Enzyme-responsive progelator cyclic peptides for minimally invasive delivery to the heart post-myocardial infarction

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Injectable biopolymer hydrogels have gained attention for use as scaffolds to promote cardiac function and prevent negative left ventricular (LV) remodeling post-myocardial infarction (MI). However, most hydrogels tested in preclinical studies are not candidates for minimally invasive catheter delivery due to excess material viscosity, rapid gelation times, and/or concerns regarding hemocompatibility and potential for embolism. We describe a platform technology for progelator materials formulated as sterically constrained cyclic peptides which flow freely for low resistance injection, and rapidly assemble into hydrogels when linearized by disease-associated enzymes. Their utility in vivo is demonstrated by their ability to flow through a syringe and gel at the site of MI in rat models. Additionally, synthetic functionalization enables these materials to flow through a cardiac injection catheter without clogging, without compromising hemocompatibility or cytotoxicity. These studies set the stage for the development of structurally dynamic biomaterials for therapeutic hydrogel delivery to the MI.

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Myocardial infarction (MI) accounts for 46% of all deaths attributed to cardiovascular disease. Within the first few days post MI, an inflammatory response causes cardiomyocyte death and degradation of the native extracellular matrix (ECM) by secreted matrix metalloproteinases (MMPs). 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.

Injectable hydrogel-based scaffolds have gained attention as a therapeutic approach to prevent negative LV remodeling by utilizing materials to stimulate cardiac repair. Materials for this purpose have included myocardial ECM, alginate, and hyaluronic acid hydrogels, or employing biomaterials as therapeutic delivery scaffolds such as naturally derived polymeric hydrogels (e.g., collagen, fibrin, heparin, and gelatin), synthetic polymeric hydrogels (e.g., poly(N-isopropylacrylamide) (PNIPAAm), ureido-pyrimidinone-modified poly(ethylene glycol) (PEG), and chitosan), and microparticles (e.g., poly(lactic-co-glycolic acid) (PLGA), and dextran). 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 identifier NCT02305602) and alginate (NCT0382508 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 bloodstream upon injection into a beating heart. One versatile class of materials that has been successfully tested in several preclinical MI models using surgical epicardial injections is self-assembling peptides (SAPs) that are attractive as they: (1) resemble native ECM, (2) require no additive reagents to induce gelation, (3) are biodegradable, (4) are biocompatible, (5) have 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 rehaeble, (8) do not suffer from batch-to-batch chemical variability, and (9) are amenable to sequence modification. However, SAPs have not demonstrated amenability to cardiac catheter delivery.

Previously, we demonstrated that soluble peptide–polymer-based nanoparticles can aggregate into macromolecular scaffolds in diseased tissue through the action of endogenously expressed inflammatory-related enzymes, MMPs, providing a viable method for targeted accumulation, prolonged tissue retention, and therapeutic delivery. However, these materials employed a non-biodegradable functionalized polyornithine backbone and did not possess viscoelastic properties. Inspired by recent efforts with enzyme-responsive peptides and a study by Nilsson and coworkers utilizing reductively triggered cyclic peptides as progelators, we focused on developing a cyclic, enzyme-triggered, responsive peptide platform that would enable minimally invasive delivery of SAPs to the heart. Specifically, MMP-2/9 and elastase, which are endogenously expressed at the site of MI during the acute inflammatory (days) and fibrotic (weeks) phases, provide a useful handle, playing key roles in the healing process through degradation of ECM and fibrogenin.

In this work, we engineer cyclic peptide progelators that flow freely in solution until proteolytically activated (Fig. 1). Model SAP sequences are prepared as water-soluble, dispersed cyclic progelators (green rings, Fig. 1a) that contain a substrate recognition sequence for MMP-2/9 and elastase (red) and a handle for fluorescent labeling. We show that enzymatic cleavage of these sterically constrained cyclic progelators results in linearization to generate SAPs (Fig. 1b) which subsequently assemble into rehaeble 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, we show that the progelators are amenable to minimally invasive catheter injection using an in vitro model system. This opens the possibility of delivering SAPs to the heart via catheter.

Results

Design of responsive SAPs for activatable gelation in vivo. SAPs undergo spontaneous assembly through electrostatic and amphiphilic interactions into ordered nanostructures. Many instances, variation of sequence and charge distribution in SAPs can influence properties such as secondary structure, fiber diameter, and bulk viscoelasticity. In our design, we began with a gellable core based on a model SAP consisting of the repeat sequence (KLDL), (referred to in this study as KLDL), which has been studied as a non-immunogenic, non-hemolytic, and anti-inflammatory scaffold for tissue engineering applications. This peptide self-assembly 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 sequence control, we chose to employ an analog of KLDL hypothesized to alter secondary structural assembly and achieve modified gel properties. For this purpose, we designed (KFD)3 (referred to as KFD), in which the incorporation of aromatic Phe residues introduce π–π interactions. From the KLDL and KFD SAPs, we engineered a small library of functionalized SAPs and progelators for studying assembly within a biological relevant environment (Fig. 2 and Supplementary Figs. 1–3). We first evaluated whether the addition of non-gelling amino acids from the MMP-2/9 recognition sequence (PLG)LAG and cysteine residues used for macrocytosis into the SAP sequences would interfere with their ability to self-assemble (Fig. 2a). Predictive computational modeling with open-access FibPred software provided initial evidence that functionalized KLDLLinear and KFDLinear 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 (Fig. 2b, c and Supplementary Tables 1–2). Notably in KFDLinear, 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 KFD SAP resulted in materials with increased mechanical strength without sacrificing re-healing capacity, inherent to the known KLDL system (Supplementary Fig. 4).

In practice, modified SAPs KLDDLLinear and KFDLinear retained the capacity to self-assemble (both experimentally and predicted) despite modest changes in secondary structure and viscoelastic properties (Fig. 3). Experimental circular dichroism (CD) demonstrated that KLDDLLinear and KFDLinear retained β-sheet conformations (n → π* transition at 215 nm). However, the positive peak at ~200 nm (π → π* transition) found in KLDL and KFD is blue-shifted to ~195 nm, indicative of a parallel to antiparallel orientation switch (Fig. 3a, b). Additionally, KFDLinear 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). This transition is predicted in theoretical spectra from DichroCalc for KFDLinear (202 nm) and not for KLDDLLinear, which contains no aromatic residues (Fig. 3c, d). Regardless of altered secondary structure, fiber morphology and bulk viscoelastic properties exhibited minimal changes to that of unmodified KLDL and KFD (Fig. 3e–h). Slight drops in storage moduli for...
KLDL and KFDF are attributed to the decreasing molar concentration of amino acids (hydrogels prepared with respect to weight at 15 mg mL\(^{-1}\)) contributing to \(\beta\)-sheet formation. Regardless, no significant disruptive effects to gelling capacity, as defined by tan\(\delta<1\) (Fig. 3g, h) or strain tolerance (Supplementary Fig. 5), 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.
Cyclization and progelator formulation. SAP steric constraint provides a simple and versatile engineering approach for preventing network assembly in a biological environment (Fig. 4). Functionalized SAP analogs containing an Fmoc protecting group or rhodamine dye on the N terminus were cyclized through oxidation of terminal cysteine residues to generate unlabelled and labeled progelators (Fig. 4a–e). Dilute solution phase macrocyclization was complete within 125 min by liquid chromatography–mass spectrometry (LCMS) (Fig. 4f, h). Combined, matrix-assisted laser desorption/ionization (MALDI), high-resolution mass spectrometry (HRMS), and tandem-mass spectrometry (MS) confirm synthesis of the cyclic products (Supplementary Fig. 6–8). Ultraviolet (UV) spectra confirm the presence of rhodamine absorbance in Rho-KLDLcyclic and Rho-KFDFCyclic progelators (Fig. 4g, i). Pure nonvicious progelators (Fig. 4j, k) were formulated with a pH switch from basic to neutral conditions into 1× Dulbecco’s phosphate-buffered saline (DPBS), pH 7.4 (10 mM peptide) to minimize aggregation, as determined by light scattering and transmission electron microscopy (TEM; Supplementary Figs. 9–11). Conformational rigidification via macrocyclization is suspected to induce limited oligomerization via intermolecular stacking of cyclic constructs into transient nanotubes (Supplementary Fig. 12), as has been reported in the literature.46,47 Despite this observed secondary structure, large assemblies were absent by TEM (Supplementary Fig. 10c). Thus, our formulated cyclic progelators persist as free-flowing solutions for easy injection in vivo.

Recognition of progelators by inflammatory enzymes. Labeled cyclic peptide progelators were tested for their responsiveness to inflammatory-related proteases overexpressed post MI (Fig. 5). The initial inflammatory response is marked by the recruitment of abundant neutrophils, which contribute to the release of proteases such as MMPs and elastase in the first days post MI.31,48 The fibrotic phase (weeks post MI) is notable for residual high MMP concentrations,2,31 and elevated sources of elastase from neutrophil extracellular traps, remaining weeks after neutrophil apoptosis.32,33

Rho-KLDLCyclic and Rho-KFDFCyclic were incubated with MMP-9 catalytic domain and porcine elastase to cleave at preferential cut sites (Fig. 5a, d). Enzymatic cleavage by active MMP-9 and elastase (Supplementary Fig. 13) induced visible aggregation in reaction vials and entangled fibrous meshes by TEM (Fig. 5b, e), whereas denatured enzymes elicited no response. 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 to assess general stability of our material to excess proteolysis and subsequent hydrogel disassembly in vivo.49 Despite the promiscuous activity of thermolysin, incubation with Rho-KLDLCyclic and Rho-KFDFCyclic induced fiber formation by TEM (Fig. 5b, e) and structural rearrangement by CD (Fig. 5c, f and Supplementary Fig. 14). Potential intramolecular30 and intermolecular31,52 steric hindrance from these labeled progelators might explain differences in the CD cleavage kinetics. The similar responsiveness of both unlabeled progelators to inflammatory enzymes and resistance to dissolution by thermolysin demonstrate the versatility of our general platform for conformational control.
**Progelators form viscoelastic and reahelable hydrogels.** The concept behind our cyclic progelator platform relies on upregulating enzymes to initiate assembly and ultimately viscoelastic gel formation at the site of MI. However, excess proteolysis in vivo from the ‘soup’ of proteases at the MI may prevent gelation or retention of hydrogels at the site of injury. To simulate this environment, we treated our material at 10 mM with piperidine in 20% DMF to create a soluble progelator. Route I uses N-terminal Fmoc-protected SAPs to improve material separation during chromatographic purification. Fmoc removal generates the intended unlabeled progelators KLDLCyclic and KFDFCyclic. Route II depicts the synthesis of labeled progelators through N-terminal modification with 5(6)-carboxytetramethyl rhodamine prior to cyclization.

Fig. 4 Facile synthesis of sterically constrained and labeled cyclic progelators. **a** Synthetic scheme for unlabeled (route I) and labeled (route II) KLDLCyclic and KFDFCyclic progelators. All self-assembling peptides (SAPs) were cyclized under dilute conditions (500 μM with 5 eq. I2 in a mixture of AcOH/MeOH/H2O to create a soluble progelator. Route I uses N-terminal Fmoc-protected SAPs to improve material separation during chromatographic purification. Fmoc removal generates the intended unlabeled progelators KLDLCyclic and KFDFCyclic. Route II depicts the synthesis of labeled progelators through N-terminal modification with 5(6)-carboxytetramethyl rhodamine prior to cyclization. **b** High-performance liquid chromatography (HPLC) monitored at 214 nm and corresponding electrospray ionization (ESI) spectra (c) of Fmoc-KLDLLinear (red), Fmoc-KLDLCyclic (blue), and KLDLLinear (green) (835.79 m/z, 781.862 m/z, and 1356.66 m/z, respectively) verify purity and mass of synthetic modifications to KLDL peptides. **d** HPLC (d) monitored at 214 nm and corresponding ESI spectra (e) of Fmoc-KFDFLinear (red), Fmoc-KFDFCyclic (blue), and KFDFCyclic (green) (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. **f** KLDLLinear SAP macrocyclization kinetics monitored by liquid chromatography-mass spectrometry (LCMS) at 214 nm reveal complete cyclization after 120 min. **g** Absorbance spectra of unlabeled (black) and labeled KLDL progelators (pink, λmax = 565 nm for rhodamine signal). **h** KFDFLinear SAP macrocyclization kinetics monitored by LCMS at 214 nm reveal complete cyclization after 120 min. **i** Absorbance spectra of unlabeled (black) and labeled KFDF progelators (pink, λmax = 565 nm for rhodamine signal). **j** Photographs of KLDLLinear precursor as a hydrogel (left) and the resulting KLDLCyclic progelator as a soluble solution (right). **k** Photographs of KLDLLinear precursor as a hydrogel (left) and the resulting KLDLCyclic progelator as a soluble solution (right).
of excess strain (100%) (Fig. 6b, c). Morphological changes from rheology samples before and after enzyme incubation are illustrated in Fig. 6d, e. Synthetic product analog SAPs in which the gelator sequence contained flanking substrate residues (KLDLControl and KFDFControl) were used for simple comparison with enzyme-induced gelation (Supplementary Figs. 15-16). Indeed, secondary assembly characteristics (CD), fibril morphology (TEM), and viscoelastic properties (rheology) of KFDFControl match closely to that of enzymatically cleaved KFDFCyclic in Fig. 6. We suspect that the slowed kinetics of gel formation for enzyme-treated KLDLCyclic vs KFDFCyclic is caused by lower specificity of thermolysin for Leu vs Phe at the P1 position. With hydrogel assembly following close behind, increased proteolytic resistance of tightly packed fibers and limited enzyme diffusion within the porous network likely has a restrictive effect on further progelator linearization and subsequent gel stiffening for all samples. Regardless, thermolysin cleavage of either progelator generates linear SAPs through the loss of Leu–Gly–Leu (Supplementary Fig. 17).

To understand the initial stages of self-assembly when progelators first encounter their target protease in vivo, slow step-wise cleavage of KFDFCyclic 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 204 nm, 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...

Fig. 5 Enzyme responsiveness of Rho-KLDLCyclic and Rho-KFDFCyclic progelators. a–c Analysis of Rho-KLDLCyclic responsiveness to matrix metalloproteinase-9 (MMP-9), elastase, and thermolysin. a Peptide sequence and theoretical enzymatic cuts sites. Gray arrow (not observed). b Photographs (top) of 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 transmission electron microscopy (TEM) images (bottom) of active enzyme cleavage products show fiber formation. Scale 100 nm. c Circular dichroism (CD) spectra of cleavage kinetics with thermolysin. Disappearance of signal at 204 nm (black arrow), corresponds to ring-opening. d–f Analysis of Rho-KFDFCyclic to responsiveness MMP-9, elastase, and thermolysin. d Peptide sequence and theoretical enzymatic cuts sites. Gray arrow (not observed). e Photographs (top) of 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. Scale 100 nm. f CD spectra of cleavage kinetics with thermolysin. Disappearance of signal at 204 nm (black arrow) corresponds to ring opening. CD of 500 µM progelator in 10 mM Tris buffer, pH 7.4. Enzyme cleavages performed at 500 µM progelator with 1:1000, 1:250, and 1:4500 enzyme/substrate molar ratio in 1× cleavage buffers (see Methods). Samples diluted to 100 µM for TEM.
Enzyme aromatic π-buffered saline (DPBS) with 1:4500 enzyme/substrate molar ratio. TEM of samples diluted to 100

reduction of progelator disulfide bond (Fig. 6f and Supplementary Fig. 18). By TEM, reduced peptides assemble as tangled fibrils, similarly to enzymatically cleaved peptides (Fig. 6g). Thus, the enzyme-induced gels observed in Fig. 6 are likely forming through the same self-assembly mechanisms predicted with KFDFControl. The rheological and spectroscopic results presented here indicate that our system has potential to tolerate excess proteolytic degradation in vivo.

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 requires that material should be able to reside within the catheter for up to hour-long procedures, yet still flow over multiple injections during this time, and finally only form a solid gel once it has entered the tissue17,55. Rapid gelation and/or high viscosity prohibit many injectable hydrogels from being considered for this minimally invasive delivery route. KLDLCyclic and KFDFCyclic progelators were amenable to cardiac catheter injection (Fig. 7). As shown in Fig. 7a, both unmodified SAPs and cyclic progelators exhibit shear-thinning behavior, but the viscosities for the latter are over 20× lower for both KLDLCyclic and KFDFCyclic. 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).

To mimic cardiac catheter injection in vivo, both cyclic progelators (2 mol% labeled for visualization) were flowed through the inner nitinol tubing of a 27 G MyoStar transendocardial injection catheter (Fig. 7c). No excess resistance was detected by manual operation following incubation in the catheter loop for 60 min for either peptide. Complex viscosity of cyclic progelators was independent of peptide concentration up to 10 mM (Supplementary Fig. 19). In contrast, unmodified KLDL 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 upon cyclic progelators during injection do not inhibit enzyme activation, peptides were injected through the catheter into a vial containing thermolysin and incubated for 3 h (37 °C), resulting in hydrogel formation (Fig. 7c and Supplementary Movie 1).

**Biocompatibility and injection in a rat MI model.** In vitro and in vivo tests were conducted to assess practical application using SAP cyclic progelators (Fig. 8). Cytotoxicity of both progelators (KLDLCyclic and KFDFCyclic) and linear SAPs (KLDLLinear and KFDFLinear) were studied with human cardiac progenitor cells (CPCs) (Fig. 8a, b). Exposure at high concentration (10 mM) for 4 h showed no difference in toxicity for all peptides (Fig. 8a). Exposure at 24 and 48 h showed an increase in metabolic activity for all peptides at each concentration and timepoint comparable to growth media only (Fig. 8b). This suggests that our various peptide modifications were not only cytocompatible but also did not influence cell metabolism and proliferation. We next examined the KFDFCyclic progelator in hemocompatibility studies and assessed in vivo gelation (Fig. 8c-i). Biomaterial leakage is a concern with transendocardial injection since injections are performed into a beating heart56. 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 protein concentrations in blood. Activated clotting times (ACT) were evaluated in the presence of increasing cyclic peptide progelator concentrations in blood. Activated clotting times (ACT) were used as a standard method57 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. 8c and Supplementary 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. 8d and Supplementary Fig. 21). In contrast, collagen and glass controls increased the clot rate.

RBC hemolysis in the presence of progelator was used to assess acute toxicity (Fig. 8e and Supplementary Table 6). Doses up to 1:10 revealed <5% hemolysis, which is below the limit for
consideration as a hemolytic biomaterial. Finally, a prothrombotic assay using platelet-poor plasma (PPP) was used to monitor non-platelet and phospholipid-specific effects on blood (Fig. 8f), 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. No changes to coagulation rate (slope), half-maximal coagulation time, and extent of coagulation (Supplementary Figs. 22-23) were observed in samples incubated with progelator up to doses of 1:100 peptide/blood.

With hemocompatibility verified, we applied the progelator in an initial proof-of-concept study in vivo. Enzyme-responsive progelator, KFDF\textsubscript{Cyclic}, doped with a small amount of Rho-KFDF\textsubscript{Cyclic} (5 mol\%) for imaging, was injected into the infarct region 7 days post MI in a rat ischemia–reperfusion model (Fig. 8g). Assessment of hearts at 24 h post injection confirmed that progelator effectively gelled at the heart (Supplementary Figs. 22-23). Histological and fluorescence analysis of heart...
sections revealed hydrogel assembly occurred at the site of MI in all rats (Fig. 8h, i). No differences were observed in cardiomyocyte apoptosis or macrophage infiltration (Supplementary Figs. 24–25) between saline- and peptide-injected infarcts at 24 h post injection, suggesting that the activation and subsequent gelation of enzyme-responsive progelators did not increase the infarct inflammatory response.

Discussion

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. In this study, we examined a platform modification strategy to enable one promising class of hydrogels, SAPs, to be injectable via a cardiac injection catheter by conformationally constraining linear SAPs into macrocyclic progelators which resist self-assembly (Fig. 1). These progelators were further modified with a substrate recognition sequence for endogenously expressed MI-associated proteases. We took the approach of designing 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 (Fig. 2). 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 sequence modifications did not interfere with self-assembly as β-sheets into fibrils and viscoelastic hydrogels (Fig. 3). It is hypothesized that any number of other SAP sequences used in biomedical applications could similarly be adapted to our structurally dynamic platform.9,60

The versatility of our 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 Fig. 4 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 (Fig. 5). 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 (Fig. 6).

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.61 With our design, mechanical differences (e.g., stiffness or healing kinetics) in the resulting hydrogel would not affect the initial progelator formulation. Importantly, we provide a demonstration of low-viscosity peptide-based progelators for use in a cardiac injection catheter with little resistance to flow (Fig. 7). The results of these experiments demonstrate that the cyclic progelator acts as a non-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. While two types of materials have been delivered via catheter to the heart in clinical trials, namely alginate and a myocardial ECM hydrogel, our approach could have some unique advantages since it is a synthetic system that can be precisely tuned, is well defined, and has limited batch-to-batch variation.

With the potential for tissue leakage into the bloodstream, thrombogenicity and toxicity of transendocardially injected biomaterials is a concern. Synthetic modifications to the SAPs were shown to imbue no cytotoxicity to human CPCs and were hemocompatible and non-thrombogenic through a comprehensive assessment of whole blood clotting times, hemostatic kinetics, RBC hemolysis, and pro-thrombotic assays (Fig. 8). A dosing analysis shows no statistically significant difference in hemostasis due to increasing progelator concentration in whole blood. The extent of coagulation in PPP was only altered at the highest peptide dose which indicates that local high concentrations of peptide have an minor anti-coagulative effect on the intrinsic coagulation (contact-based) pathway; furthermore, the impact that our peptide dose has on coagulation was shown to be independent of platelet-dependent thrombus formation, a key player in the pathogenesis of acute MI.62 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.63 Given a standard clinical concentration of 1:10,000, which assumes that no peptide was injected into the bloodstream, all materials injected into the bloodstream, we observed no adverse effects 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. Our platform enables facile delivery of a free-flowing progelator, and on-demand gelation at the site of inflammation (Fig. 8). In vivo analysis of KFD0Cyclic progelator revealed successful gelation of activated hydrogel scaffolds in a rat MI model, without inducing an inflammatory response or cardiomyocyte apoptosis. As such, we have demonstrated a simplistic strategy that uses the naturally complex 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 our progelator system for cardiac injection in vivo. Additionally, there exists the potential to use this platform in conjunction with various therapeutics. Herein, we observed that covalent conjugation of a rhodamine dye to cyclic progelators did not disrupt gelation. We envision 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., cell therapies or growth factors), injecting a mixture with the cyclic progelators and subsequent encapsulation within locally activated networks might provide a means for targeted delivery without the need for complex synthetic chemistries. Ultimately, our simple strategy for structural control of self-assembling peptides could be employed for minimally invasive delivery of promising therapeutic peptides to other forms of injury or disease where MMPs are upregulated, including osteoarthritis,64 cartilage tissue repair,61 nerve damage,65 and acute brain injury.66

Methods

Peptide synthesis. Peptides were synthesized in an AAPPTec Focus XC 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. HFTRU (N,N,N’,N’-tetramethyl-O-(1H-benzo[d][1,2]oxazine-5(4H)-one hexafluorophosphate) was used as the general coupling agent. When included, 5(6)-carboxytetramethyl rhodamine was incorporated during the synthesis at the N terminus. General peptide cleavage and deprotection was performed in 95:2.5:2.5 (v/v) tri-fluoroacetic acid (TFA), triisopropyl silane, and H2O, respectively, for 2 h. Cleaved peptides were precipitated in cold anhydrous ether (3×) to yield solid crude. Semi-protected peptides containing Cys(acm) were purified prior to cyclization. Fmoc was temporarily used for unlabeled progelators to improve peak separation during purification and to monitor cyclization by high-performance liquid chromatography (HPLC) and electrospray ionization (ESI; Fig. 4b–e). To synthesize cyclic progelators, iodine was used to simultaneously deprotect Cys(acm) and initiate disulfide bond formation under dilute conditions to favor intramolecular macrocyclization. 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 s). The reaction was vigorously stirred at room temperature for 2 h and reaction completion was confirmed by LCMS. After 2 h of reaction, Amberlite IRA-400 Resin (chloride form) was stirred in the solution for 1 h to quench excess iodine and absorb reacted iodide ions. Filtrated 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 (Fig. 4f, h), the N-terminal Fmoc was temporarily left on unlabeled peptides for improved peak separation during purification.

Peptide purification and analysis. Analytical scale RP-HPLC analysis of peptides was performed on a Jupiter Proteo90A Phenomenex column (150 x 4.6 mm) using a Hitachi Elite Lachrom L2130 pump with ultraviolet–visible (UV–Vis) detector (Hitachi-Elite LaChrom L-2420) monitoring at 214 nm, 256 nm, 290 nm, and 365 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 Armenian Glide 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 prepared for purification. For 5:25:70 acetonitrileBuffer A/Buffer B via initial dialysis in 5% acetic acid with sonication, followed by addition of acetonitrile and 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 preparative phase HPLC, respectively. Following purification, product was analyzed by ESI to verify identity (see Supplementary Fig. 3). Due to the large number of cis/trans configurations isomers that our unlabeled progelators could adopt, 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 isolated materials.

UV absorbance spectra. UV absorbance spectra of peptide labeling were measured on a ThermoScientific Nanodrop 2000c.

Photographs of samples and illustrations. Photographs of vial flaps, samples, and experimental setups were taken with an Android Samsung S7. Adapted clipart from Servier Medical Art is licensed under a Creative Commons Attribution 3.0 Unported License.

Progelator sterilization and formulation for in vivo analysis. HPLC purified peptides were dialyzed with 1 kDa molecular weight cutoff tubing into milliQ H₂O, sterile filtered through a 0.2 µm PES filter, and lyophilized to a powder. Peptide was reconstituted prior to injection with sterile pH 9 H₂O to form a clear solution (111 mM), then diluted further with sterile 10× DPBS to a final concentration of 10 mM peptide in 1× DPBS (pH 7.4). Solutions for in vivo experiments contained 5 mol % rhodamine-labeled progelator (0.5 mM labeled + 9.5 mM unlabeled) for fluorescence imaging purposes.

Transmission electron microscopy. Formvar/Carbon-coated 400 mesh Cu grids (Ted Pella, Inc.) were glow discharged for 90 s and spotted with 4–5 µL peptide sample (100 µM) 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.

Experimental CD. Peptides were dissolved at a final concentration of 125 mM (labeled peptides) or 500 mM (unlabeled peptides) in 10 mM Tris buffer or HClO (for TCEP reduction only) at pH 7.4 to reduce signal interference seen with DPBS at lower wavelengths. UV–Vis CD was measured on a Jasco J-810 Spectro-polarimeter to evaluate the secondary structure of peptide samples. Measurements were taken using the following conditions: scan range was 190–260 nm, scanning speed was 50 nm min⁻¹, response time was 2 s, data pitch was 1 nm, bandwidth was 1 nm, accumulations = 3, pathlength = 1 mm, and temperature = 37 °C. Spectra are presented as an average of all accumulations. Sample volume 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 [θ] using the following equation:

\[ [\theta] = \text{mdeg} \times \frac{M}{C \times l \times 10} \]

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⁻¹, and L is the cell pathlength in cm.

Computational modeling with FibPredictor. FibPredictor was utilized as a commercially available software for generating native-like amyloid fibril structures. 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 prediction and statistical scoring functions with 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 SCWR4 to predict the energetically lowest side-chain orientations. This conformational energy function was used to calculate the mini total energy of the entire model and output coordinates for the structure as a PDB file (see Supplementary Data) 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 Amber (computer) and computational methods (more efficient) and GOAP (more accurate) statistical scoring functions were used to calculate total energies of the protein structure. Reported energies are unisland as they are empirically derived scores, and thus it is not advisable to interpret results as kcal mol⁻¹. Normalized GOAP scores are useful for comparing fibrils of different sizes. Three generations of modeling were performed with literature-recommended parameters shown in Supplementary Table 1. Results of top model for each peptide sequence from the third-generation analysis are reported in Supplementary Table 2. Corresponding structures and raw PDB files are provided in the main text Fig. 2 and as Supplementary Data. Output PDB files, containing the atomic coordinates of the chromophores, from top models were used to generate theoretical CD spectra with DichroCalc.

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. These parameters include the peptide chromosome in the far-UV, charge-transfer between neighboring peptide groups and aromatic interaction, and aromatic interaction in chromating with the molar in the near-UV. Full analysis CD spectra were output in units of molar ellipticity [θ] vs wavelength.

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 1× enzyme cleavage buffers. MMP-9 cleavages were performed at 1:1000 enzyme/substrate molar ratio in 1× buffer (50 mM Tris-HCl, 200 mM NaCl, 5 mM CaCl₂, 1 mM ZnCl₂, pH 7.5) for 5 h. Elastase cleavages were performed at 1:250 enzyme/substrate molar ratio in 1× buffer (100 mM Tris-HCl, 0.2 mM Na₂SO₄, pH 8.0) and thermolysin (50 mM Tris-HCl, 0.5 mM CaCl₂) for 5 h. Thermolysin cleavages were performed at 1:4500 enzyme/substrate molar ratio in 1× buffer (1× DPBS, pH 7.4) for 15 min. Analysis of unlabeled cyclic peptide progelator cleavage by CD (Fig. 6f) was conducted with serial additions of thermolysin (4 × 180,000 enzyme/substrate) over a period of 7 h in 10 mM Tris buffer. Control samples utilized denatured enzymes under the same conditions. MMP and elastase were heat denatured with 10 min of incubation at 65 °C. Thermolysin was inactivated by incubation with 10% (v/v) EDTA (0.5 M, pH 8.0).

Rheological characterization. All peptide solutions were prepared at 10 mM peptide, except for those used to assess the effect of SAP functionalization with respect to weight (15 mg mL⁻¹). 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 and time course measurements) 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, strain sweeps were performed between 0.05 and 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 0.1–100 rad s⁻¹ at a strain of 0%. 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). 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 0.1–10 s⁻¹ (5% tolerance). For time course measurements of enzyme activation, a stock of cyclic peptide (1 mM, 10 mL, 1× DPBS, pH 7.4) was prepared and incubated at 37 °C. A sample from this stock was applied to the rheometer for storage and loss moduli measurements (2.5 rad s⁻¹, 0.5% strain) over a period of 35–40 min period. This sample was removed and replaced with an identical sample mixed via pipetting for 5 s with thermolysin (1:4500 enz/peptide) followed by immediate addition to the rheometer. Delay time between enzyme addition and rheology measurements was ~30 s.

In vitro catheter injections and effect on assembly. Peptide solutions (0.6–0.8 mL) were prepared at 10 mM in 1× 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⁻¹. Peptide
was injected through the 27 G inner nitinol tubing of a MyoStar catheter that was immersed in a 37°C water bath.

**Hemocompatibility analysis.** For all measurements, peptide stocks in 1x DPBS were prepared such that the final concentrations (v/v) in blood or plasma were 1:10, 1:100, 1:500, 1:1000, 5:1000, and 10,000, and 1:20,000 (peptide volume) were through incubation at 37°C immediately prior to use. Plate reader measurements were conducted on a Microplate Reader with 96-well tissue culture plates (TCP). All blood studies were done in compliance with the Northwestern University Bloodborne Pathogen Exposure Control Plan for human whole blood. Citrate (BSC, LS 358A), was handled under US FDA registration #2577632 and pre-screened for viral contaminants (Title 21 CFR PART 610.40).

**ACT with whole human blood.** A Hemochron 801 instrument calibrated with an electronic system verification (ESV) device was used to measure ACT of whole human blood. ACTs were determined using recalcified citrated whole human blood to minimize variability in starting time points for clotting in all assays. Each Hemochron P214 tube with glass beads was added 4 mL 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 for 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⁻¹) was used as a positive control to decrease clotting time. Vehicle (1x DPBS) served as a positive control for blood without additive. Samples without calcium, serving as the negative control, exceeded instrument maximum time range (>1500 s). Each experiment was performed n = 6 times with averages and standard error of the mean (SEM) plotted.

**Whole blood hemostasis kinetics.** Changes to time-dependent hemostasis were monitored using a non-activated whole blood clotting assay. Briefly, 100 µL of citrated whole human blood 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⁻¹) 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 room temperature for 5, 15, 30, and 45 min. Each sample was prepared in triplicate. At the end of every timepoint, 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. Release of hemoglobin from lysis was detected by measuring the 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 SEM are plotted.

**Hemolysis assay.** RBCs were isolated by centrifugation of 40 mL citrated whole human blood at 500 × g for 5 min, followed by gentle washes with 150 mM NaCl (×1) and 1x DPBS (×3). Isolated RBCs were diluted 1:50 to 1x DPBS (pH 7.4) and lightly agitated to prevent settling. Briefly, 190 µL of dilute RBCs and 10 µL of peptide stock or additive (50× final concentration) were mixed and added to a 96-well plate (n = 4 repeats), covered, and incubated at 37°C for 1 h. Plates were centrifuged at 500 × g to pellet intact RBCs using a centrifuge equipped with a microplate rotor. Supernatant (100 µL) was transferred to a new 96-well plate, taking care not to disturb the pellet. Absorbance of the supernatant was measured at 540 nm to detect released hemoglobin. To calculate % hemolysis, absorbances were corrected for background absorbance from untreated vehicle and then normalized to 1% Triton X-100-treated RBCs to represent 100% hemolysis. % Hemolysis was calculated according to the following equation:

\[
\%\text{Hemolysis} = \frac{(A_{\text{Experimental}} - A_{\text{Negative control}})}{(A_{\text{Positive control}} - A_{\text{Negative control}})} \times 100, \tag{2}
\]

where A_{\text{Experimental}} is the average well absorbance pertaining to RBCs pertaining to the same sample as that being analyzed. A_{\text{Negative control}} is the average well absorbance for RBCs incubated with vehicle (1x DPBS), and A_{\text{Positive control}} is the average well absorbance for RBCs containing 1% Triton X-100.

**Pro-thrombotic assays.** PPP was isolated from citrated whole human blood through two rounds of centrifugation at 2000 × g for 10 min and collection of the upper two-third layer. Briefly, 100 µL of PPP, 50 µL of peptide stock or additive (4× final concentration), and 50 µL of CaCl₂ (50 mM) were mixed and added to a 96-well plate and incubated at 37°C for kinetics measurements. For each sample, n = 6 repeats were conducted. Coagulation profiles were obtained by measuring well absorbance at 405 nm at 30 s intervals for 60 min. Onset to clotting was detected as a sharp increase in sample turbidity.

**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 (UCSD), and the Association for the Assessment and Accreditation of Laboratory Animal Care and approved by the Institutional Animal Care and Use Committee at the UCSF (A3033-01). Female Sprague-Dawley rats (225–250 g) were used in all studies.

**Intramyocardial injections of peptide into infarcted rats.** MI was performed via 35 min ischemia–reperfusion and intramyocardial injections were performed under anesthesia. Briefly, cyclic peptide (75 µL, 0.75 μmol, 10 min) 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⁻¹) at 24 h post injection (n = 5).

**Cryosectioning, 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 identify the infarct region or stained for immunofluorescence analysis. H&E slides were analyzed on an Aperio ScanScope CS at ×20 magnification. For immunofluorescence, tissue sections were permeabilized in acetone (−20°C) for 1.5 min and blocked with 2% bovine serum albumin and 0.3% Triton X-100 in 1x PBS. Myocardium was labeled using mouse anti-rat α-actinin antibody (1:800 dilution in blocking buffer, 1 h of incubation, Sigma A7811) and an Alexa Fluor 488 goat-anti-mouse IgG secondary antibody (1:500 dilution in blocking buffer, 30 min of incubation, Thermo Fisher A-11001). Nuclei were labeled using Hoechst 33422 (1:10,000 dilution in deionized water, 10 min of incubation, Life Technologies). Tissue sections were mounted with Fluoromount (Sigma) and imaged using a Leica Arioi slide scanner with Arioi software at ×20 magnification.

**Human cardiac progenitor cell culture.** Human fetal CPCs were isolated from tissue harvested under standard informed consent procedures and prior approval of the institutional ethics committee of the University Medical Center Freiburg. All cell studies were done in compliance with the UC San Diego Human Research Protections Committee. CPCs were isolated from human fetal hearts for selection with Sca-1⁺ magnetic bead sorting (Miltenyi Biotech, 130-091-176) and cultured on 1% gelatin in growth media. Growth media consists of 25% EGM-2 (Cambrex, CC-3156) supplemented with EGM-2 Single Quots (Cambrex, CC-4176), 10% fetal bovine serum, 1x penicillin/streptomycin (Sigma, P4458), and 1x minimum essential medium (MEM) non-essential amino acids (BioWhittaker, BE11-114E) in media. Cells at passages 3–5 after isolation were tested for mycoplasma, showing no contamination. Cells at passages 17–21 were used for all experiments.

**Cytotoxicity assays.** Material acute cytotoxicity was tested using human CPCs. The cells were stained with calcine-AM cell viability dye (25 nm; ebioScience) for 30 min and 500,000 cells were resuspended in 50 µL of each peptide (10 mM). After 15 min, 1 mL of growth media was added to the peptide-cell mixture and 50 µL was transferred into a 24-well plate for imaging. Five to six images were taken per condition using a fluorescence microscope (Carl Zeiss, Dublin, CA, USA). Cells resuspended in media only were used as a control. The percentage of viable cells was determined by counting the stained cells over the total number of cells. Three experiments were performed (n = 8–12 repeats in duplicate). Alternatively, CPCs were also cultured up to 4 days with or without peptides, and cytotoxicity was evaluated by an Alamar blue metabolic activity assay. In all, 3500 hCPCs were plated into each well of a 96-well plate in growth media. At 24 h after cell seeding, baseline metabolic activity was taken by incubating the cells in Alamar blue (1:10 in medium, Invitrogen) for 3 h and analyzing at 550/585 nm with a Synergy 4 Multi-Mode Microplate Reader (Biotek). Peptide was added to cells and cultured for 4 days. Alamar blue assay was repeated after 24 and 96 h, and growth media was also used as a control (n = 3 repeats in quadruplicate). Metabolic activity reported as fold increase vs baseline values.

**Statistical analysis.** All statistical results are expressed as mean ± SEM. Ordinary one-way analysis of variance (ANOVA) tests were used for multiple comparisons of the mean in each group with that of the standard. Tukey’s 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 ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, and ****p ≤ 0.0001.

**Reporting summary.** Further information on experimental design is available in the Nature Research Reporting Summary linked to this article.
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Author contributions

A.S.C., N.C.G., and K.L.C. designed the research and analyzed the data. A.S.C. performed synthetic, computational, biochemical, and animal experiments, and drafted the paper. R. L.B. and C.L. performed direct intramyocardial injections and myocardial infarction surgeries, respectively. R.G. performed cell viability assays and toxicity staining. N.C.G. and K.L.C. provided financial support. K.L.C. and N.C.G. directed the research. All authors edited the manuscript.

Additional information

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Competing interests: K.L.C. is co-founder, board member, consultant, and holds equity interest in Ventrix, Inc. The other authors declare no competing interests.

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