication, followed by addition of ACN then H₂O. Unless otherwise stated, peptides were purified using a gradient of 25-45% Buffer B over 30 min and 50 min for analytical HPLC and preparatory phase HPLC, respectively. Following
5 purification, product was analyzed by ESI to verify identity (see **Figs. 11a-11m**). Due to the large number of cis/trans configurational isomers that our cyclic progelators could adopt,^{66,67} and amphiphilic self-assembling nature of our peptides, peak resolution via HPLC was low in many instances. We relied on various mass spectrometry techniques (ESI, MALDI, HRMS, and Tandem-MS) to verify purity of
10 isolated materials.

[0091] Mass spectrometric (MS) analysis of peptides. General mass spectra were obtained at the UCSD Chemistry and Biochemistry Molecular Mass Spectrometry Facility using a Micromass Quattro Ultima Triple Quadrupole Electrospray Ionization (ESI) mass spectrometer or Bruker Biflex IV MALDI-TOFMS using HCCA (1:1 v/v) as
15 the matrix. HRMS on a Thermo LTQ Orbitrap XL MS and Tandem-MS on a Thermo LCQdeca-MS were collected for cyclic progelators to discern conformation as cyclic or linear. DisConnect software⁶⁸ was used to unambiguously characterize disulfide connectivity. The following settings were used: MS² fragmentation ions, monoisotopic mass calculations, tolerance $\pm$ 3Da, normal peptide fragments (no losses), and
20 calculations based on ion-trap filtering.

[0092] UV Absorbance Spectra. UV absorbance spectra of peptide labeling was measured on a ThermoScientific Nanodrop 2000c.

[0093] Progelator sterilization and formulation for *in vivo* analysis HPLC purified peptides were dialyzed with 1 kDa MW cutoff tubing into milliQ H₂O, sterile
25 filtered through a 0.2 μ m PES filter, and lyophilized to a powder. Peptide was reconstituted prior to injection with sterile pH 9 H₂O to form a clear solution (11.11 mM) then diluted further with sterile 10x DPBS to a final concentration of 10 mM peptide in 1x DPBS (pH 7.4). Solutions for *in vivo* analysis contained 5 mol% rhodamine labeled progelator (0.5 mM labeled + 9.5 mM unlabeled) for fluorescence imaging purposes.

[0094] Transmission electron microscopy (TEM). Formvar/Carbon-coated 400
30 mesh Cu grids (Ted Paella, Inc.) were glow discharged for 90 s and spotted with 4-5 μ L

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peptide sample (100 μ M) and set for 5 min. Grids were washed with distilled H₂O (10 drops), stained with 1% w/w uranyl acetate (3 drops), and wicked dry with filter paper. TEM images were acquired on an FEI Tecnai G2 Sphera at 200 kV.

[0095] **Static and dynamic light scattering (SLS and DLS).** Light scattering measurements were performed on a Wyatt DynaPro NanoStar at 37 °C (λ = 657 nm). For each DLS measurement, 10 acquisitions for 10 sec were taken. Samples were prepared at 1 mg/mL in either H₂O, 1x DPBS, or 295 mM sucrose (pH 7.4). Experiments with enzyme activation used 1:4500 enzyme/substrate molar ratio. **Experimental circular dichroism (CD).** Peptides were dissolved at a final concentration of 125 μ M (labeled peptides) or 500 μ M (unlabeled peptides) in 10 mM Tris buffer or H₂O (for TCEP reduction only) at pH 7.4, to reduce signal interference seen with DPBS at lower wavelengths. UV-Vis circular dichroism (CD) was measured on a Jasco J-810 Spectropolarimeter to evaluate the secondary structure of peptide samples. Measurements were taken using the following settings: wavelength range = 190-260 nm, scanning speed = 50 nm/min, response time = 2 s, data pitch = 1 nm, band width = 1 nm, accumulations = 3, pathlength = 1 mm, and temperature = 37 °C. Spectra are presented as an average of all accumulations. Sample voltage was monitored to verify the signal intensity did not exceed 800 mV within the wavelength range. Spectra were converted from units of millidegrees (mdeg) to molar ellipticity $[\theta]$ using the following equation:

$$[\theta] = \frac{mdeg * M}{C * L * 10},$$

where mdeg is the measured absorbance of circularly polarized light, C is concentration in g/L, M is the mean residual weight in g/mol, and L is the cell pathlength in cm.

[0096] **Computational modeling with FibPredictor.** FibPredictor was utilized as a commercially available software for generating native-like amyloid fibril structures.⁴² This software was chosen for our study due to its unique ability to reliably model all classes of amyloid fibrils starting from sequence-only input. The algorithm combines β -sheet model building, β -sheet replication, and symmetry operations with side-chain prediction and statistical scoring functions, for computational predictions on amyloid fibril structures of self-assembling peptides utilized in this study. From each random model generated, a SCRWL internal scoring function was used by SCWRL4 to predict the energetically lowest side chain orientations. This scoring function was used to calculate

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the minimal total energy of the entire model and outputs coordinates for the structure as a PDB file with predicted side chains using the same residue numbering and chain identifiers as the input structure. To identify the most energetically favorable candidate structures in the ensemble, both Amb_3b (computationally more efficient)⁶⁹ and GOAP (more accurate)⁷⁰ statistical scoring functions were used to calculate total energies of the protein structure. Reported energies are unitless as they are empirically derived scores, thus it is not advisable to interpret results as kcal/mol. Normalized GOPA scores are useful for comparing fibrils of different sizes. Three generations of modeling were performed with literature-recommended parameters shown in **Table 1**. Results of top model for each peptide sequence from the third-generation analysis are reported in **Table 2**. Corresponding structures and raw PDB files are provided in the main text **Fig. 2**. Output PDB files, containing the atomic coordinates of the chromophores, from top models were used to generate theoretical CD spectra with DichroCalc.⁴⁵

[0097] Theoretical CD spectra with DichroCalc. DichroCalc was used to calculate CD spectra using predicted PDB from FibPredictor. This web interface predicts secondary structure type using a variety of matrix method parameters, which have been derived from *ab initio* calculations.⁴⁵ These parameters include the peptide chromophore in the far-UV, charge-transfer between neighboring peptide groups in the deep-UV, and aromatic side chain chromophores with transitions in the near-UV. Full analysis CD spectra were output in units of molar ellipticity [θ] vs wavelength.

[0098] *In vitro* enzyme cleavage of cyclic peptide progelator. Unless otherwise stated, enzyme cleavage experiments were performed on progelator at a final concentration of 500 μM in 1x enzyme cleavage buffers. MMP-9 cleavages were performed at 1:1000 enzyme/substrate molar ratio in 1x buffer (50 mM Tris-HCl, 200 mM NaCl, 5 mM CaCl₂, 1 mM ZnCl₂, pH 7.5) for 5 hr. Elastase cleavages were performed at 1:250 enzyme/substrate molar ratio in 1x buffer (100 mM Tris-HCl, 0.2 mM NaN₃, pH 8.0), and thermolysin (50 mM Tris-HCl, 0.5 mM CaCl₂) for 5 hr. Thermolysin cleavages were performed at 1:4500 enzyme/substrate molar ratio in 1x buffer (1x DPBS, pH 7.4) for 15 min. Analysis of unlabeled cyclic peptide progelator cleavage by CD (Fig. 4c) was conducted with serial additions of thermolysin (4 x 1:18,000 enzyme/substrate) over a period of 73 hr. Control samples utilized denatured enzymes under the same conditions.

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MMP and elastase were heat denatured with 10 min incubation at 65 °C. Thermolysin was inactivated by incubation with 10% (v/v) EDTA (0.5 M, pH 8.0)

[0099] **Rheological characterization.** All peptide samples were prepared at 10 mM peptide, except for those used to assess the effect of SAP functionalization with respect to weight (15 mg/mL). Viscous and viscoelastic properties were assessed using a stress-controlled rheometer (TA Instruments AR-G2) equipped with a Peltier plate to control temperature and a 20 mm diameter parallel plate geometry. Unless otherwise stated all measurements were taken at an angular frequency of 2.5 rad s^{-1} , strain of 0.5%, and temperature of 37 °C. Measurements were performed with a gap height of 1000 μm and repeated three times (except for step-strain tests) to ensure reproducibility. To prevent water evaporation, mineral oil was wrapped around the edge of the geometry at the air-sample interface. For viscoelastic measurements the apparatus was used in oscillatory mode. To ensure the measurements were made in the linear viscoelastic regime (LVR), strain sweeps were performed between 0.05-50 % strain and showed no variation in storage (G') and loss (G'') moduli up to a strain of 0.5%. The dynamic moduli of the hydrogels were measured as a function of frequency in the range $0.25\text{--}100 \text{ rad s}^{-1}$ at a strain of 0.5%. Continuous step-strain oscillations were used to monitor hydrogel healing through disruption (3 min, 100% strain) and recovery (15 min, 0.5% strain) cycles ($n=3$). Temperature sweeps were measured by increasing the apparatus temperatures from 21-40 °C at a ramp rate of 0.5 °C/min. For viscosity measurements the apparatus was used in steady state flow mode. The viscosity of the samples was measured as a function of shear rate in the range $0.1\text{--}10 \text{ s}^{-1}$ (5% tolerance).

[00100] ***In vitro* catheter injections and effect on assembly.** *In vitro* injection of peptide systems through the 27G inner nitinol tubing of a MyoStar catheter was performed, as previously described.⁵¹ Peptide solution (0.6-0.8 mL) was prepared at 10 mM in 1x DPBS (pH 7.4) and loaded into a 1 mL Leur Lock syringe attached to a syringe pump set to a flow rate of 0.6 mL/min. Catheters were inspected for potential clogging during injection.

[00101] **Hemocompatibility analysis.** For all measurements, peptide stocks in 1xDPBS were prepared such that the final concentration (v/v) in blood or plasma were 1:10, 1:100, 1:500, 1:1000, 1:5000, 1:10000, and 1:20000 (peptide:fluid volume). Controls with collagen as a procoagulant initial platelet-adhesive surface (0.095 mg/mL as per

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literature⁷¹ or 1:240 dilution) or with calcium chelated by sodium citrate utilized 1xDPBS as a vehicle to ensure consistent blood dilution in all experiments. Peptide stocks, whole human blood, isolated red blood cells (RBCs), and platelet poor plasma (PPP) were warmed to 37 °C immediately prior to use. Plate reader measurements were conducted on an EnSpire Multimode Plate Reader with 96-well tissue culture plates (TCP).

[00102] Activated clotting times (ACT) with whole human blood. A Hemochron 801 instrument calibrated with an electronic system verification (ESV) device was used to measure activated clotting time (ACT) of whole human blood. Activated clotting times (ACT) were determined using recalcified citrated whole human blood to minimize variability in starting time points for clotting in all assays.⁷¹ To each Hemochron P214 tube with glass beads was added 4 µL CaCl₂ (1.1 M) and 36 µL peptide stock or additive (12.2x final blood concentration). Samples were mixed thoroughly for 30 s to soak the glass beads and incubated 30 s at 37 °C. Citrated whole human blood (400 µL) was then added (t=0 s), mixed by hand for 10 s, and added to the instrument. Time points at which the magnet was displaced by clot formation were recorded by the instrument. Collagen (0.095 mg/mL) was used as a positive control to increase clotting time. Vehicle (1x DPBS) served as a standard for blood without additive. Samples without calcium, serving as the negative control, exceeded instrument maximum time range (>1500 sec). Each experiment was performed n=6 times with averages and standard error of the mean (SEM) plotted.

[00103] Whole blood hemostasis kinetics. Changes to time-dependent hemostasis were monitored using a non-activated whole blood clotting assay.⁷² Briefly, 100 µL of citrated whole human was mixed with 10 µL of peptide or collagen stock (11.5x final blood concentration) and 5 µL of CaCl₂ (230 mM). Collagen (0.095 mg/mL) and glass coverslips were used as positive controls to increase clotting rates from an additive and contact initiated perspective, respectively. Samples without calcium, where no clot was observed at any timepoint, served as negative controls. Aliquots (100 µL) were transferred to a 12-well TCP, covered, and incubated at r.t. for 5 min, 15 min, 30 min, and 45 min. Each sample was prepared in triplicate. At the end of each time point, RBCs not caught in the thrombus were lysed by gently adding 3 mL distilled H₂O and incubating for 5 min. Each well was sampled (200 µL), taking care not to disturb the clot, and transferred to a 96 well plate for analysis. Released hemoglobin from lysis was detected by measuring the

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absorbance at 405 nm (height 4.0 mm, 100 flashes) using a plate reader. Extent of clotting is inversely proportional to measured absorbance. Averages (n=3) and standard error of the mean (SEM) are plotted.

[00104] **Hemolysis assay.** Acute toxicity of cyclic peptide progelator was measured with a RBC hemolysis assay specific to biomacromolecular drug analysis, as previously described.⁷³ Averages (n=4) and standard error of the mean (SEM) for absorbance measurements (at 540 nm) were plotted.

[00105] **Pro-thrombotic assays.** The intrinsic coagulation pathway was assessed using a pro-thrombotic assay, as previously described.⁷² Collagen (0.095 mg/mL), which interacts with platelets, was used to confirm proper preparation of PPP to study platelet-independent effects of our peptide. Samples without calcium, where no clot was observed at any timepoint, served as negative controls. Untreated vehicle (1x DPBS) samples were used as a standard to compare peptide dosing impacts. For each sample, n=6 repeats were conducted. Coagulation profiles were obtained by measuring well absorbance at 405 nm (height 7.0 mm, 100 flashes) at 30 sec intervals for 60 min. Onset to clotting was detected as a sharp increase in sample turbidity.

[00106] **In vivo studies.** All animal experiments were conducted in accordance with the guidelines established by the Institutional Animal Care and Use Committee at the University of California, San Diego and the Association for the Assessment and Accreditation of Laboratory Animal Care and approved by the Institutional Animal Care and Use Committee at UCSD (A3033-01). Female Sprague Dawley rats (225 – 250 g) were used in all studies.

[00107] **Intramyocardial injections of peptide into infarcted rats.** MI was performed via 35-min ischemia-reperfusion and intramyocardial injections were performed under isoflurane, using a previously described procedure.^{74,75} Briefly, cyclic peptide (75µL, 0.75 µmol) was administered as a single injection into the infarct with a 27 G needle at 7 days post-MI. Animals were euthanized with an overdose of pentobarbital (200 mg/kg) at 24 hours post-injection (n=5).

[00108] **Cryo-sectioning, histology, and immunofluorescence imaging.** After euthanasia, hearts were resected, fresh frozen in TissueTek OCT, and cryosectioned for histological analysis. Slides were either stained with hematoxylin and eosin (H&E) to

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identify the infarct region or stained for immunofluorescence analysis. H&E slides were imaged on an Aperio ScanScope CS² at 20x magnification. For immunofluorescence, tissue sections were permeabilized in acetone (-20 °C) for 1.5 min and blocked with 2% BSA and 0.3% Triton X-100 in PBS. Myocardium was labeled using mouse anti- α -actinin antibody (1:800 dilution in blocking buffer, 1 hr incubation, Sigma-Aldrich) and an Alexa Fluor 488 goat anti-mouse IgG secondary antibody (1:500 dilution in blocking buffer, 30 min incubation, Life Technologies). Nuclei were labeled using Hoechst 33342 (0.1 μ g/mL in DI water, 10 min incubation, Life Technologies). Slides were then imaged using a Leica Ariol slide scanner with Ariol software at 20x magnification.

10 [00109] **Statistical analysis.** All statistical results are expressed as mean $\pm$ standard error (SEM). Ordinary one-way ANOVA tests were used for multiple comparisons of the mean in each group with that of the standard. Tukey corrections with 95% confidence intervals and significance were used. Two-way ANOVA tests without matching were used for multiple comparisons of the mean in each group at each timepoint with that of the standard. Holm-Sidak test was used for multiple comparisons. Statistical significance was defined as follows: *ns* ($p > 0.05$), *** ($p \leq 0.001$), **** ($p \leq 0.0001$).

15 [00110] These and other embodiments of the invention will be apparent to one skilled in the art upon a review of the present invention.

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Table. 1. FibPredictor experimental setup over three generations.

[00111] Input included the peptide sequence, number of strands per sheet (x 2 sheets total) to model, and selection criterion about scoring functions, and rotation operations performed on the second β -spine relative to the first β -spine for generating all classes of amyloid fibrils. The number of random models, and distances angle variation between sheets, were also selected to improve modeling conditions. Changes to parameters with each future generation were a result of model analysis from the (n-1) generation.

	Generation 1	Generation 2	Generation 3
Number of strands per sheet	2	4	4
Sense of β -sheet orientation	Both	Antiparallel or parallel	Selected in Gen 2
Scoring function(s)	Amb_3b_score only	Amb_3b and GOAP score	Amb_3b and GOAP score
Rotations	x,z,zx,no rotation	x,z,zx,no rotation	x,z,zx,no rotation
Random models	30	60	200
Distance variation between the sheets	7 Å	7 Å	6 Å
Minimum distance between the sheets	3 Å	3 Å	4 Å
Angle variation between the sheets	45°	45°	10°

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Table. 2. Experimental CD and theoretical modeling of gelators.

- [00112] Supplementary data to **Fig. 2-3** in main text. Observed shift in high energy minimum in CD spectra indicate reorientation in 2° structure. Calculated orientations for minimal total energy structures generated in FibPredictor agree with observed inferences.
- 5 Internal scoring SCWRL4 energies and corresponding empirical (Amber_3b) and statistical (SCWRL4) scores for minimal energy models are displayed for the top model of each sequence. Normalized GOAP scores are provided for comparison of fibrils of different sizes. Scores are unitless.

Peptide	Sequence	Obs. CD $\pi \rightarrow \pi^*$ Peak	Obs. CD Inference	Theor. CD Orientation	SCWRL4 energy	Amb_3b score	GOAP score	GOAP normalized
KLDL	(KLDL) ₃ (SEQ ID NO: 3)	---	antiparallel	antiparallel	353.796	-96.984	7271.93	-75.7493
KLDL _{Control}	LAG(KLDL) ₃ PLG (SEQ ID NO: 8)	Blue-shifted	Parallel	Parallel	351.78	112.363	7675.04	-53.2989
KLDL _{Linear}	C(KLDL) ₃ PLGLAGC (SEQ ID NO: 6)	Blue-shifted	Parallel	Parallel	370.407	128.576	8610.19	-53.8137
KFDF	(KFDF) ₃ (SEQ ID NO: 4)	----	Antiparallel	Antiparallel	621.622	-53.771	7179.08	-74.7821
KFDF _{Control}	LAG(KFDF) ₃ PLG (SEQ ID NO: 9)	Blue-shifted	Parallel	Parallel	262.448	158.008	7755.24	-53.8558
KFDF _{Linear}	C(KFDF) ₃ PLGLAGC (SEQ ID NO: 7)	Blue-shifted	parallel	parallel	340.488	158.782	11096.7	-69.3543

Table 3. DisConnect analysis of Rho-KL_{DL}Cyclic progelator.

[00113] Data from DisConnect software displayed in tables with peak labels corresponding to **Figs. 16a-16d**, MS² fragmentation sequences, mass and charge values, and conclusions about cysteine connectivity. B is Rhodamine (413.15 m/z), | indicates separate peptides linked through a cysteine disulfide bond.

Peak	Sequence	SEQ ID NO(S)	m/z	Z (+)	Conclusion
A	BC1 KL _{DL} PLGLAGC2	15	806.36 / 1612.72	1 and 2	All Cys connected
A	BC1K LDLPLGLAGC2	16	806.36 / 1612.72	1 and 2	All Cys connected
A	BC1KL DLPLGLAGC2	57	806.36 / 1612.72	1 and 2	All Cys connected
A	BC1KLD LPLGLAGC2	18&19	806.36 / 1612.72	1 and 2	All Cys connected
A	BC1KL _{DL} PLGLAGC2	20&21	806.36 / 1612.72	1 and 2	All Cys connected
B	DLKLDLKL _{DL} PLGLAG	22	837.98	2	0 0 0 0
B	KL _{DL} KL _{DL} KL _{DL} PLG	23	837.995	2	0 0 0 0
C	BC1K DLKLDLPLGLAGC2	58	984.46	2	All Cys connected
C	BC1KLD KL _{DL} PLGLAGC2	18&15	984.46	2	All Cys connected
C	BC1KL _{DL} K DLPLGLAGC2	24&59	984.46	2	All Cys connected
C	BC1KL _{DL} KL _{DL} PLGLAGC2	25&21	984.46	2	All Cys connected
D	BC1 KL _{DL} KL _{DL} PLGLAGC2	60	1041	2	All Cys connected
D	BC1K LDLKL _{DL} PLGLAGC2	61	1041	2	All Cys connected
D	BC1KL DLKLDLPLGLAGC2	62	1041	2	All Cys connected
D	BC1KLD LKLDLPLGLAGC2	18&63	1041	2	All Cys connected
D	BC1KL _{DL} KL _{DL} PLGLAGC2	20&64	1041	2	All Cys connected
D	BC1KL _{DL} K LDLPLGLAGC2	26&65	1041	2	All Cys connected
D	BC1KL _{DL} KL DLPLGLAGC2	27&66	1041	2	All Cys connected
D	BC1KL _{DL} KL _{DL} LPLGLAGC2	28&19	1041	2	All Cys connected
D	BC1KL _{DL} KL _{DL} PLGLAGC2	29&21	1041	2	All Cys connected
E	BC1K LKLDLKL _{DL} PLGLAGC2	67	1161.59	2	All Cys connected
E	BC1KL KL _{DL} KL _{DL} PLGLAGC2	68	1161.59	2	All Cys connected
E	BC1KL _{DL} K LKLDLPLGLAGC2	30&69	1161.59	2	All Cys connected
E	BC1KL _{DL} KL KL _{DL} PLGLAGC2	31&70	1161.59	2	All Cys connected
E	BC1KL _{DL} KL _{DL} K LPLGLAGC2	32&71	1161.59	2	All Cys connected
E	BC1KL _{DL} KL _{DL} KL PLGLAGC2	33&72	1161.59	2	All Cys connected
F	BC1KL _{DL} KL _{DL} KL _{DL} LGLAGC2	34&73	1170.58	2	All Cys connected

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F	BC1KLDLKLKLDL GLAGC2	35&74	1170.58	2	All Cys connected
G	BC1KL LKLDLKLPLGLAGC2	75	1218.13	2	All Cys connected
G	BC1KLDLKL LKLDLPLGLAGC2	36&76	1218.13	2	All Cys connected
G	BC1KLDLKLKLDL LPLGLAGC2	37&77	1218.13	2	All Cys connected
H	BC1KL LGLAGC2	78	1287.56	1	All Cys connected
H	BC1KLD GLAGC2	18&79	1289.51	1	All Cys connected
H	BC1KLDLK GC2	38	1289.54	1	All Cys connected
I	BC1KLD LGLAGC2	18&80	1402.59	1	All Cys connected
I	BC1KLDL GLAGC2	20&81	1402.59	1	All Cys connected
I	BC1KLDLKL GC2	39	1402.62	1	All Cys connected
J	BC1KL LPLGLAGC2	82	1497.69	1	All Cys connected
J	BC1K DLPLGLAGC2	83	1499.64	1	All Cys connected
J	BC1KLD PLGLAGC2	18&84	1499.64	1	All Cys connected
K	BC1KLD DLPLGLAGC2	18&85	1727.75	1	All Cys connected

Table 4. DisConnect analysis of Rho-KFDF_{Cyclic} progelator.

[00114] Data from DisConnect software displayed in tables with peak labels corresponding to **Figs. 16a-16d**, MS² fragmentation sequences, mass and charge values, and conclusions about cysteine connectivity. B is Rhodamine (413.15 m/z), | indicates separate peptides linked through a cysteine disulfide bond.

Peak	Sequence	SEQ ID NO(S)	m/z	Z (+)	Conclusion
A	BC1K DFKFDFPLGLAGC2	86	1035.445	2	All Cys connected
A	BC1KFD KFDFPLGLAGC2	40&87	1035.445	2	All Cys connected
A	BC1KFDFK DFPLGLAGC2	41&88	1035.445	2	All Cys connected
A	BC1KFDFKFD PLGLAGC2	42&89	1035.445	2	All Cys connected
B	KFDFKFDFKFDFPLGLAGC2	43	1104.035	2	69 0 0 0
C	BC1 DFKFDFKFDFPLGLAGC2	90	1240.03	2	All Cys connected
C	BC1KFD FDFKFDFPLGLAGC2	44&91	1240.03	2	All Cys connected
C	BC1KFDF DFKFDFPLGLAGC2	45&92	1240.03	2	All Cys connected
C	BC1KFDFKFD FDFPLGLAGC2	46&93	1240.03	2	All Cys connected
C	BC1KFDFKFDF DFPLGLAGC2	47&94	1240.03	2	All Cys connected
D	BC1KFDFKFDFKFDF GLAGC2	48&95	1272.545	2	All Cys connected
E	BC1KFDFKFDFKFDFP LAGC2	49&96	1292.56	2	All Cys connected
E	BC1KFDFKFDFKFDFPL AGC2	50	1292.56	2	All Cys connected
F	BC1K DFPLGLAGC2	97	1533.63	1	All Cys connected
F	BC1KFD PLGLAGC2	51&98	1533.63	1	All Cys connected
G	BC1 KFDFPLGLAGC2	99	1680.7	1	All Cys connected
G	BC1K FDFPLGLAGC2	100	1680.7	1	All Cys connected
G	BC1KF DFPLGLAGC2	101	1680.7	1	All Cys connected
G	BC1KFD FPLGLAGC2	52&102	1680.7	1	All Cys connected
G	BC1KFDF PLGLAGC2	53&103	1680.7	1	All Cys connected
H	BC1KFD DFPLGLAGC2	54&104	1795.73	1	All Cys connected
I	BC1 DFKFDFPLGLAGC2	105	1942.8	1	All Cys connected
I	BC1KFD FDFPLGLAGC2	55&106	1942.8	1	All Cys connected
I	BC1KFDF DFPLGLAGC2	56&107	1942.8	1	All Cys connected

Table. 5. Activated clotting times (ACT) in human blood.

[00115] Supplemental data corresponding to **Fig. 8a** in main text. Average times reported $\pm$ standard error of mean (n=6 per group). Ordinary one-way ANOVA for comparison with vehicle standard. Values are mean $\pm$ SEM.

[illegible]

Table. 6. Hemolysis of red blood cells (RBCs).

[00116] Supplemental data corresponding to **Fig. 8c** in the main text. Nonhemolytic biomaterials classified by < 5% hemolysis. (n=4 per group). ns ($p > 0.05$), *** ($p \leq 0.001$), **** ($p \leq 0.0001$). Ordinary one-way ANOVA for comparison with vehicle standard. Values are mean $\pm$ SEM.

	standard		peptide in blood dilutions						positive control
	vehicle	1:20000	1:10000	1:5000	1:1000	1:500	1:100	1:10	1% Triton X-100
%Hemolysis	0.00	0.20	0.37	0.30	0.13	0.26	0.40	4.43	100.00
SEM	(± 0.07)	(± 0.06)	(± 0.12)	(± 0.08)	(± 0.07)	(± 0.26)	(± 0.12)	(±0.64)	(± 18.17)
P Value		> 0.9999	> 0.9999	> 0.9999	> 0.9999	0.9999	> 0.9999	0.9968	< 0.0001
Summary		ns	ns	ns	ns	ns	ns	ns	****

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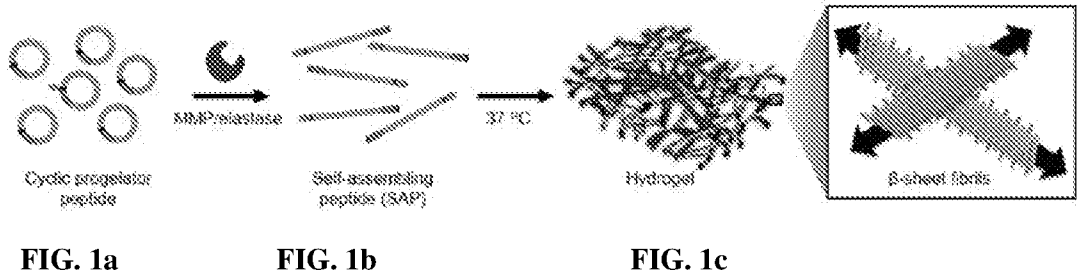
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CLAIMS

1. A tissue scaffolding composition comprising a pharmaceutically acceptable carrier and a cyclic peptide having a substrate recognition sequence that when enzymatically cleaved linearizes and self-assembles with like-kind peptides into a hydrogel.
2. The composition of Claim 1, wherein the cyclic peptide is disulfide bonded by terminal cysteine residues and comprises repeating amino acid sequence KFDF (SEQ ID NO:1) and wherein the peptide self-assembles into a hydrogel via beta-sheets.
3. The composition of Claim 1, wherein the substrate recognition sequence is specific for inflammatory-related enzymes, matrix metalloproteinases (MMP)-2/9 and/or elastase.
4. The composition of Claim 1, wherein therapeutic peptide comprises a MMP-2/9 cleavable substrate recognition amino acid sequence PLGLAG (SEQ ID NO:2).
5. The composition of Claim 1, wherein the peptide is labeled for detection.
6. The composition of Claim 1, wherein the composition further comprises a therapeutic agent, a chemotactic agent, an antibiotic, or a growth factor.
7. The composition of Claim 1, wherein the peptide comprises the amino acid sequence CKFDFKFDFKFDFPLGLAGC (SEQ ID NO:7).
8. A method of creating a scaffold for tissue repair in a patient in need thereof comprising administering to the patient a cyclic peptide having a substrate recognition sequence that when enzymatically cleaved linearizes and self-assembles with like-kind peptides into a hydrogel.
9. The method of Claim 8, wherein the cyclic peptide has a disulfide bonded by terminal cysteine residues and comprises repeating amino acid sequence KFDF (SEQ ID NO:1) and wherein the peptide self-assembles into a hydrogel via beta-sheets.
10. The method of Claim 8, wherein the substrate recognition sequence is specific for inflammatory-related enzymes, matrix metalloproteinases (MMP)-2/9 and/or elastase.

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11. The method of Claim 8, wherein the peptide comprises a MMP-2/9 cleavable substrate recognition amino acid sequence PLGLAG (SEQ ID NO:2).
12. The method of Claim 8, wherein the peptide is labeled for detection.
13. The method of Claim 8, wherein the composition further comprises a therapeutic agent, a chemotactic agent, an antibiotic, or a growth factor.
14. The method of Claim 8, wherein the tissue being repaired is ischemic tissue.
15. The method of Claim 8, wherein the tissue being repaired is left ventricular tissue for remodeling post-myocardial infarction.
16. The method Claim 8, wherein the administration is via a catheter, parenteral injection, or implantation.
17. The method of Claim 8, wherein the composition is a liquid when administered and gels upon *in situ* enzymatic cleavage.
18. The method of Claim 8, wherein the peptide comprises the amino acid sequence CKF DFKDFKDFPLGLAGC (SEQ ID NO:7).
19. A peptide comprising the amino acid sequence CKFDFKDFKDFPLGLAGC (SEQ ID NO:7).
20. The peptide of Claim 19, wherein the peptide is cyclic.



KLDL SAPs and Progelators		KFDF SAPs and Progelators		Diagrammatical Representations
Name	Sequence	Name	Sequence	
KLDL	Ac-NQDFKQDLS-CO ₂ NH ₂	KFDF	Ac-NQDFKDFDF-CO ₂ NH ₂	
KLDL _{Loop}	NH ₂ -Glaaa(NQDLSKLDLPLGLAGGaa)-CO ₂ NH ₂	KFDF _{Loop}	NH ₂ -Glaaa(NQDFKDFDFPLGLAGGaa)-CO ₂ NH ₂	
Rho-KLDL _{Loop}	Rho-Glaaa(NQDLSKLDLPLGLAGGaa)-CO ₂ NH ₂	Rho-KFDF _{Loop}	Rho-Glaaa(NQDFKDFDFPLGLAGGaa)-CO ₂ NH ₂	
KLDL _{Disulfide}	 NH ₂ -Cys(NQDLSKLDLPLGLAGG)-CO ₂ NH ₂	KFDF _{Disulfide}	 NH ₂ -Cys(NQDFKDFDFPLGLAGG)-CO ₂ NH ₂	
Rho-KLDL _{Disulfide}	 Rho-Cys(NQDLSKLDLPLGLAGG)-CO ₂ NH ₂	Rho-KFDF _{Disulfide}	 Rho-Cys(NQDFKDFDFPLGLAGG)-CO ₂ NH ₂	

Fig. 2a

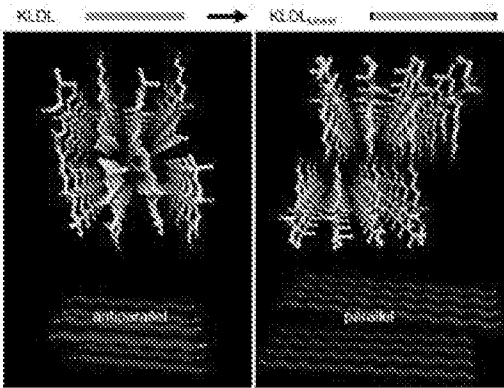


Fig. 2b

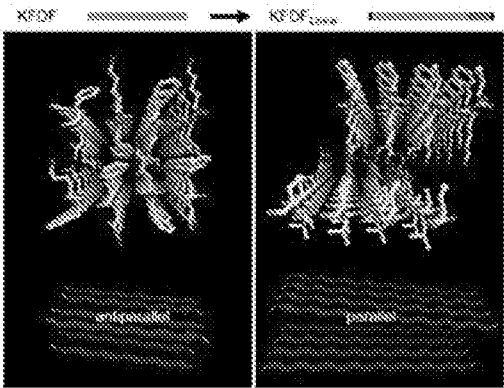
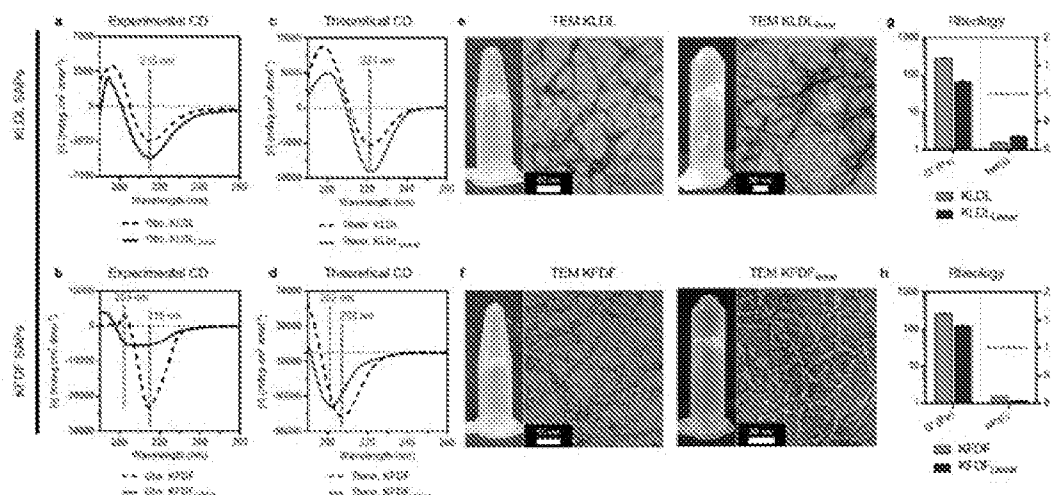
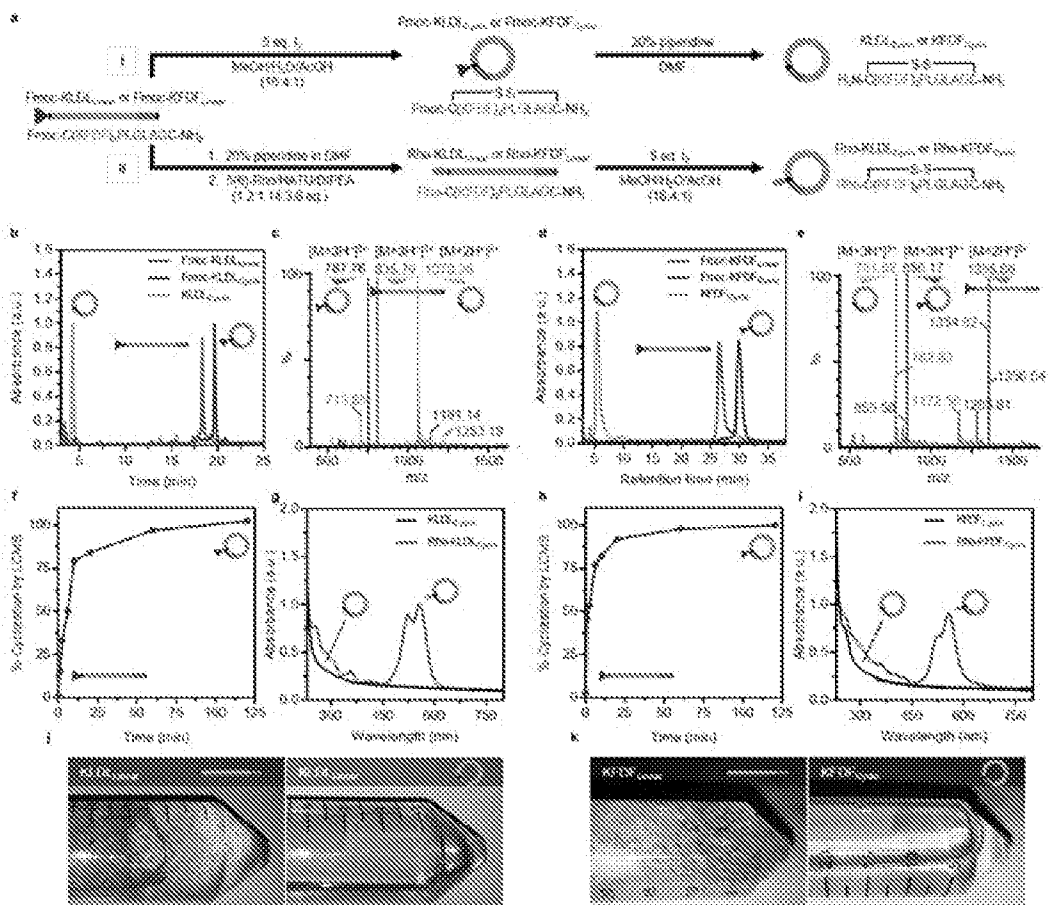


Fig. 2c

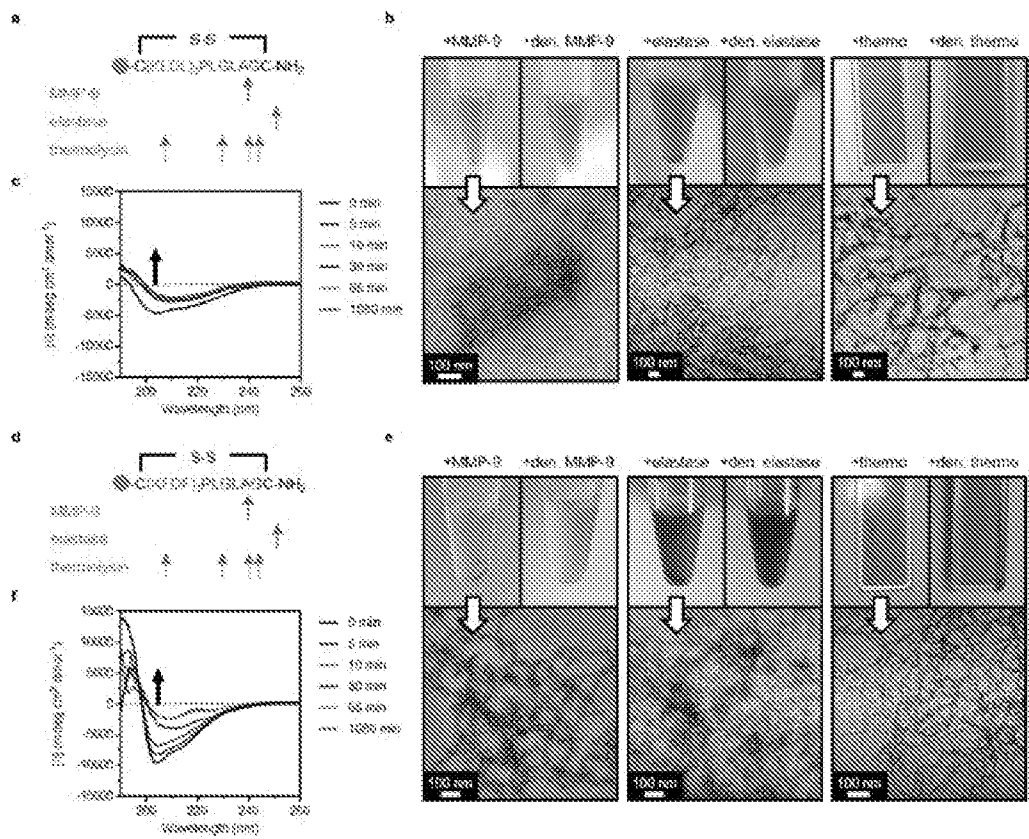
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Figs. 3a-3h

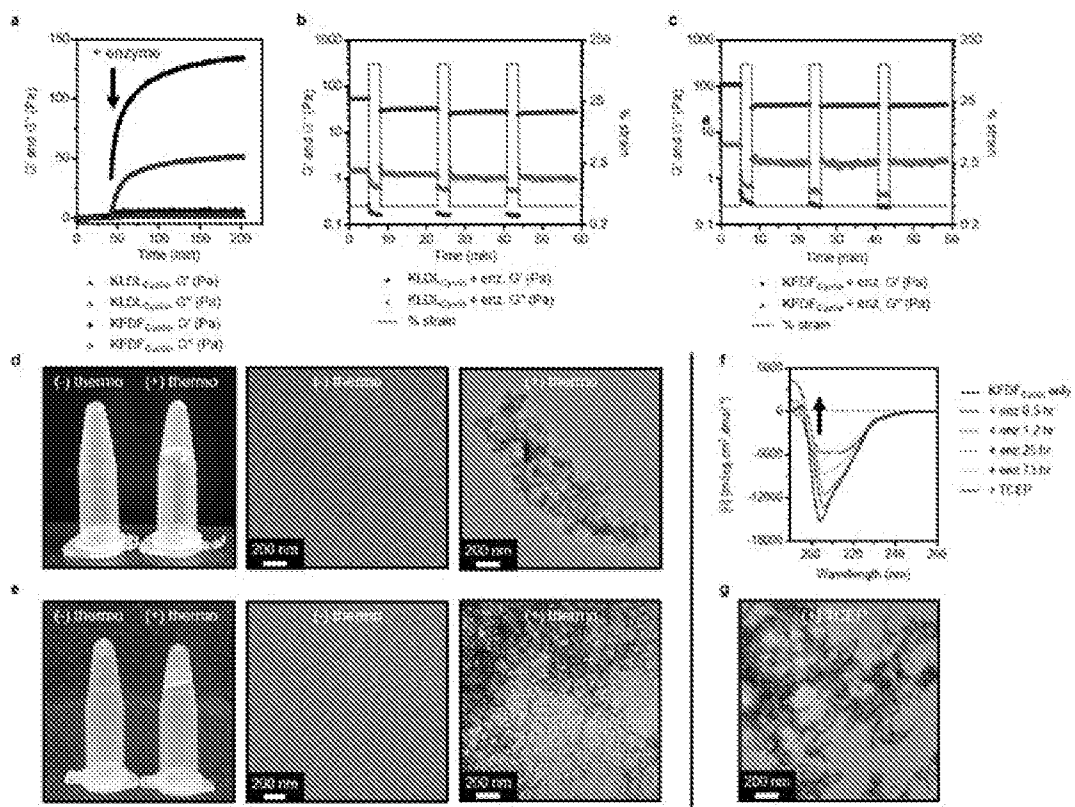


Figs. 4a-4k

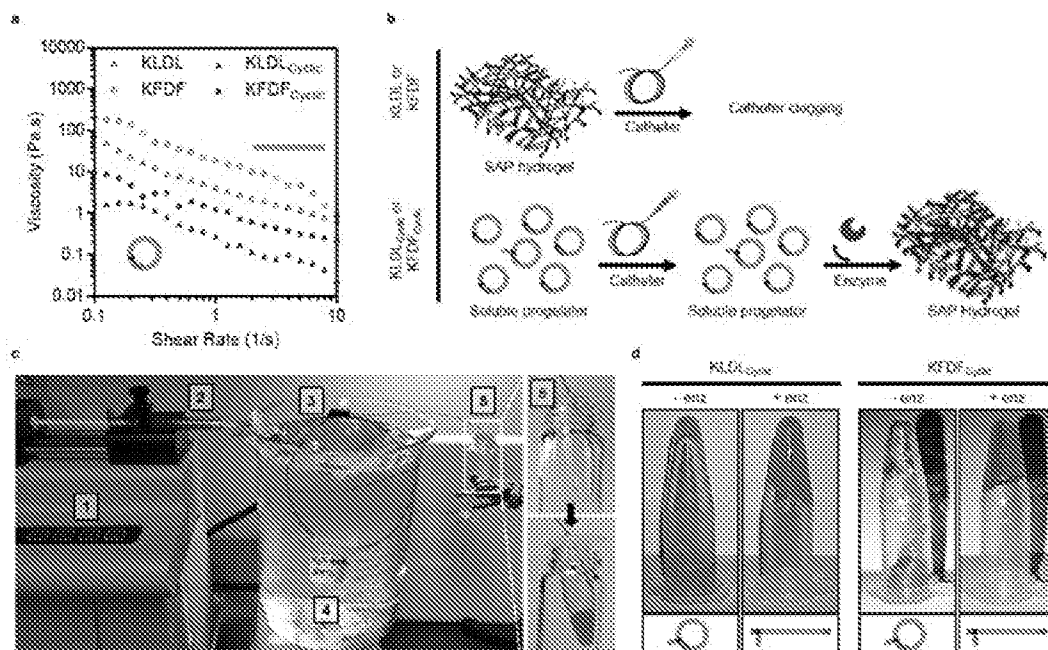


Figs. 5a-5f

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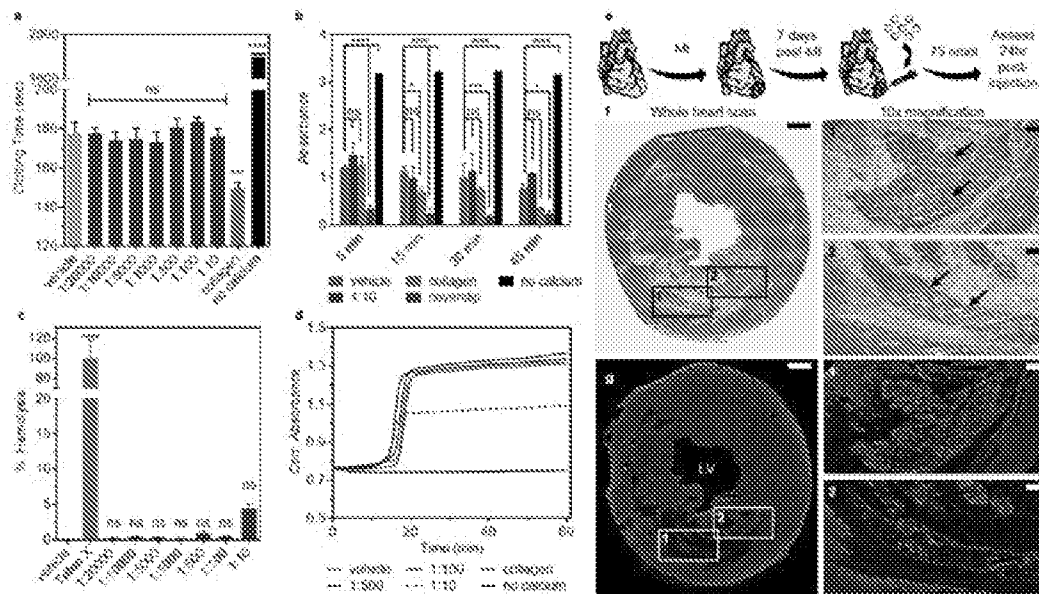


Figs. 6a-6g



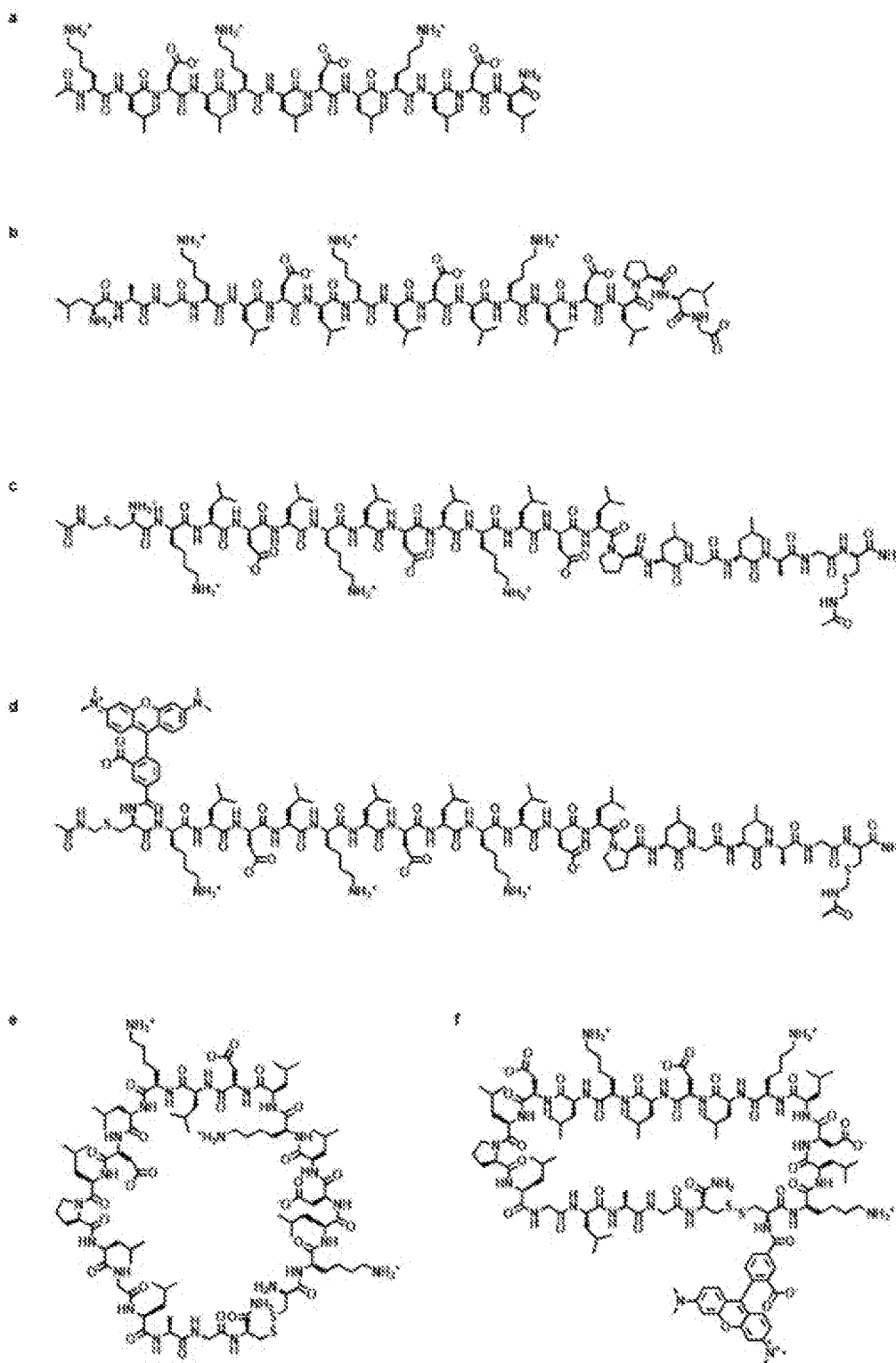
Figs. 7a-7d

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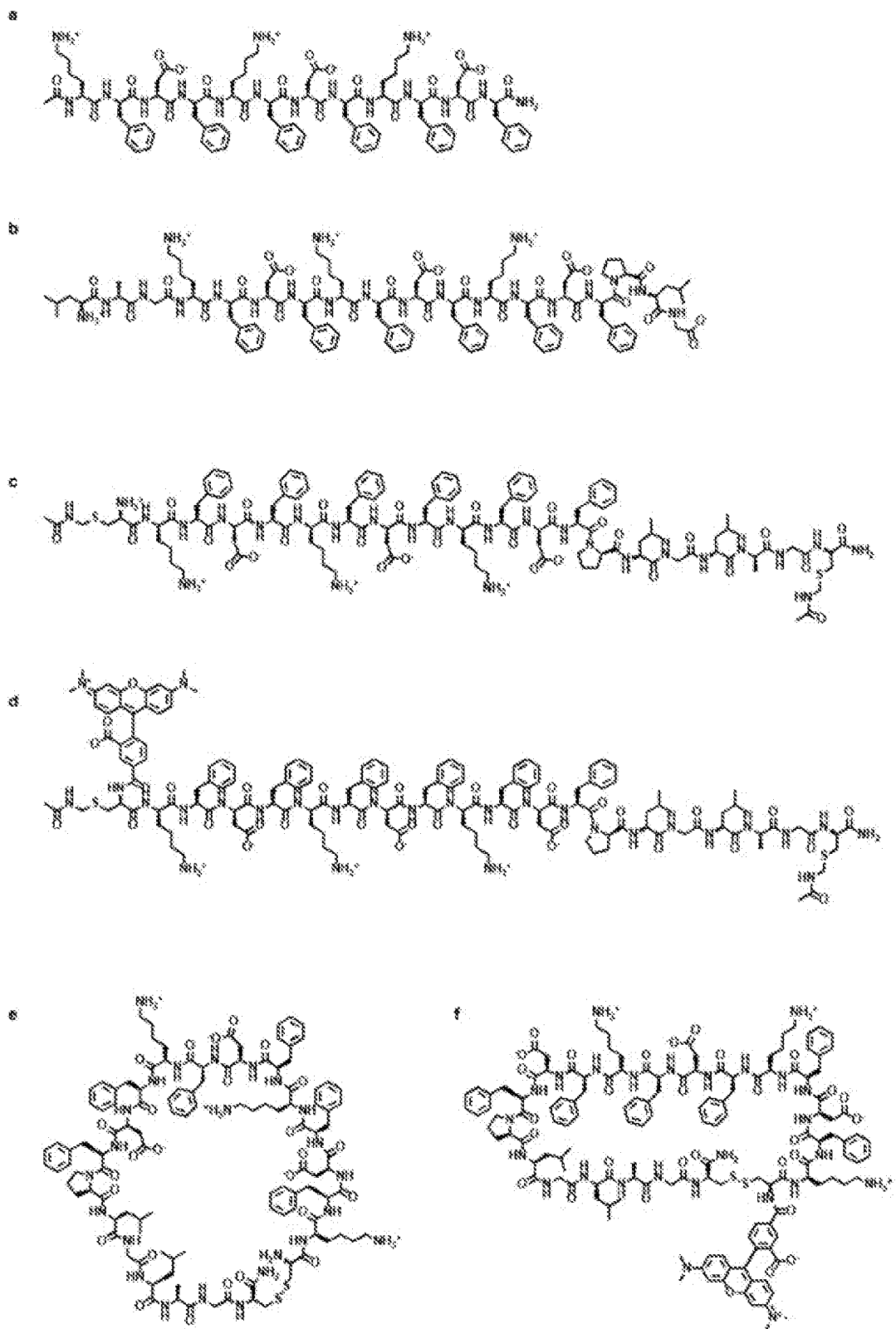
Figs. 8a-8g

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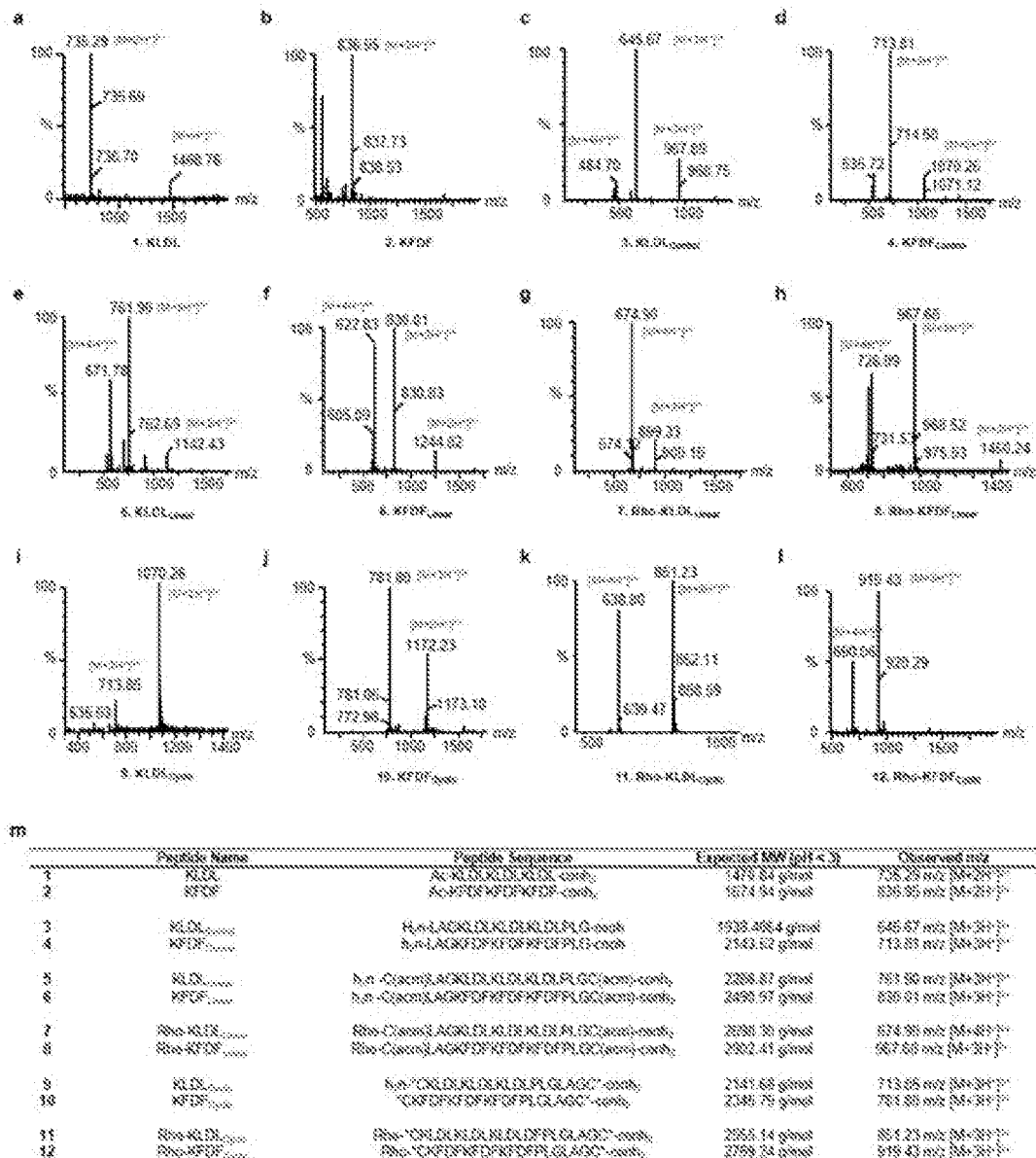
Figs. 9a-9f

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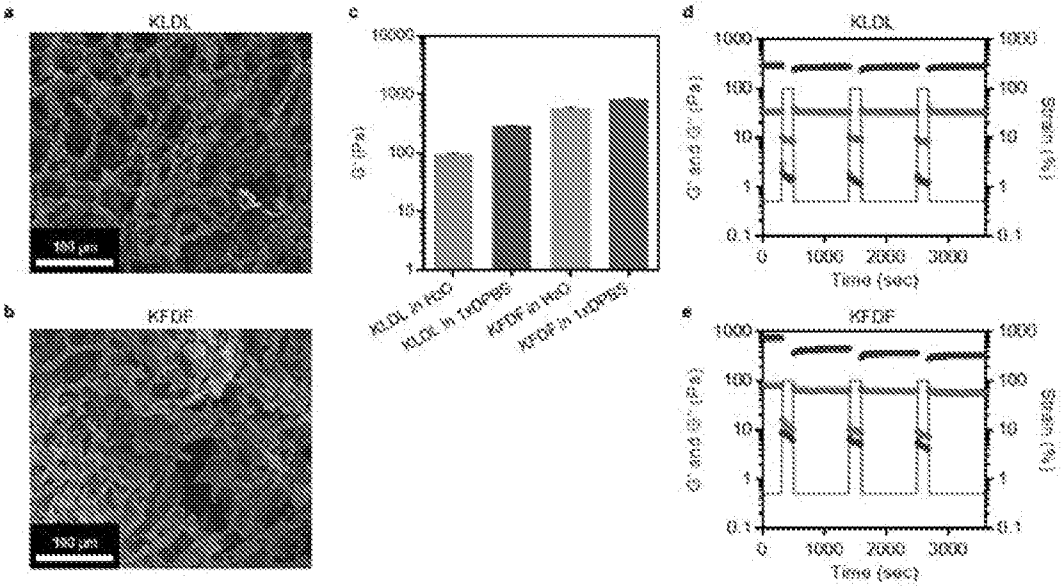


Figs. 10a-10f

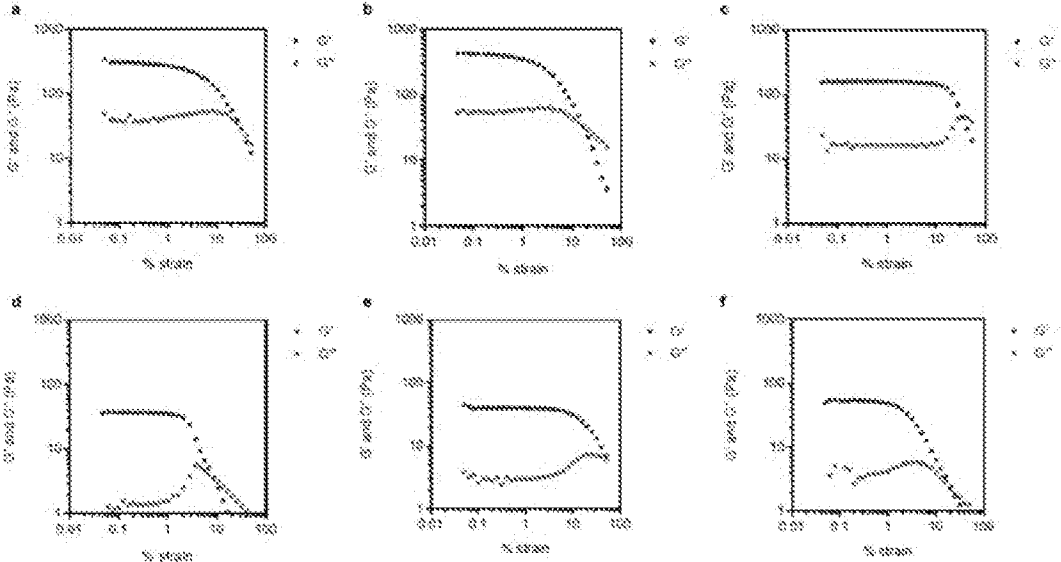
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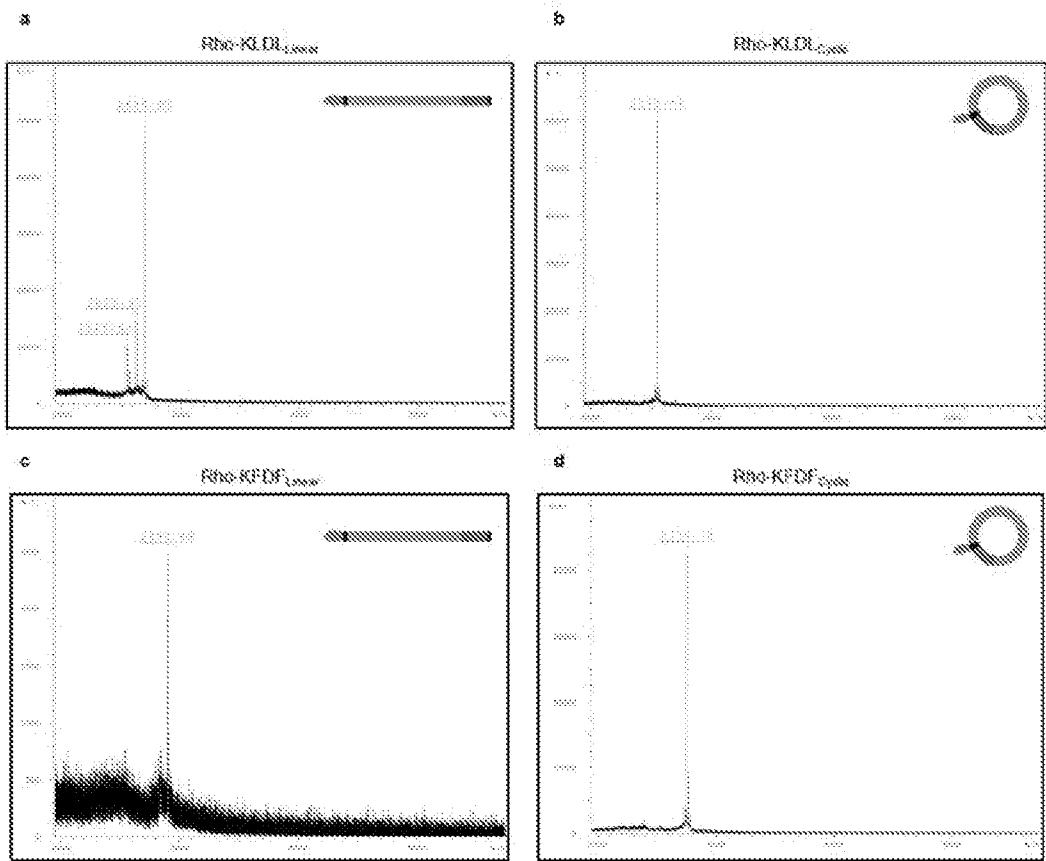
Figs. 11a-11m



Figs. 12a-12e

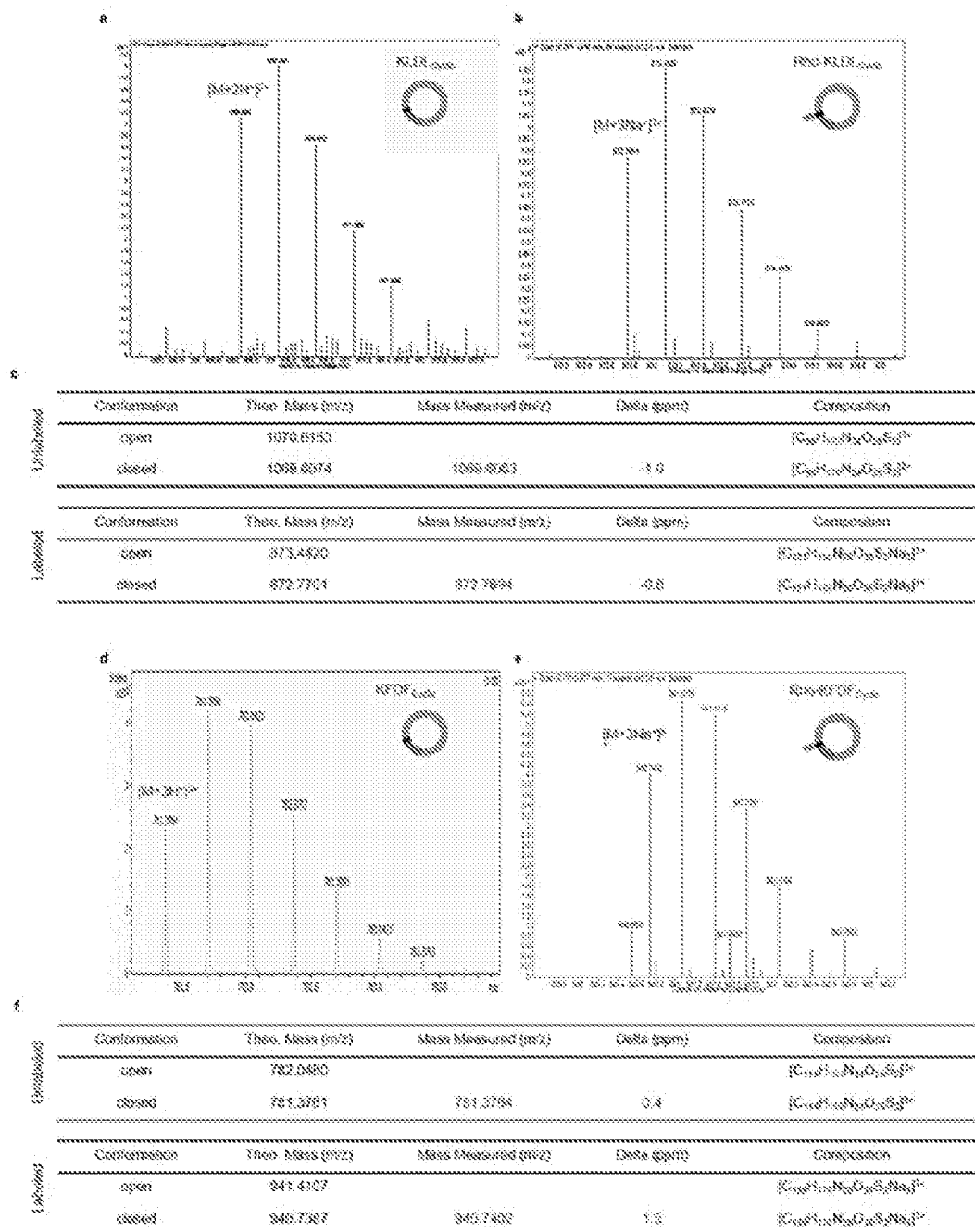


Figs. 13a-13f

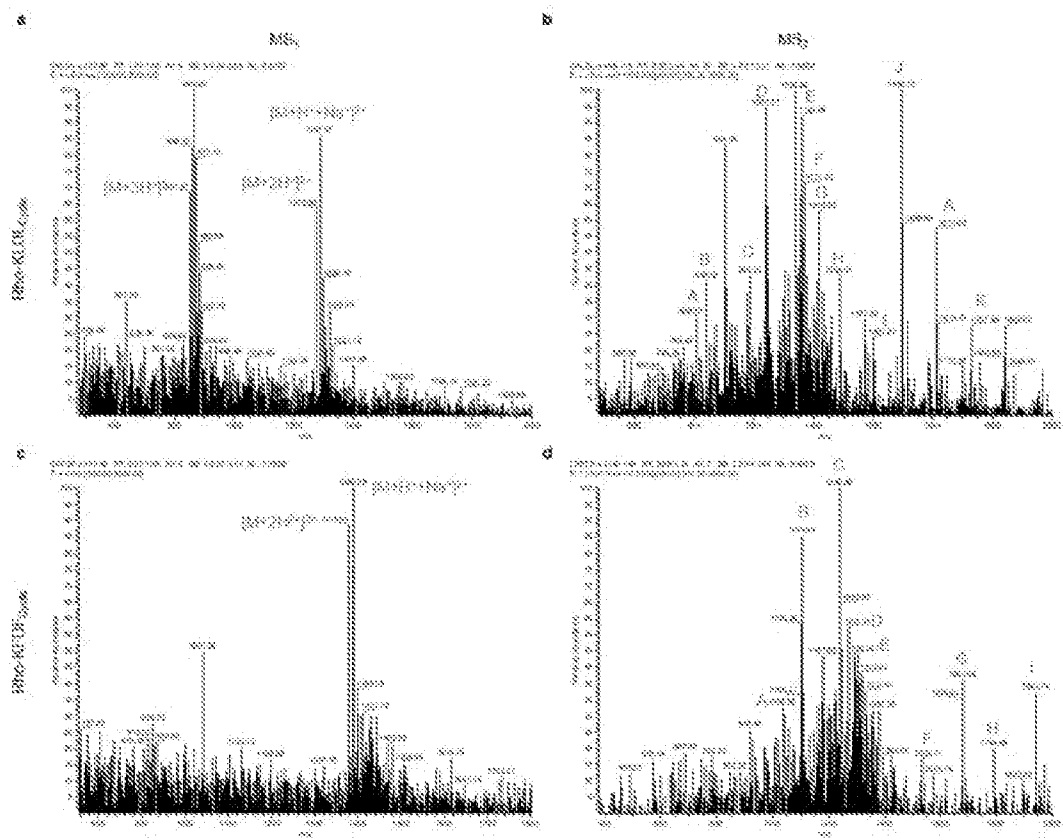


Figs. 14a-14d

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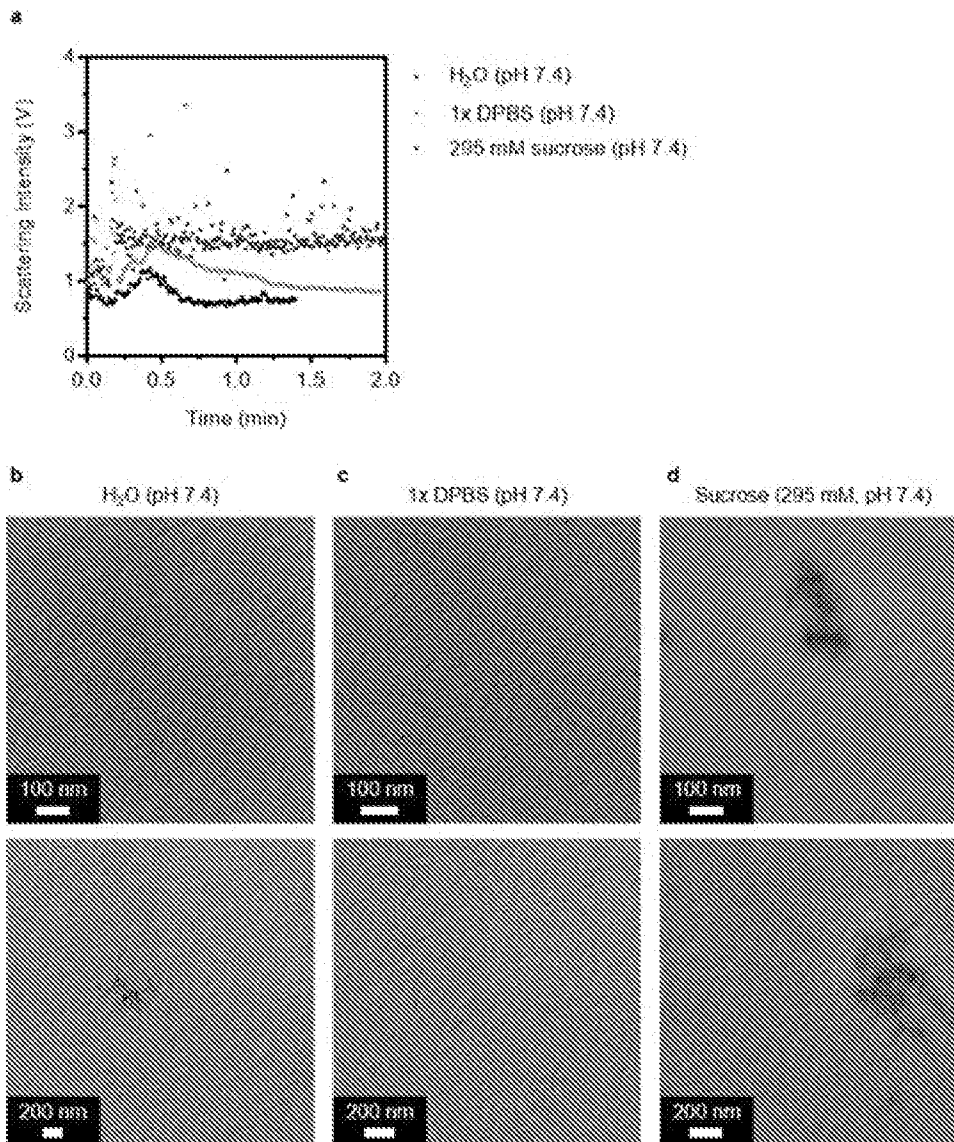


Figs. 15a-15f

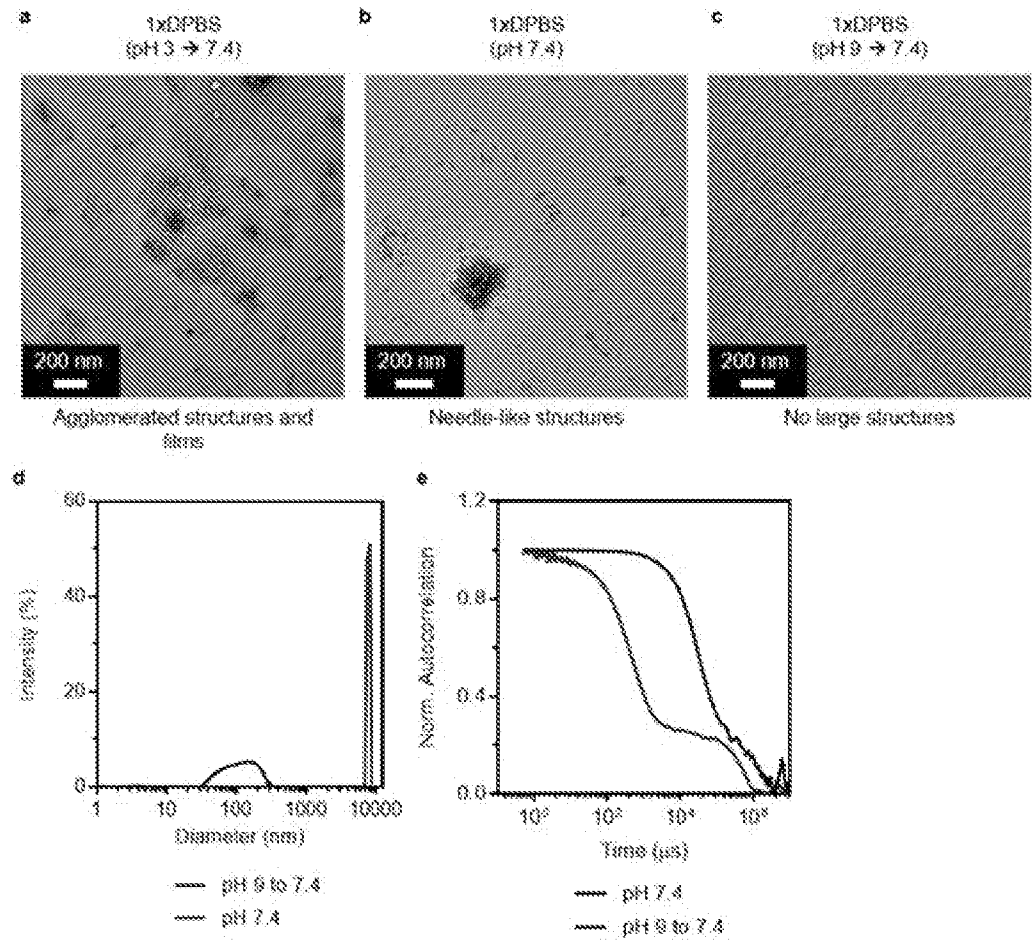


Figs. 16a-16d

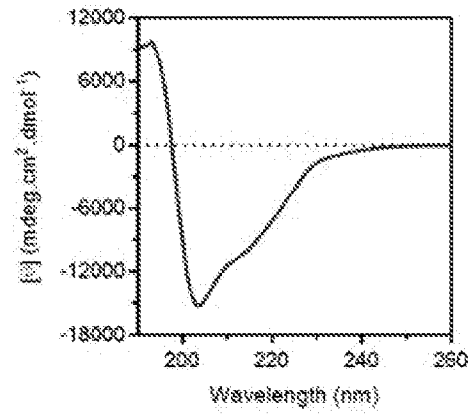
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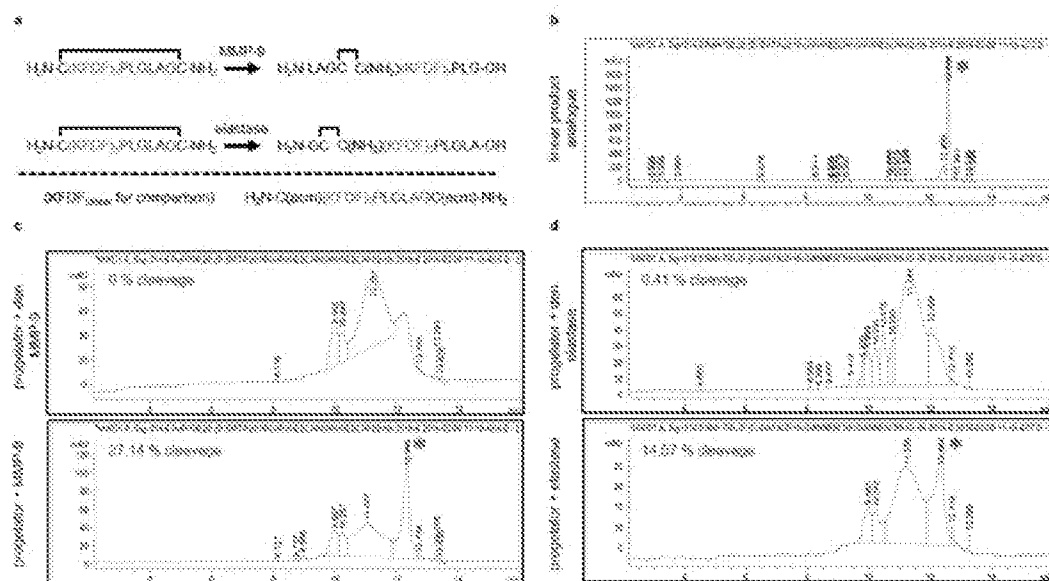


Figs. 17a-17d

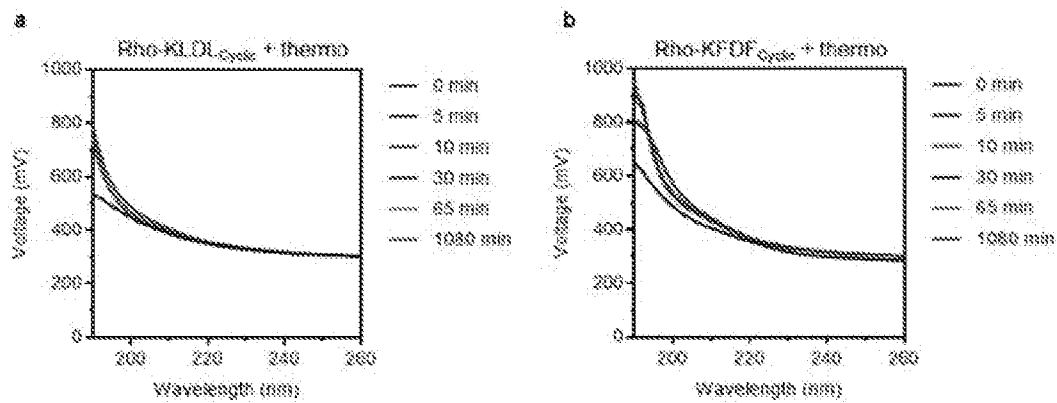


Figs. 18a-18e

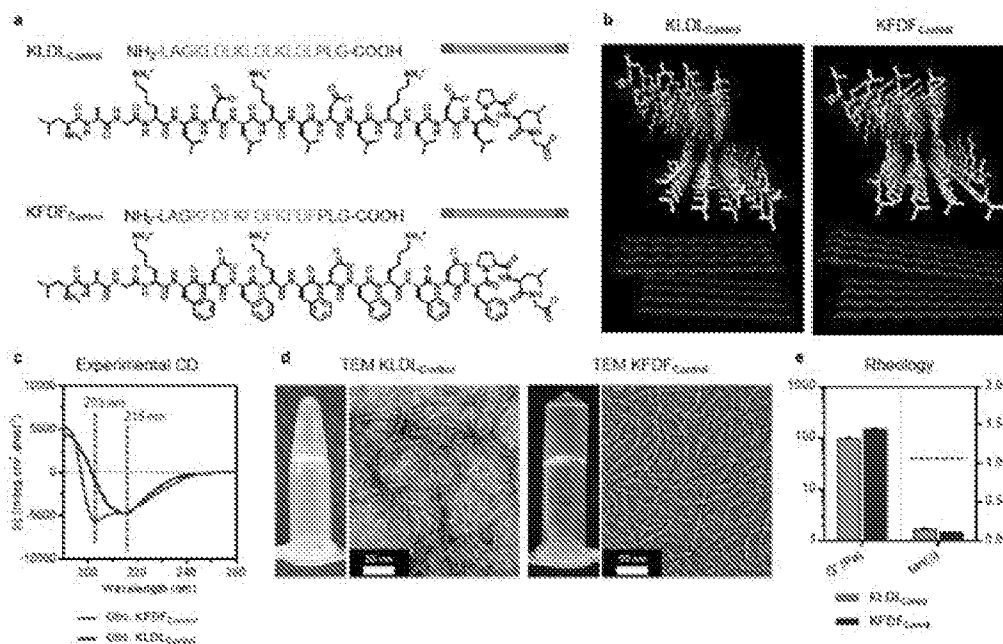




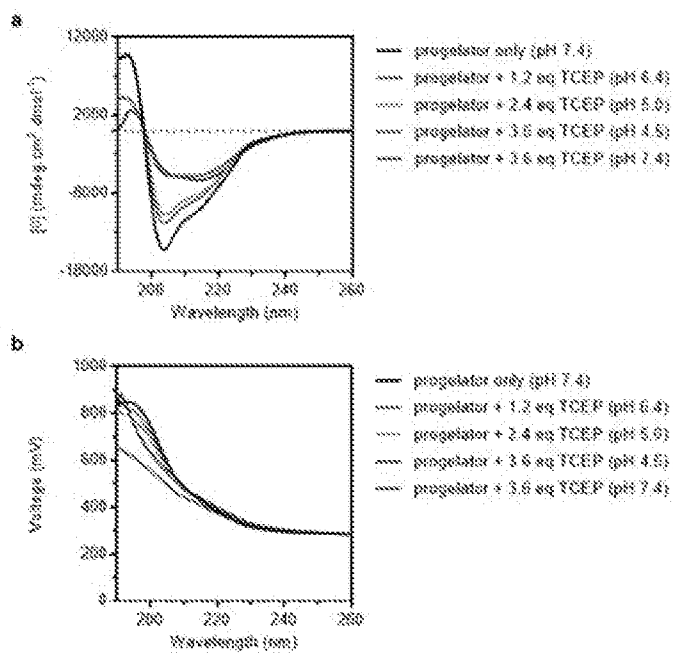
Figs. 20a-20d



Figs. 21a-21b



Figs. 22a-22e



Figs. 23a-23b

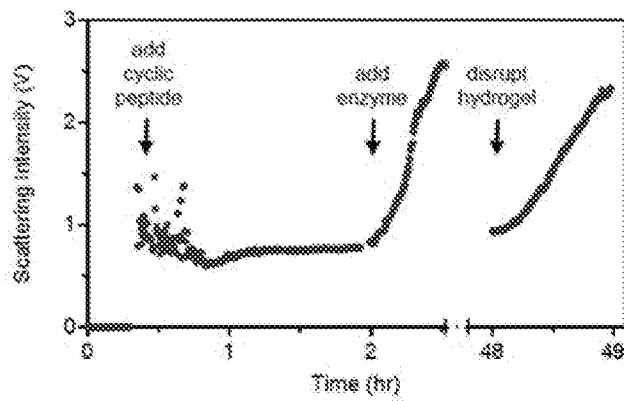


Fig. 24

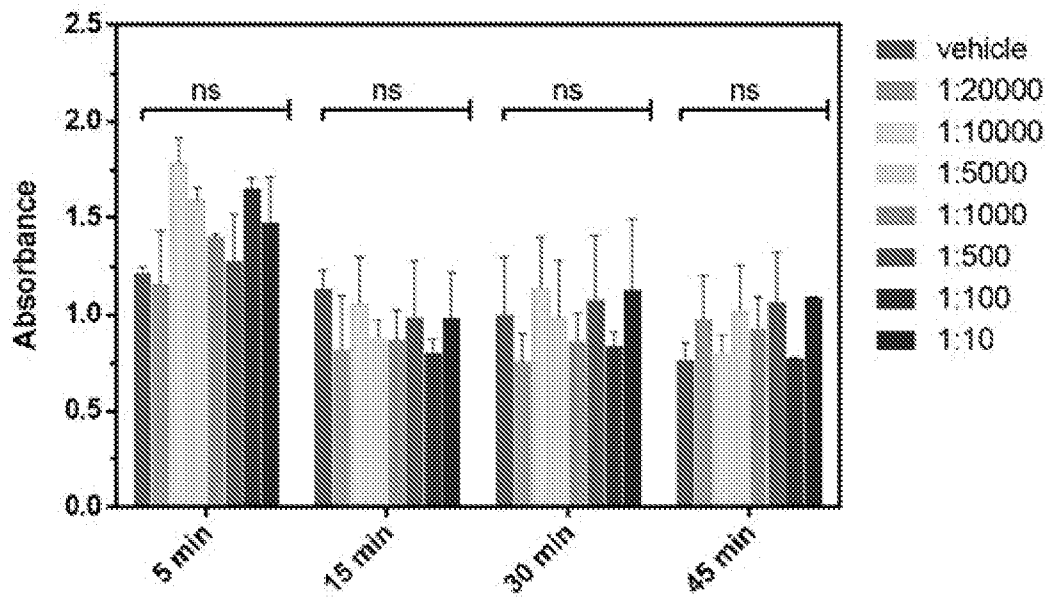
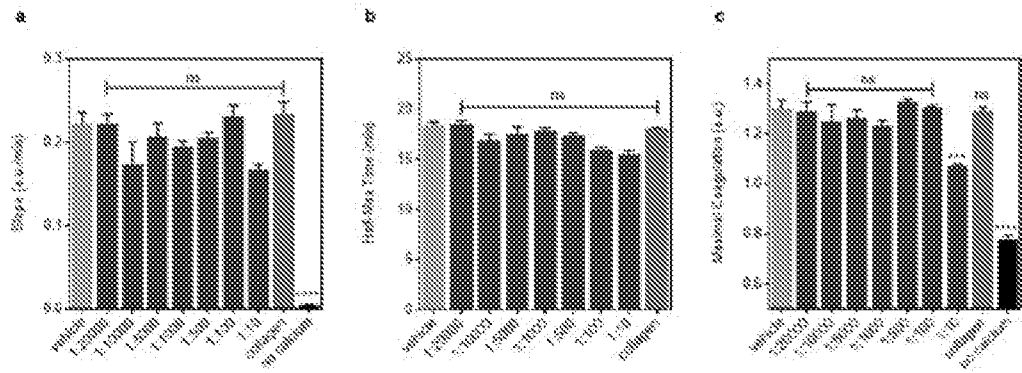
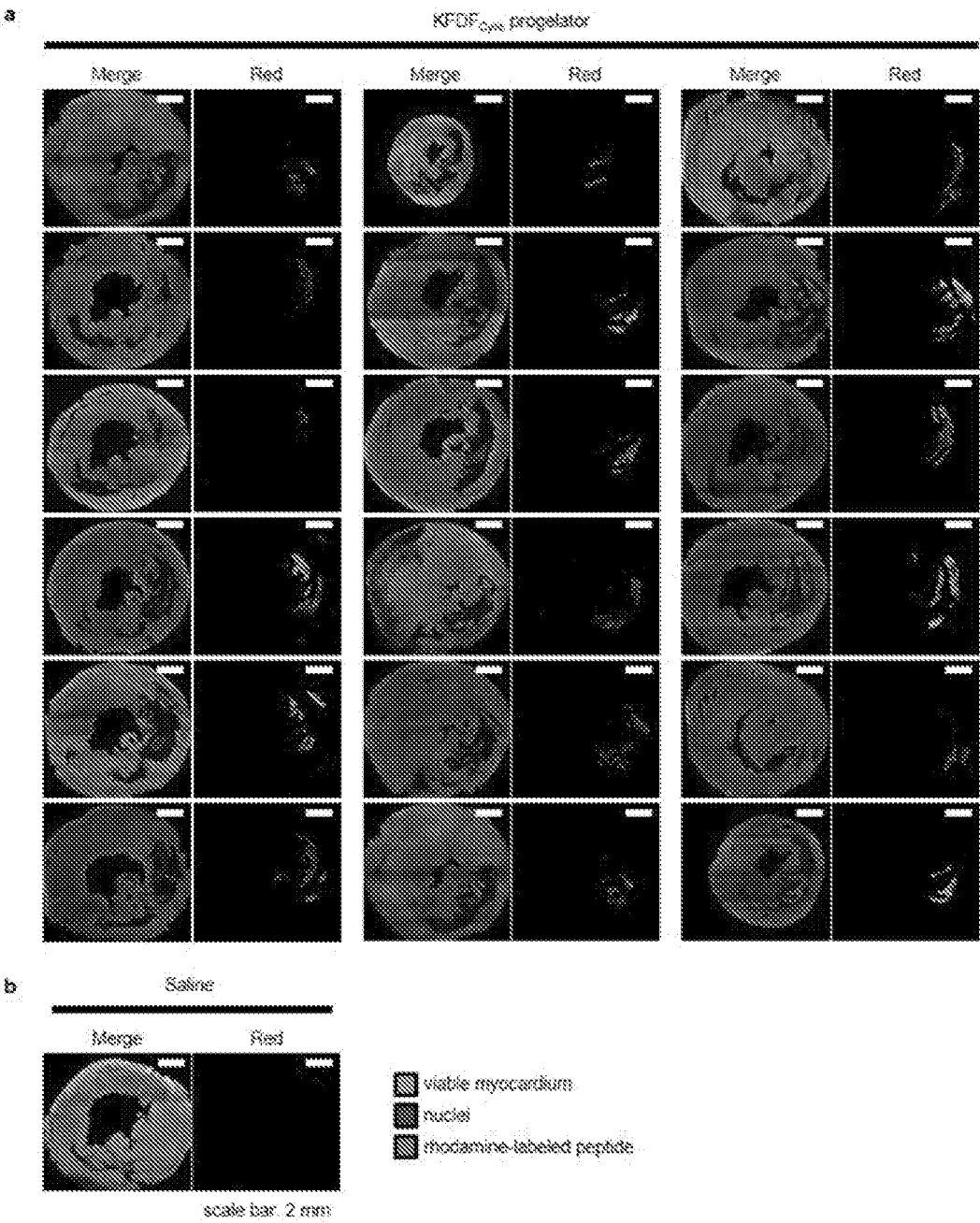


Fig. 25



Figs. 26a-26c



Figs. 27a-27b

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US18/36901

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A61K 38/00; A61L 27/52; C07K 7/08, 7/64 (2018.01)

CPC - A61K 38/00; A61L 27/52, 24/0031; C07K 7/08, 7/64

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

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

See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2016/159886 A1 (AGENCY FOR SCIENCE, TECHNOLOGY AND RESEARCH) October 6, 2016; figure 1; page 2, paragraph 4; page 8, paragraph 1; page 14, paragraph 4; page 15, page 1; page 17, paragraph 1; claims 35, 36, 49	1, 3-6, 8, 10-17
Y	US 2016/0220687 A1 (NATIONAL GUARD HEALTH AFFAIRS et al.) August 4, 2016; paragraph [0079]; claims 1, 7	1, 3-6, 8, 10-17
Y	US 2013/0338633 A1 (ABBOTT CARDIOVASCULAR SYSTEMS, INC) December 19, 2013; paragraphs [0028], [0044]	14-15
Y	US 2011/0165242 A1 (SU et al.) July 7, 2011; abstract; paragraphs [0016], [0063]	17
A	US 2015/0274789 A1 (NANYANG TECHNOLOGICAL UNIVERSITY OF SINGAPORE et al.) October 1, 2015; abstract; paragraphs [0060], [0137], [0155]	2, 9
A	US 2009/0060924 A1 (KORYTKO et al.) March 5, 2009; paragraph [0007]	2, 9
A	US 2015/0197359 A1 (3D MATRIX LTD) July 16, 2015; table 1	7, 18-20
A	US 2010/0016548 A1 (YOKOI et al.) January 21, 2010; table 8	7, 18-20

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

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14 August 2018 (14.08.2018)

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