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A tough endothelium-like dressing for vascular stents

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Vascular stent is viewed as one of the greatest advancements in interventional cardiology. However, current approved stents suffer from in-stent restenosis associated with neointimal hyperplasia or stent thrombosis. To address this issue, we developed an endothelium-like (EL) dressing for vascular stents inspired by the importance and biological functions of native endothelium for cardiovascular system. Our EL dressing is based on a *de novo* designed hydrogel that is mechanically tough and could preserve integrity on stents during angioplasty. Due to its physiochemical similarities to subendothelial extracellular matrix, the EL dressing facilitated the adhesion and growth of endothelial cells. Besides, it is non-thrombotic and capable of inhibiting smooth muscle cells thanks to the capacity to catalyze nitric oxide generation. Transcriptome analysis further unraveled the EL dressing could modulate the inflammatory response and induce the relaxation of smooth muscle cells, while potentially promoting angiogenesis by stimulating the expression of angiogenic factors. *In vivo* study demonstrated vascular stents encapsulated by it promoted rapid restoration of native endothelium and persistently suppressed in-stent restenosis in both leporine and swine models. We expect such EL dressing will open a new avenue to the surface engineering of vascular implants for better clinical outcomes.

Vascular stent, which is implanted into a narrowed blood vessel through guided balloon dilation, is regarded as the most effective means for treating coronary artery disease¹. Since its introduction in 1980s, vascular stent has been widely employed in interventional cardiology. Compared to the earlier plain balloon angioplasty, the use of first-generation bare-metal stents (BMSs) has already presented remarkable benefits in terms of less acute vessel closure and constrictive remodeling². Despite these advantages, the drawbacks of BMSs were soon reported, including acute inflammation elicited by foreign-body reaction and in-stent restenosis (ISR) induced by neointimal hyperplasia (NIH)³. As an alternative, drug-eluting stents (DESs) with a polymer coating carrying anti-cell proliferative drugs were developed and became the standard of care in percutaneous coronary intervention (PCI)⁴. Although DES has successfully alleviated inflammation and dramatically reduced the rate of early ISR, the released drugs also suppress endothelial cells, thereby increasing the risk of late NIH and stent thrombosis due to impaired endothelialization⁵.

To address these complications associated with vascular stent, it is advisable to learn from nature. The inner lining of blood vessel is a monolayer of tightly connected endothelial cells called as endothelium⁶. Native endothelium is covered by a highly hydrated layer of glycocalyx that can lubricate it and reduce its interaction with blood components⁷. In addition, it generates versatile biomolecules such as nitric oxide (NO), prostacyclin, thrombomodulin, heparin-like molecules, tissue factor pathway inhibitors and tissue plasminogen activators⁸. These molecules play important roles in normal endothelial function, including prevention of thrombosis, regulation of vasomotion, promotion of endothelial regeneration, and modulation of inflammatory response⁸. As a result, native endothelium is the best antithrombotic material in nature, which maintains the patency of blood vessel.

With the knowledge in native endothelium, we envisaged an endothelium-mimetic coating might solve the issue of ISR for vascular stents. Such coating should be capable of preventing thrombosis, inhibiting smooth muscles, and providing a microniche favored by endothelial cells so that native endothelium could rapidly form to replace it. To achieve this goal, hydrogels seem to be the best

candidate compared to direct surface engineering, polymeric coatings and ceramic films because of several reasons. First, they are three-dimensionally (3D) crosslinked aqueous materials with the best resemblance to native tissues^{9,10}. Second, they allow for versatile physical and chemical modifications for special purposes. Last but not least, as bulk materials, they can be tailored as highly efficient carriers for therapeutics. In fact, many hydrogels have been exploited as carriers of drugs^{11,12}, biomolecules^{13,14} or cells¹⁵⁻¹⁷ for various applications. However, developing a hydrogel coating for vascular stents is challenging because it must be biocompatible, endothelial cell-adhesive, convenient for handling and mechanically strong to withstand balloon dilation during angioplasty. Unfortunately, few of the hydrogels reported so far met all these requirements.

Herein, we developed an endothelium-like (EL) dressing for vascular stents using a *de novo* designed hydrogel. This hydrogel is primarily composed of alginate and gelatin, which are analogs to hyaluronic acid and collagen in extracellular matrix (ECM)¹⁸. Such combination enables it to resemble the subendothelial ECM that is favorable for endothelial cells. By tuning the proportions of these two biopolymers and the interaction between them, it can become mechanically tough. We endowed endothelial function to it by conjugating an organoselenium species to alginate, which is capable of persistently catalyzing the generation of NO that participates in nearly all important biological processes of native endothelium¹⁹. We call this hydrogel-based coating an EL dressing because of its high resemblance to native endothelium. Such EL dressing was expected to withstand the balloon dilation during angioplasty, prevent thrombosis, promote rapid restoration of native endothelium, and effectively suppress ISR.

Design, synthesis and optimization of the hydrogel

To generate a uniform hydrogel coating on vascular stents, a good strategy is implementing *in situ* gelation. One possible approach to achieving this is dip-coating the stents with the hydrogel precursor solution that cures subsequently. At first glance, it seems the sol-gel transition of gelatin in the solution

can be utilized for that purpose, which liquefies when the temperature is above the melting point (T_m) of gelatin and solidifies after cooling. However, the T_m of gelatin ($\sim 30\text{ }^{\circ}\text{C}$) is much lower than that of our body temperature ($37\text{ }^{\circ}\text{C}$)²⁰, which suggests chemical crosslinking needs to be introduced in the hydrogel. The reaction of such chemical crosslinking must be cytocompatible and mild. Considering cytocompatibility, Michael-addition reaction is one of the best crosslinking methods as it produces no byproducts²¹. However, conventional thiol-maleimide addition proceeds too fast at physiological conditions²², which is inconvenient for our application. In our previous work, we reported Michael-addition reaction between maleimide and amine could be leveraged for crosslinking a hydrogel^{23,24}. Compared to thiol-maleimide addition, the maleimide-amine addition is milder, allowing for more maneuverability during the preparation of hydrogel. Based on our findings, we envisioned that a hybrid hydrogel formed by the crosslinking between maleimide-modified alginate (alginate maleimide, A-M for short) and gelatin could be tailored as the EL dressing for vascular stents (Fig. 1a).

Fig. 1 | Development of a tough EL dressing for vascular stents using a *de novo* designed hydrogel. **a**, Schematic for the design of our EL dressing. **b**, Shear moduli (at 37 °C) of the hydrogels formulated with A-M of varying degrees of maleimidyl modification (DMM) and gelatin at different mass ratios. **c**, Comparison in shear modulus among the selected hydrogels at 25 °C and 37 °C ($n = 6$). **d**, Tensile testing of them at ambient temperature. The insets on the right exhibit the photographs of A-M(9.3)/G_4/6 hydrogel prior to and during extension. (Scale bar: 1 cm) **e**, Radar charts showing their scores in shear modulus (at 37 °C) (A), fracture strength (B), fracture strain (C), toughness (D), and capacity for further modification (E). Dashed frameworks represent the average values. **f**, Photographs of a vascular stent coated with A-M(9.3)/G_4/6 hydrogel before (left) and after (right) balloon dilation in PBS at 37 °C. **g**, Fluorescence images of it before (left) and after (right) balloon dilation. The hydrogel coating was labeled with FITC.

A-M was synthesized by the coupling reaction between pristine alginate and N-(2-aminoethyl)maleimide trifluoroacetate (AEM.TFA) via aqueous carbodiimide chemistry (Supplementary Fig. 1). By tuning the feed ratio between alginate and AEM.TFA, A-Ms with varying degrees of maleimidyl modification (DMM) were obtained. In our nomenclature, A-M(*x*) indicates that *x*% structural units in alginate are coupled by maleimide. In total, three variants of A-M were prepared, including A-M(4.8), A-M(9.3), and A-M(15.9), as characterized by proton nuclear magnetic resonance (¹H-NMR, Supplementary Fig. 2). In a preliminary trial to explore the gelation process, we mixed the precursor solutions (10 w/v%, pH~7.5) of A-M(9.3) and gelatin at different mass ratios and cured them at 37 °C. The cure kinetics of them were investigated by measuring the changes of their shear moduli at 37 °C after cure for different durations. As shown in Supplementary Fig. 3, although these hydrogels with varying formulations had different stiffness, all of them exhibited similar cure kinetics and reached full mechanical strength within 36 h. Among them, A-M(9.3)/G_4/6 was the stiffest hydrogel with a shear modulus of 732 Pa at 37 °C after cure for 72 h. However, we considered even this hydrogel was not strong enough to be applied as a coating material. To further enhance their strength, we increased the mass concentration of the precursor solutions. In the beginning, we tried to prepare A-M solutions of 20 w/v%, but we failed because A-Ms were not fully soluble in water at such high concentration. As a tradeoff, we ended up with using precursor solutions of 15 w/v%. At this mass concentration, both alginate, A-Ms and gelatin can be readily manipulated. At this time, pristine alginate and all three variants of A-M were tested together and a prolonged gelation time (72 h) was assumed to ensure all hydrogels were fully cured.

As anticipated, pure gelatin was unable to maintain solid state and pristine alginate could not form a hybrid hydrogel with it at 37 °C (Fig. 1b). Nevertheless, with the use of A-M, hybrid hydrogels were successfully generated at 37 °C. Notably, gelation only occurred when the proportions of A-M and gelatin were in proper ranges. A-M with a higher DMM tends to give rise to gelatin in a broader range and stiffer hydrogels. In addition, the higher the DMM of A-M is, the more gelatin is required to

achieve an optimum crosslinking density. Subsequently, we selected the hydrogels with shear moduli beyond 1.0 kPa at 37 °C (encompassed by the dashed framework in Fig. 1b) for further investigation. Additional dynamic mechanical test (Fig. 1c) suggests that the hydrogels are much stronger at ambient temperature (~25 °C), which is in accordance with our intuition as gelatin itself forms a physical hydrogel below its T_m . The ratios of shear moduli at 37 °C and 25 °C ($G_{37\text{ °C}}/G_{25\text{ °C}}$) lie between 0.19 and 0.48, and A-M/G hydrogel with a higher content of A-M is consistently more refractory to softening at 37 °C given the DMM of A-M is constant.

To further unravel the mechanical behaviors of the hydrogels, tensile testing was conducted. The stress-strain curves (Fig. 1d) of them are diverse that some hydrogels are weak, while others are strong and can tolerate high strain (Supplementary Video 1). Nonetheless, stiffening at higher strain was common in all groups. Quantitative assessment (Supplementary Fig. 4) indicated that the Young's modulus, fracture strength, fracture strain and toughness of them were in the ranges of [12.4, 27.3] kPa, [10.4, 60.7] kPa, [84.3, 271.4]% and [4.2, 69.2] kJ m⁻³, respectively. For comparison, a comprehensive scoring system concerning the mechanical properties of the hydrogels and their capacity for further modification was established (Fig. 1e). Among them, A-M(9.3)/G_4/6 demonstrated the best overall performance with full scores in fracture strength, fracture strain and toughness. In particular, this hydrogel was remarkably strong and flexible to be consecutively bent, twisted and knotted without damage at ambient temperature (Supplementary Fig. 5). Consequently, we selected it as the base material to make our EL dressing on vascular stents. BMSs of 316L stainless steel were assumed as the platform for our initial attempt. Since stainless steel contains no amino groups, a film of poly(dopamine-co-hexanediamine) (P(DA-co-HDA))²⁵ pre-deposited onto the BMSs to facilitate the bonding of our hydrogel to it. To assess the strength of the hydrogel on the stent, we encapsulated it with A-M(9.3)/G_4/6 and simulated the process of angioplasty (Fig. 1f) in phosphate buffered saline (PBS, pH 7.4) at 37 °C. Fortunately, no clear damage was identified in the hydrogel coating (Fig. 1g; Supplementary Fig. 6 to Fig. 8) even if it had endured a very high pressure (up to 8

MPa) for one minute. Such preliminary result was promising and warranted further investigation. Before preparing the EL dressing, we also noted Jayakrishnan et al.²⁶ had reported another hybrid hydrogel formed with alginate dialdehyde and gelatin (A-D/G). However, the crosslinking of this hydrogel is implemented by Schiff base reaction between the aldehyde groups of oxidized alginate and the amino groups of gelatin, which generates imine and is reversible²⁷. Therefore, the stability of it might be very poor. We systematically compared A-M(9.3)/G_4/6 hydrogel with A-D/G hydrogel. Our analyses demonstrated A-M(9.3)/G_4/6 had better performance in the aspects of mechanical strength, chemical stability, optical property and biocompatibility (Supplementary Fig. 9 to Fig. 15; see Supplementary Information for full discussion).

Mechanism on the toughness of the hydrogel

Conventional hydrogels are normally weak because they are crosslinked by pure chemical bonds or physical interactions. However, some of our A-M/G hydrogels displayed good mechanical properties at ambient temperature. In particular, A-M(9.3)/G_4/6 could be extended by nearly three times with a stiffness comparable to that of muscles²⁸. Understanding such character is important for the application of our materials and the design of new hydrogels with better mechanical performance. Extensive studies have shown that a highly stretchable hydrogel generally has some mechanism to dissipate the energy built up during its deformation. In our case, we hypothesized the physical interaction within gelatin or between gelatin and A-M played the role of energy dissipation. To unravel this, we started our investigation by altering the chemistry of gelatin, which generated gelatin glycinamide (GelGA or GG in short) and gelatin methacrylate (GelMA or GM in short) (Supplementary Fig. 16 and 17).

Gelatin exists as random coils in an aqueous solution above its T_m ²⁹. The solution spontaneously transforms into a hydrogel when it cools. At the same time, the random coils bind to form ordered triple helices that function as physical crosslinks for hydrogel formation²⁹. This coil-helix transition is reversible and readily affected by many factors, including pH, ionic strength, and chemical

modification to gelatin³⁰. Circular dichroism (CD) was employed to study the influence of chemical modification to gelatin on the molecular structure of its hydrogel at ambient temperature. The CD spectra show a strong negative peak around 240 nm (Fig. 2a) for pristine gelatin hydrogel, which was assigned to the triple helices. The same peak was found for GelGA hydrogel, but the intensity of it decreased with a 2 nm blue shift, implying the reduction of triple helices in it. For GelMA hydrogel, the peak was almost gone, indicating the predominance of random coils in it. A direct consequence of this phenomenon one would anticipate is the loss of mechanical strength for the generated hydrogels. Our data reveal the shear modulus of pristine gelatin hydrogel is 8.9 kPa at ambient temperature, but it significantly declines to 3.3 kPa ($P<0.0001$) and 1.3 kPa ($P<0.0001$) for GelGA and GelMA hydrogels, respectively (Supplementary Fig. 18a). Moreover, the T_m of gelatin hydrogel was 30.6 °C, but it decreased to 29.4 °C of GelGA hydrogel and 27.9 °C of GelMA hydrogel, respectively (Fig. 2a and Supplementary Fig. 18b). In particular, the T_m of GelMA hydrogel is almost 3 °C lower than that of pristine gelatin hydrogel. Tensile testing of these hydrogels (Fig. 2a and Supplementary Fig. 19) disclosed similar trends, with the Young's modulus, fracture strength, fracture strain and toughness of pristine gelatin hydrogel significantly larger than those of GelGA and GelMA hydrogels. In summary, all information reflects the physical interaction within gelatin hydrogel was destroyed with the imposed chemical modifications.

Fig. 2 | Mechanism on the toughness of A-M(9.3)/G₄/6 hydrogel. **a**, Effects of different chemical modifications to gelatin on the molecular structure, melting point and tensile behavior of its hydrogel. **b**, Comparison in tensile behavior among A-M(9.3)/G₄/6, A-M(9.3)/GG₄/6 and A/G₄/6 hydrogels. **c**, Schematic illustration for the mechanism on the toughness of A-M(9.3)/G₄/6 hydrogel.

On top of these findings, we further mixed A-M with GelGA or GelMA to make A-M(9.3)/GG₄/6 and A-M(9.3)/GM₄/6 hydrogels. As a negative control, we prepared alginate/gelatin (A/G₄/6) hydrogel as well. Tensile testing was conducted on these hydrogels except for A-M(9.3)/GM₄/6 because it was too brittle and repeatedly broke during demolding. Indeed, this hydrogel transformed into a liquid upon heating above the T_m of GelMA, suggesting no chemical crosslink had formed in it. Such phenomenon verified the necessity of amino groups for the chemical crosslinking of our hydrogel since all of them had been amidated in GelMA (Supplementary Fig. 17). The mechanical strength of A/G₄/6 is also very weak and almost identical to that of pristine gelatin hydrogel except that the

Young's modulus of it is even lower ($P < 0.0001$) (Supplementary Fig. 19 and 20). As a matter of fact, there is no chemical crosslink in A/G_{4/6} as well since pristine alginate does not have any maleimidyl group. The fracture strength, fracture strain and toughness of A/G_{4/6} are merely 11.2%, 19.1% and 3.8% as those of A-M(9.3)/G_{4/6}, though the Young's modulus of it is slightly larger (Fig. 2b and Supplementary Fig. 20). The difference in tensile behavior between A/G_{4/6} and A-M(9.3)/G_{4/6} manifests the importance of chemical crosslinking in the mechanical strength of the hydrogels. Nevertheless, this does not imply that the physical interaction is less important, as the mechanical strength of A-M(9.3)/G_{4/6} is much lower than that of A-M(9.3)/G_{4/6} even if the triple-helix structure of GelGA is only slightly impaired. We believe both interactions are equally important for producing a hydrogel that is tough and highly stretchable. In fact, we compared the robustness of A-M(9.3)/G_{4/6}, gelatin and photocrosslinked GelMA (UV illumination for 30 min) on metal springs by performing 1,000 cycles of stretching and compressing. Our result (Supplementary Fig. 21) demonstrated that only A-M(9.3)/G_{4/6} preserved integrity after the test even if the Young's modulus of the photocrosslinked GelMA is nearly two magnitudes higher than that of A-M(9.3)/G_{4/6} (Supplementary Fig. 22). In addition, the photocrosslinked GelMA hydrogel was severely fractured and peeled off the struts of the vascular stent after balloon dilation in PBS at 37 °C (Supplementary Fig. 23).

Based on the results above, we can rationally describe the mechanism on the toughness of A-M(9.3)/G_{4/6} (Fig. 2c). When the tensile force was exerted on this hydrogel, it was gelatin that felt the stress in the first place as itself had formed a 3D crosslinked network through the triple helices. The stress quickly propagated to the chemical crosslinks between A-M(9.3) and gelatin upon the full stretch of the random coils within gelatin. At this stage, apparently more and more crosslinks were involved, which gave rise to the stiffening of the hydrogel as observed in the stress-strain curve. In the meantime, the triple helices underwent disassembly, dissipating the energy built up during deformation until the fracture strain of the hydrogel. On the one hand, without the existence of chemical crosslinks,

A/G_4/6 fractured at low stress upon triggering the disassembly of the triple helices. On the other hand, due to the damage to the triple-helix structure, the capacity for energy dissipation was reduced in A-M(9.3)/GG_4/6, resulting in lower mechanical strength of it as well. It is at the synergism between the physical interaction and chemical crosslinking that a tough and highly stretchable hybrid hydrogel of alginate and gelatin can be produced.

Preparation of the EL dressings with the capacity to catalyze NO generation

In cardiovascular system, NO plays critical roles in nearly all important biological functions of native endothelium¹⁹. Consequently, we decided to prepare the EL dressings by rendering our hydrogel the capacity to catalyze NO generation. There are two known pathways responsible for the production of NO *in vivo*. One is through endothelial nitric oxide synthase (eNOS), which catalyzes the degradation of L-arginine into NO¹⁹. The other is through glutathione peroxidase-3 (GPx-3), which metabolizes endogenous nitrosated thiols (RSNO) to generate NO³¹. The pathway of eNOS is complex and involves many components so that harnessing it is difficult. In contrast, NO generation catalyzed by GPx-3 is relatively simple. The selenocysteine residue of this enzyme is believed to be the catalytic center³². In fact, many selenium species have shown the capacity to catalyze the degradation of RSNO into NO. For instance, selenocystamine (SeCA) is capable of catalyzing the production of NO in a mechanism proposed by Meyerhoff et al.³³ (Fig. 3a). This pathway can be readily exploited so that we made use of it in our EL dressings by conjugating SeCA to A-M(9.3). Inductively coupled plasma mass spectrometry (ICP-MS, Supplementary Fig. 24a) disclosed the content of conjugated SeCA was about 0.016 mmol g⁻¹ in it. By tuning the proportions of SeCA-conjugated A-M(9.3) and blank A-M(9.3), EL dressings conjugated with varying contents (0.2 to 1.0 mM) of SeCA were prepared.

Fig. 3 | Catalytic generation of NO from the EL dressings. **a**, Mechanism on the catalytic generation of NO from NO donors ($\text{R}'\text{S}-\text{NO}$) by SeCA conjugated to alginate. **b**, Representative curves of NO generation from GSNO (10 μM) in PBS (pH 7.4) at 37 $^{\circ}\text{C}$ with the blank hydrogel or EL dressings conjugated with varying contents of SeCA (0.2 to 1.0 mM). **c**, Correlation between the fluxes and rates of NO generation catalyzed by the EL dressings. **d**, Summary on the release features of NO from the EL dressings conjugated with varying contents of SeCA ($n = 6$). **e**, Quantification of the immobilized SeCA on the EL dressings after catalytic generation of NO. **f**, Release rates of NO from GSNO catalyzed by the EL dressing conjugated with 1.0 mM SeCA after pre-incubation in PBS at 37 $^{\circ}\text{C}$ for different durations ($n = 6$). One-way analysis of variance (ANOVA) with Tukey post-hoc test was performed to determine the difference among various groups. (n.s., not significant; ##### $P < 0.0001$ compared to other groups; ** $P < 0.01$ between two groups)

In most studies, the flux of NO, defined as the production of it per unit area per unit time, was measured for a coating. Since the weight of an EL dressing on a substrate might vary significantly, we assumed the release rate of NO (production of NO per unit mass per unit time) to accurately reflect the NO-generating capacity of it. We detected the release rates of NO (Fig. 3b) catalyzed at 37 $^{\circ}\text{C}$ by the EL dressings submerged in PBS containing 10 μM S-nitrosoglutathione (GSNO, an endogenous NO donor) and 30 μM glutathione (GSH). As anticipated, the blank hydrogel was unable to catalyze the generation of NO from GSNO. With the conjugation of SeCA, a burst release of NO followed by

gradual decline until steady state was observed. To our delight, the flux of NO was nearly proportional to the release rate of it, implying the uniform coating density of the EL dressings on the substrates (Fig. 3c). At the average coating density of 22.9 mg cm^{-2} , the flux of NO catalyzed by the EL dressing containing 1.0 mM SeCA was $6.19 \times 10^{-10} \text{ mol cm}^{-2} \text{ min}^{-1}$, approaching the normal level from native endothelium³⁴. Though the steady release rate of NO was proportional to the content of conjugated SeCA, the peak value presented a quadratic relationship with it (Fig. 3d), leading to the highest burst release ratio of NO (3.48) in the EL dressing containing 1.0 mM SeCA (Supplementary Fig. 24b). Nevertheless, this value is still much smaller than those of many NO-eluting coatings³⁵. The transient burst of NO ($26.3 \times 10^{-10} \text{ mol cm}^{-2} \text{ min}^{-1}$ at maximum) is unlikely to be detrimental for endothelial cells as it only lasts for a few minutes. When an EL dressing was removed from the reaction solution, NO release did not go back to the initial baseline, suggesting some organoselenium species had diffused into the reaction solution. Quantitative analysis (Fig. 3e) uncovered about 41% of SeCA was still linked to the EL dressings after catalytic generation of NO. The organoselenium species in the solution came from several sources, including remnant free SeCA in the EL dressings, SeCA conjugated to uncrosslinked A-M(9.3) molecules, and derivatives of SeCA as byproducts (Fig. 3a). In the third scenario, every SeCA molecule linked to the EL dressings with merely one amino group lost half of its constituent part after catalysis. Finally, we measured the release rates of NO from the EL dressings after they had been pre-incubated in PBS for different durations. Our result shows that their catalytic potency could last for more than two weeks (Fig. 3f).

Effects of the EL dressings on cellular behaviors *in vitro*

The integration of a vascular implant into the blood vessel is featured by the formation of neointima predominantly consisting of smooth muscle cells and/or endothelial cells. To prevent NIH, an ideal vascular stent must be capable of inhibiting vicinal smooth muscle cells while promoting rapid recruitment of endothelial cells. Previous studies have reported NO at the physiological level can

inhibit smooth muscle cells while such effect does not act on endothelial cells^{36,37}. To corroborate this, we started our investigation by conducting a competitive adhesion test between human umbilical vein endothelial cells (HUVECs) and human umbilical artery smooth muscle cells (HUASMCs) on our coatings in the medium supplemented with GSNO (10 μ M) and GSH (30 μ M). Our results (Fig. 4a and 4b) demonstrate the number of HUASMCs adhering on the blank hydrogel within three hours was less than a half of that on bare stainless steel. For the EL dressings, the cell densities were even lower. In contrast, no significant difference in the density of adhering HUVECs was found among bare stainless steel and these coatings. Taken together, it can be concluded the blank hydrogel coating selectively facilitated the adhesion of endothelial cells, while NO generation catalyzed by the EL dressings further inhibited the attachment of smooth muscle cells.

To further evaluate the potential of our EL dressings to promote endothelial regeneration, we seeded HUVECs onto them and cultured the cells in the presence of GSNO for prolonged time. Most of them attached onto the substrates within 6 h (Fig. 4c). Quantitative analyses (Fig. 4d to 4f) revealed no significant difference in the proliferation, coverage and spreading of HUVECs among the blank hydrogel and EL dressings, suggesting NO had little influence on their behaviors. In detail, the density and coverage of HUVECs on them increased from 147 ± 20 cells mm^{-2} to $1,010 \pm 61$ cells mm^{-2} , and $12.8 \pm 2.7\%$ to $96.9 \pm 3.0\%$, respectively in one week while the individual cell area almost unchanged. Compared to these coatings, the endothelial cells were much more spread on bare stainless steel even at early time. The individual cell area were $2,126 \pm 385$ μm^2 after 6 h and $2,295 \pm 243$ μm^2 after 3 days on it, while the values of these indices for our coatings were barely 863 ± 97 μm^2 and 990 ± 101 μm^2 , respectively. However, no significant difference in cell proliferation rate was noted among them. The direct consequence of better cell spreading on bare stainless steel was the higher cell coverage ($29.9 \pm 5.0\%$ after 6 h and $73.8 \pm 6.3\%$ after 3 days). Nonetheless, these indices became almost identical in one week among all groups.

Fig. 4 | Effects of the EL dressings on cellular behaviors *in vitro*. **a**, Fluorescence images exhibiting the competitive adhesion between HUVECs and HUASMCs on various substrates. The cell growth medium was supplemented with GSNO (10 μ M) and GSH (30 μ M). (Scale bar: 500 μ m) **b**, Quantitative analyses on the competitive adhesion between HUVECs and HUASMCs ($n = 6$). **c**, Confocal laser scanning microscopy (CLSM) images displaying the adhesion, spreading and proliferation of HUVECs seeded onto various substrates in the presence of GSNO. (Scale bar: 100 μ m) **d-f**, Summary of cell density, cell coverage and individual cell area on those substrates ($n = 6$). **g**, CLSM images showing the formation of adherens junctions (VE-cadherin) between HUVECs grown on the EL dressing containing 1.0 mM SeCA. (Scale bar: 20 μ m) **h**, Proliferation assay of HUASMCs co-cultured with the blank hydrogel or EL dressings containing varying contents of SeCA in the presence of GSNO. **i**, Migrations of HUASMCs on bare stainless steel, the blank hydrogel and EL dressing containing 1.0 mM SeCA. One-way ANOVA with Tukey post-hoc test was performed to determine the difference among various substrates and two-tailed Student's *t*-test was assumed to determine the difference between the two types of cells on the same substrate. (#### $P < 0.0001$ compared to other groups; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ and **** $P < 0.0001$).

It is worthy of note HUVECs gathered as colonies and then propagated to form confluent monolayers on our coatings whereas those grown on bare stainless steel dispersed evenly and proliferated until the formation of an intact cell sheet. Mauck et al.³⁸ have unraveled that cells are regulated by the interplay between cell-cell and cell-ECM interactions. On a stiff substrate like bare stainless steel in our case, the traction force sensed by HUVECs is relatively large, guiding them into a more spread phenotype through activating mechanotransduction pathways such as YAP/TAZ³⁹. On the contrary, those grown on our soft coatings were governed by cell-cell interaction due to the relatively low cell-ECM interaction, thereby leading to the formation of cell colonies and collective cell migration. At this stage, we could not conclude which scenario is more favorable for endothelial regeneration *in vivo*, but we observed that adherens junctions (VE-cadherin), which is necessary for healthy endothelium, had already formed between HUVECs grown on our coatings (Fig. 4g).

The competitive adhesion test implied our EL dressings could inhibit smooth muscle cells. To verify this point of view, we co-cultured HUASMCs with our coatings in the presence of GSNO. Cell proliferation assay (Fig. 4h) suggested GSNO alone had little influence on the cells, while their viability was significantly reduced when co-cultured with an EL dressing or even the blank hydrogel. The anti-proliferative effect of the blank hydrogel might come from uncrosslinked A-M(9.3) molecules in it (Supplementary Fig. 13), and the cell proliferation was further inhibited upon the generation of NO from the EL dressings. Indeed, the cell viability declined monotonically with the content of SeCA in them. Specifically, after incubation with the EL dressing containing 1.0 mM SeCA for 5 days, the viability of HUASMCs decreased by 30% compared to those supplemented with GSNO alone. However, the EL dressings were unable to stop the proliferation of smooth muscle cells due to the unsustainable generation of NO *in vitro*. Nonetheless, this may not be an issue *in vivo* since the volumes of blood in experimental rabbits and pigs are two to three magnitudes larger.

We continued to evaluate the migration of HUASMCs on our coatings in 24 h according to a published protocol⁴⁰. The experimental data (Fig. 4i) demonstrate GSNO alone has little influence on

the migration of HUASMCs since the distance traveled by them on bare stainless steel was unaffected by it (1.83 ± 0.16 mm with GSNO vs. 1.86 ± 0.17 mm without GSNO). To our delight, the movement of HUASMCs on our coatings was dramatically slowed down in comparison to those on bare stainless steel. The cells traveled 0.60 ± 0.33 mm on the blank hydrogel and 0.31 ± 0.17 mm on the EL dressing containing 1.0 mM SeCA, respectively. These results indicate the hydrogel material itself possesses some repressive effect on the migration of smooth muscles, while NO generation catalyzed by SeCA conjugated to the EL dressing could further retard this progress.

Transcriptome analysis of HUASMCs

To figure out why HUASMCs were inhibited by the blank hydrogel and EL dressings, we performed a transcriptome analysis after the cells had been co-cultured with them. The EL dressing containing 1.0 mM SeCA was selected as the delegate since it was most efficient in inhibiting smooth muscle cells. Principal component analysis (PCA) and clustering assay (Fig. 5a and Supplementary Fig. 25) revealed all three independent replicates in each group correlated well and GSNO alone barely had any impact on the gene expression of HUASMCs. However, with the co-culture of the blank hydrogel plus GSNO, the phenotypic change of these cells became dramatic. For those incubated with the EL dressing plus GSNO, such change was largest. We set a threshold of $|\log_2\text{fold change (FC)}| > 1$ and $P < 0.05$ to screen out genes with significantly differential expression. Our results (Fig. 5b to 5d) disclose that 26, 244 and 565 genes presented significantly differential expression in HUASMCs after incubation with GSNO, the blank hydrogel plus GSNO, and the EL dressing plus GSNO, respectively for 6 h. Intriguingly, most of the alterations were up-regulated gene expression. Such data validate GSNO alone had bare any influence on the phenotype of HUASMCs again, while the effects of the other two treatments were prominent. Compared to the blank hydrogel, the EL dressing could catalyze the generation of NO from GSNO. The larger variation in gene expression of this group demonstrates that NO greatly affected the behavior of smooth muscle cells. To unveil the effects of altered genomic

expression profiles on cell behavior, these genes were analyzed in terms of inflammation, proliferation, and apoptosis.

Inflammation is a localized protective response elicited by the stimulation or injury to a tissue^{41,42}. However, dysregulated inflammation is disastrous as it may lead to either hyperplasia or excess destruction. Our analysis (Fig. 5e) unraveled tens of pro-inflammatory genes were significantly up-regulated in both cases. At the same time, numerous anti-inflammatory genes such as SOD2⁴³ and TSG6⁴⁴ were also activated after both treatments (Fig. 5f). The inflammatory response was likely to be elicited by gelatin in our coatings since it was derived from animal tissues that might contain pro-inflammatory substances. Similar observations were also reported by others⁴⁵, yet the mechanism is not understood. In contrast, alginate might function as an anti-inflammatory mediator⁴⁶ so that the process of inflammation was constrained. In the case of HUASMCs co-cultured with the EL dressing plus GSNO, the total number (50) of significant anti-inflammatory alterations was even larger than that (42) of pro-inflammatory ones. Obviously, NO molecules generated from the EL dressing contributed extra anti-inflammatory modulation since some anti-inflammatory genes such as NUR77⁴⁷ were exclusively up-regulated (Fig. 5f). Regulated inflammation is beneficiary since it can help reconstruct the damaged tissue. In this study, we noted that pro-angiogenic cytokines such as VEGF, PDGFA and PDGFB were activated in HUASMCs after incubation with EL dressing plus GSNO, implying such coating might accelerate endothelial regeneration *in vivo*.

Fig. 5 | Transcriptome analysis of HUASMCs. **a**, Principal component analysis (PCA) representing the general variations in gene expression of HUASMCs among different groups. The EL dressing containing 1.0 mM SeCA was selected as the delegate. **b-d**, Differential gene expression heat maps of HUASMCs after various treatments when compared to the blank control group. The gene expression levels for each set of comparison were normalized to the mean values within those two groups. **e**, Pie charts displaying the changes and numbers of significantly differential gene expression related to inflammation, proliferation, or apoptosis. The area of a pie represents the number of genes involved in. The gridded region represents the total number of anti-inflammatory, anti-proliferative, or anti-apoptotic alterations in gene expression. **f**, Heat map showing the relative changes in expression level for selected genes when compared to the blank control group. **g**, Schematic illustration of NO signaling pathway.

With respect to proliferation, anti-proliferative alterations exceeded pro-proliferative ones for both groups. In the group of EL dressing plus GSNO, the number of implicated genes was more than doubled compared to that in the group of hydrogel plus GSNO, and the anti-proliferative alterations

became dominant. Many anti-proliferative alterations were unique in this group, such as the down-regulation of proto-oncogenes CCND2⁴⁸ and SKP2⁴⁹, as well as the up-regulation of tumor suppressor genes PER2⁵⁰, GADD34⁵¹ and FOXO1⁵². These results confirmed that the EL dressing exerted intensified inhibitory effects on smooth muscle cells through the generation of NO.

When it comes to apoptosis, pro-apoptotic alterations prevailed over anti-apoptotic ones in both groups. Besides, the EL dressing with NO release also affected more genes. Although we did not observe the significant up-regulation of canonical pro-apoptotic makers such as CYC and CASP3, many anti-proliferative genes were found to be pro-apoptotic as well, including aforementioned PER2 and GADD34.

It is well known NO affects smooth muscle cells through the canonical cGMP/PKG pathway^{19,36,37} (Fig. 5g). It activates soluble guanylate cyclase (sGC), which subsequently catalyzes the transformation of guanosine triphosphate (GTP) into cyclic guanosine monophosphate (cGMP). cGMP can induce the relaxation of smooth muscle cells by interacting with cGMP-gated ion channels or play other biological functions through activating phosphate kinase G (PKG). In this study, we did observe the significant and unique up-regulation of guanylate cyclase 1 soluble subunit alpha 2 (GUCY1A2) in HUASMCs after the treatment of EL dressing plus GSNO. Besides, HMOX1⁵³ and PTGER4⁵⁴, which are two mediators for vascular relaxation, were also up-regulated dramatically. These results confirm that our EL dressing induced the relaxation of smooth muscle cells. Such phenomenon is highly desired because it can help the blood vessel to main vasodilation, thereby preventing the occlusion of stented artery.

Vascular stent deployment in rabbit iliac arteries

Encouraged by the results above, we continued to construct our EL dressing on BMSs, and then test them in animals. Before that, the mechanical stability and thrombogenicity of the EL dressing were examined. Firstly, we conducted a mock angioplasty by dilating an EL dressing-coated stent in a plastic

catheter perfused with PBS (37 °C). Our results (Supplementary Fig. 26) demonstrate that the EL dressing was still intact even after being flushed by PBS for 1 week ($Q = 120 \text{ mL min}^{-1}$ or $v = 28.3 \text{ cm s}^{-1}$). Thereafter, we carried out a thrombogenicity test in an *ex vivo* arteriovenous shunt model (Supplementary Fig. 27). Our data show both bare stainless steel and the blank hydrogel triggered severe clotting. In contrast, the EL dressing could effectively retard blood coagulation or even completely inhibit it depending on the content of conjugated SeCA (see Supplementary Information for detailed discussion). Indeed, the EL dressing containing 1.0 mM SeCA could be regarded as non-thrombogenic. In view of its excellent biological performance, the EL dressing containing 1.0 mM SeCA was selected as the coating material for BMSs.

We started our preclinical study by implanting the EL dressing-coated stents into the right iliac arteries of rabbits (Fig. 6a and Supplementary Fig. 28). BMSs of 316L stainless steel was used as the control due to its wide application in clinical practice, and were implanted into their left iliac arteries. At the designated time points, the stented arteries were harvested. Van Gieson staining of their cross-sections showed all vascular stents were fully expanded, but neointimal growth varied dramatically among different groups (Fig. 6b). Quantitative analyses (Fig. 6c) suggested the stent diameters were nearly identical and matched well with the reference value (2.7 mm) provided by the manufacturer of BMS. The neointimal thickness (NT) and ISR of them showed no difference within 1 week of implantation. However, neointima grew fast on BMS with NT increased from 118 μm to 295 μm , and ISR from 9.1% to 34.0% in 3 months. In contrast, these indices of EL dressing-coated stent slowly increased to 200 μm and 21.1%, respectively, which were significantly smaller ($P < 0.0001$) than those of BMS. Besides, the neointimal growth rate on EL dressing-coated stent decreased from 160 $\mu\text{m month}^{-1}$ in the first month to 20 $\mu\text{m month}^{-1}$ in the next two months, whereas these values for BMS were 217 $\mu\text{m month}^{-1}$ and 39 $\mu\text{m month}^{-1}$, respectively.

Fig. 6 | Vascular stent deployment in rabbits. **a**, Schematic illustration for vascular stent deployment in rabbit iliac arteries. **b**, Optical images showing the cross-sections of stented arteries after van Gieson staining. (Scale bar: 500 μ m) **c**, Quantitative analyses on the cross-sections ($n = 12$). **d**, CLSM images unveiling the endothelialization on the stents (outlined by the dashed lines). The right panels present the fluorescence intensities of different cell components along the line segments (OA, O'B) in the images. (Blue: cell nucleus, green: CD31, red: F-actin; scale bar: 50 μ m). **e**, SEM images showing the luminal faces of stented arteries at 3 months post stent deployment. One-way ANOVA with Tukey post-hoc test was performed to determine the difference among various groups and Student's t -test was assumed to determine the difference between two groups. (n.s., not significant; * $P < 0.05$ and **** $P < 0.0001$)

As aforementioned, our EL dressing was expected to promote rapid restoration of native endothelium. To assess that, we utilized confocal laser scanning microscope (CLSM) to examine the luminal faces of the stented arteries. CLSM (Fig. 6d and Supplementary Video 2) showed that some struts of BMS were not fully covered by endothelial cells in 1 week, which were also corroborated by

SEM (Supplementary Fig. 29). In addition, clusters of giant flat or small granular cells that seemed to be inflammatory cells, were found on the non-endothelialized region. In contrast, EL dressing-coated stent was encapsulated by intact endothelium in 1 week and hardly any sign of inflammation was observed (Fig. 6d and Supplementary Video 3). After implantation for 1 month, both types of stents were completely endothelialized (Supplementary Fig. 30). Nevertheless, the endothelial cells adhering on EL dressing-coated stent presented a more mature phenotype compared to BMS, featured by elongated morphology and high degree of orientation (Fig. 6e and Supplementary Fig. 30).

In cardiovascular system, the coordination between coagulation and fibrinolysis are critical for maintaining the intactness of blood vessels. During angioplasty, the vessel wall is injured inevitably, thereby releasing tissue factors that trigger coagulation. The ensuing formation of thrombus not only activates fibrinolysis, but also recruits inflammatory cells⁵⁵ as observed on BMS in our case. However, NO can suppress clotting cascade by preventing platelets from activation and may potentially inhibit thrombin through up-regulation of thrombomodulin in smooth muscle cells (Fig. 5f). Besides, the EL dressing could provide a highly hydrated lubricating interface between the stent and blood, thereby reducing the turbulence of blood flow compared to BMS. It is reasonable to believe thrombus formation was repressed on EL dressing-coated stent as proved by the *ex vivo* thrombogenicity test. Consequently, the inflammation elicited by acute thrombosis was effectively mitigated on EL dressing-coated stent. In addition, the EL dressing inhibited the proliferation of smooth muscle cells through the combinational effects of NO gas and A-M molecules. At the same time, it mimicked the subendothelial ECM, thereby providing a favorable microniche for endothelial cells. Thanks to these factors, EL dressing-coated stent effectively suppressed ISR and presented faster restoration of native endothelium in comparison to BMS.

Vascular stent deployment in swine coronary arteries

Although EL dressing-coated stent demonstrated satisfactory outcomes in leporine model, those data

might still be inadequate in predicting its performance in human being since rabbits are herbivorous. Among large experimental animal species, the coronary artery system and physiology of pigs are very similar to those of human being, making them an ideal model for coronary stenting⁵⁶. Consequently, we continued to evaluate EL dressing-coated stent in a swine model. We compared it with an everolimus-eluting DES, which represents the gold standard for coronary stents. Both of them were constructed on the same kind of cobalt chromium alloy (CoCr) stents, and the anti-restenotic drug of DES was loaded in its polymer coating (see Experimental Section for details). In addition, blank hydrogel-coated and blank polymer-coated stents were included as two negative controls (see Experimental Section for details). These four types of stents were randomly implanted in three or four coronary arteries of individual Bama miniature pigs (Fig. 7a and Supplementary Fig. 31) under the guidance of digital subtraction angiography (DSA).

Fig. 7 | Vascular stent deployment in pigs. **a**, Schematic illustration for vascular stent deployment in swine coronary arteries. **b**, Digital subtraction angiography prior to the harvest of stented arteries. The white arrows indicate the sites of implanted stents. The yellow arrow refers to severe restenosis occurring in a polymer-coated stent. (Scale bar: 1 cm) **c**, Photographs displaying the luminal faces of stented coronary arteries at 2 weeks and 3 months post stent deployment. (left to right: EL dressing-coated stent, blank hydrogel-coated stent, DES and polymer-coated stent; scale bar: 5 mm) **d**, Optical images showing the cross-sections of stented arteries after van Gieson staining. (Scale bar: 500 μm) **e**, Quantitative analyses on the cross-sections ($n = 6$). **f**, CLSM images unveiling the endothelialization on the stents (outlined by the dashed lines). (Blue: cell nucleus, green: CD31, red: F-actin). **g**, SEM images showing the luminal faces of stented arteries at 2 weeks and 3 months post stent deployment. Student's t -test was performed to determine the difference. (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ and **** $P < 0.0001$ between two groups; ##### $P < 0.0001$ compared to other groups)

DSA was conducted again prior to the harvest of stented arteries, which revealed none of the stented arteries was occluded in 2 weeks. However, severe narrowing occurred in polymer-coated stents (3 out of 6) while the blood flow was almost unaffected in others at 3 months post stent deployment (Fig. 7b;

Supplementary Video 4 and 5). We harvested the stented arteries and examined them with naked eyes. Our photographs (Fig. 7c) show thick neointima grew on polymer-coated stent while neointimal formation was much slower or even negligible on other stents. The cross-sections of stented arteries were also stained (Fig. 7d). Quantitative analyses (Fig. 7e) revealed the diameter of polymer-coated stent was significantly smaller (0.2~0.4 mm) than those of others, suggesting the occurrence of recoil to it due to the strong radial force produced by NIH. The NT value of it was $203\pm34\ \mu\text{m}$ at 2 weeks and reached $268\pm40\ \mu\text{m}$ at 3 months since implantation, while ISR increased from $29.2\pm5.4\%$ to $39.1\pm4.6\%$ duration that time. Upon the loading of everolimus in the polymer coating, DES efficiently inhibited neointimal growth. The NT and ISR for DES were barely $116\pm15\ \mu\text{m}$ and $8.7\pm2.0\%$, respectively after implantation for 2 weeks. However, such inhibitory effect was unsustainable as we found these indices increased to $165\pm28\ \mu\text{m}$ and $19.1\pm5.1\%$ in 3 months. In contrast, EL dressing-coated stent presented both efficient and sustained suppression of NIH. Though this type of stent was slightly inferior in the short term, it defeated DES in the long run since the NT and ISR increased merely to $135\pm15\ \mu\text{m}$ and $13.5\pm1.7\%$, respectively in 3 months. Notably, the NIH on blank hydrogel-coated stent was not severe when compared to polymer-coated one. In fact, the ISR of blank hydrogel-coated stent even approached that of DES in 3 months.

To explain the difference among various stents, we also examined the status of endothelialization on them. CLSM and SEM images (Fig. 7f and 7g; Supplementary Fig. 32 and 33) disclose endothelialization was dramatically delayed on DES compared to that of other stents. In fact, the progress of endothelialization on it was still incomplete even in 3 months. In stark contrast, both EL dressing-coated and blank hydrogel-coated stents promoted rapid restoration of endothelium in 2 weeks. However, the endothelial cells on blank hydrogel-coated stent displayed loose contact with each other and were less oriented compared to those on EL dressing-coated one. Besides, the strut profile was not visible for blank hydrogel-coated stent due to the thick neointima. When it comes to polymer-coated stent, though high degree of endothelialization was observed in 2 weeks, the

endothelial cells were highly elongated and oriented in the direction of blood flow. In addition, the newly formed endothelium was dispersed with holes. At 3 months post implantation, the endothelium on polymer-coated stent became intact, but the endothelial cells were further stretched due to the large shear stress of blood flow caused by ISR.

Based on the observations above, we can rationally deduce polymer-coated stent is highly pro-inflammatory, thereby stimulating NIH in the stented artery. In contrast, blank hydrogel-coated stent is more biocompatible so that the neointimal formation was much slower on it. In the case of DES, though everolimus released from the polymer coating inhibited neointimal growth, such effect was unsustainable. Besides, the anti-restenotic drug also impaired the regeneration of native endothelium. Consequently, inflammatory response was still elicited upon the depletion of the drug, causing NIH on DES at late stage. Fortunately, EL dressing-coated stent not only provided a temporary endothelial function, but also promoted rapid restoration of native endothelium to replace it. As a result, EL dressing-coated stent suppressed ISR persistently.

To present the advancement of our EL dressing more convincingly, we did a literature review and compared EL dressing-coated stent with other stents deployed in the iliac arteries of healthy, balloon injured, or high-fat diet fed rabbits. Our summary (Supplementary Table 1) reflects conventional DESs are potent in suppressing ISR in general. However, the progress of endothelialization on them was markedly delayed, and it was still incomplete in 3 months on some DESs. BMSs are favorable for the restoration of endothelium, but they normally possess high thrombogenicity and induced thicker neointimal formation. The FDA-approved fully bioresorbable stent Absorb BVS® failed in all terms of thrombogenicity, endothelialization and ISR. Other stents are either thrombogenic, inefficient in promoting endothelialization, or incompetent in suppressing ISR. In stark contrast, EL dressing-coated stent is non-thrombogenic and achieved complete endothelial regeneration in 1 week. In the meantime, it is comparable to DESs in view of anti-restenosis. Taking thrombogenicity, endothelialization and ISR into consideration together, EL dressing-coated stent is best in overall performance.

Outlook

The emergence of vascular stents has saved millions of patients with coronary artery disease. However, in-stent restenosis, as a result of neointimal hyperplasia or stent thrombosis, is a great challenge for the approved stents. The EL dressing developed in this study shed a new light on the resolution of this issue. It mimicked the physiological characteristics of native endothelium and promoted rapid endothelial regeneration, while providing temporal artificial endothelial function to inhibit neointimal hyperplasia and thrombosis. At the same time, it may also act as a carrier for therapeutic. For instance, our EL dressing can be encapsulated with small organic or large protein-based medicine (Supplementary Fig. 34) to afford extra therapy for coronary artery disease. Definitely, such EL dressing should be applicable to other vascular implants such as artificial valves and blood vessels. More importantly, the hydrogel designed for fabricating the EL dressing is self-crosslinking, highly biocompatible, and mechanically tough. Besides, it can be conjugated with various molecules for special goals due to the abundant functional groups. Thanks to these advantages, we expect such elastic hydrogel will find wide applications in biomedical engineering, including scaffolds for tissue engineering, dressings for wound healing, and interfaces for implantable medical devices.

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Author contributions

Y.C., Z.Y. and H.W. conceived the idea for this study. Y.C. designed, prepared and characterized the new materials with the aid of X. D.. Y.C. and L.H. undertook the *in vitro* study. Y.C., P.G., X.T., N.Z., T.Y. and H.Q. carried out the *ex vivo* and *in vivo* experiments. Y.C. performed the transcriptome analysis with the aid of P.G.. S.M., Q.T., N.H. and Z.G. contributed technical supports. Y.C., P.G., L.H., J.Z., Z.Y. and H.W. analyzed the data and discussed the results. Y.C. wrote and edited the manuscript. J.Z., Z.Y. and H.W. supervised the study. All authors participated in revising the manuscript and agreed on the final version of it.

Competing Interests

The authors declare no competing interests for this work.

Materials

Alginate (MW: 10~20 kDa) and gelatin (Type A, Cat. No.: G1890) were purchased from Qingdao Hyzlin Biology Development Co., Ltd. (China) and Sigma-Aldrich (USA), respectively. N-(2-aminoethyl)maleimidetrifluoroacetate salt (AEM.TFA), N-hydroxysulfosuccinimide (Sulfo-NHS) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC.HCl) were supplied by Suzhou Highfine Biotech (China). Sodium periodate (Cat. No.: S817518), glycine hydrochloride (Cat. No.: 459977) and methacrylic anhydride (Cat. No.: L14357) were obtained from Macklin (China), J&K Scientific (China) and Alfa Aesar (USA), respectively. Selenocystamine dihydrochloride (SeCA.2HCl, Cat. No.: S0520), S-nitrosoglutathione (GSNO, Cat. No.: N4148) and L-glutathione (reduced, GSH, Cat. No.: G4251) were bought from Sigma-Aldrich. Polydimethylsiloxane (PDMS) prepolymer (SYLGARD® 184) was provided by Dow Corning (USA). Pentobarbital sodium salt (Cat.

No.: P0776), benzylpenicillin sodium salt (Cat. No.: 150061248) and heparin (Cat. No.: BP252410) were acquired from TCI (Japan), Shandong Shengwang Pharmaceutical Co., Ltd. (China) and Thermo Fisher Scientific (USA), respectively. Other chemicals were purchased from Sigma-Aldrich and used without further purification unless stated otherwise.

Syntheses of A-M and A-D

To synthesize A-M, pristine alginate (4.2 g) was dissolved in PBS (100 mL) at 50 °C and then centrifuged at 6,000 g for 15 min to deposit insoluble impurities. Subsequently, the supernatant was pushed through 0.45 µm and 0.22 µm filters (Minisart®, Cat. No.: 16555 and 16532, Sartorius, Germany) consecutively, and collected in a clean glass bottle (250 mL). For the coupling of maleimide to alginate, AEM.TFA (508.4 mg, 1016.8 mg, or 1525.2 mg; 2 mmol, 4 mmol, or 6 mmol), Sulfo-NHS (521.2 mg, 1042.4 mg, or 1563.6 mg; 2.4 mmol, 4.8 mmol or 7.2 mmol), and EDC.HCl (1.38 g, 2.76 g, or 4.14 g; 7.2 mmol, 14.4 mmol or 21.6 mmol) were added into the solution and mixed by magnetic stirring. To obtain A-M with the potency to catalyze NO generation, the corresponding solution was also supplemented with SeCA (42.4 mg; 0.133 mmol). The reaction was carried out at ambient temperature in the dark for the designated period of time (6 h, 8 h or 10 h). Afterwards, the pH value of the solution was adjusted to about 4.5 by HCl solution (1 M, 3 mL), and then the reaction mixture was dialyzed (MWCO: 12~14 kDa, Spectra/Por 4, Spectrum Laboratories Inc., USA) against diluted HCl solution (pH 4.5) for two days. Finally, the solution was flash frozen in liquid nitrogen, lyophilized and then stored in a -80 °C freezer until use after being pushed through a 0.22 µm filter.

The synthesis of A-D was implemented according to the published protocol²⁶. Briefly, pristine alginate (4 g) was dispersed in ethanol (20 mL) and then added with sodium periodate (0.428 g, 1.070 g, or 2.139 g; 2 mmol, 5 mmol, or 10 mmol) dissolved in water (20 mL). The reaction was performed at ambient temperature in the dark for 6 h and then the DO of A-D was determined based on iodine test using starch as the indicator. Afterwards, the suspension was dialyzed against water for two days.

The obtained clean solution was also flash frozen in liquid nitrogen, lyophilized and then stored in the -80 °C freezer after being pushed through a 0.22 µm filter.

Syntheses of GelGA and GelMA

To synthesize GelGA, pristine gelatin (2.56 g) was dissolved in PBS (40 mL) at 45 °C. Subsequently, glycinamide hydrochloride (4.42 g; 40 mmol), Sulfo-NHS (0.87 g; 4 mmol), and EDC.HCl (2.30 g; 12 mmol) were added into the solution and mixed by magnetic stirring. The reaction was carried out at 45 °C in the dark for 5 h after the pH value of the solution had been adjusted to about 7.5 by NaOH solution (1M, 8 mL). After that, the biopolymer was precipitated in ethanol (500 mL). The precipitant was collected by filtration, redissolved in hot water (40 mL), and then dialyzed against warm water (35 °C) for two days. Finally, the purified solution was flash frozen in liquid nitrogen, lyophilized and then stored in a -20 °C freezer after being pushed through a 0.22 µm filter.

The synthesis of GelMA was also conducted according to the published protocol⁵⁷. Briefly, pristine gelatin (5 g) was dissolved in PBS (50 mL) at 50 °C. Subsequently, methacrylate anhydride (4 mL) was added dropwise to the gelatin solution while being stirred. After 6 h, the reaction was stopped and the solution was dialyzed against warm water (35 °C) for five days to remove unreacted monomers and salts. The purified solution was also flash frozen in liquid nitrogen, lyophilized and then stored in the -20 °C freezer after being pushed through a 0.22 µm filter.

¹H-NMR spectroscopy for the biopolymers

To determine the ¹H-NMR spectra of A-M, GelGA and GelMA, the biopolymers were dissolved in D₂O at 40 °C to the mass concentration of around 10 mg mL⁻¹. Afterwards, the freshly prepared specimens were analyzed by a 400 MHz NMR spectrometer (Avance II, Bruker), with scan times being at least 32 to achieve high signal-to-noise (SNR) ratios. The spectra were calibrated by setting the chemical shift (δ) of HOD to be 4.8 ppm.

Preparation of the hydrogels

The precursor solutions of the biopolymers were prepared by dissolving them in phosphate buffer (PB, 10 mM, pH 7.4) to the mass concentration of 150 mg mL⁻¹ or 100 mg mL⁻¹ with final pH adjusted to about 7.5 using NaOH solution (1 M). To fabricate the hydrogels of A-M/G, the corresponding precursor solutions (at 60 °C) were mixed at the designated mass ratios using a pipette gun and then cured in a humidified atmosphere at 37 °C and under 5 % CO₂. The pristine gelatin, GelGA and GelMA hydrogels were made by simply cooling down the precursor solutions (at 60 °C) in a humidified atmosphere at ambient conditions. The photocrosslinked GelMA hydrogels were produced by further illuminating the physically crosslinked GelMA hydrogels (containing 0.3 wt% Irgacure 2959) with UV light (365 nm, 8 W, ENF-280C, Spectronics Corporation, USA) for 10 min or 30 min.

Dynamic mechanical analysis of the hydrogels

Hydrogels in disc shapes (diameter: 8 mm, thickness: 1 mm) were prepared in a homemade well plate formed by bonding a perforated plastic plate to a piece of polydimethylsiloxane (PDMS) membrane. At the designated time points, the hydrogel disc was carefully demolded and transferred to a parallel plate fixture (8 mm in diameter) mounted on a rheometer (ARES, TA Instruments, USA). The upper plate was slowly moved down until close contact with the hydrogel. For strain sweep (1% to 100%), dynamic mechanical test was carried out at 1 rad s⁻¹ angular frequency. The cure kinetics for the hydrogels was investigated in time sweep mode at 5 % shear strain and 1 rad s⁻¹ angular frequency.

Tensile experiment of the hydrogels

To prepare hydrogels for tensile experiment, the precursor solutions were injected into homemade dumbbell-shape PDMS molds (middle part: 20 mm × 5 mm, end parts: 5 mm × 9 mm; depth: 2 mm). After cure, the hydrogel was carefully demolded and transferred onto a thin film fixture mounted on the rheometer. Two homemade PDMS clamps with the complementary shape to the ends of the

hydrogel were used to fix it to the fixture. Thereafter, the tensile test was conducted on the hydrogel by stretching it at the rate of 0.1 mm s^{-1} until fracture.

CD spectroscopy for the gelatin-based hydrogels

Pristine gelatin, GelGA or GelMA solution (150 mg mL^{-1}) was cured in the well (path length: 0.1 mm , volume: $16 \text{ }\mu\text{L}$) of a quartz plate and then examined by a spectropolarimeter (Chirascan, Applied Photophysics, UK) at ambient temperature. The CD spectra (range: 200 to 320 nm , interval: 1 nm) of the gelatin-based hydrogels were generated from the averaged value of three scans (20 nm min^{-1}), which were smoothened by the software provided by the manufacturer of the equipment.

Melting points of the gelatin-based hydrogels

To determine the melting points of the gelatin-based hydrogels (150 mg mL^{-1}), dynamic mechanical test was performed in temperature sweep mode ($23 \text{ }^{\circ}\text{C}$ to $40 \text{ }^{\circ}\text{C}$) at 5% shear strain and 1 rad s^{-1} angular frequency. The ramp of temperature was set as $2 \text{ }^{\circ}\text{C min}^{-1}$ and the sampling frequency was between 0.008 Hz and 0.016 Hz . We defined the melting point of a hydrogel as the temperature at which the shear modulus was reduced by a half from its initial value.

Swelling and degradation of the hybrid hydrogels

A-D(49.1)/G_4/6 and A-M(9.3)/G_4/6 precursor solutions (150 mg mL^{-1} , $\sim 24 \text{ }\mu\text{L}$) were coated onto 316L stainless foils ($15 \text{ mm} \times 8 \text{ mm} \times 0.02 \text{ mm}$) pre-deposited with a film of poly(dopamine-co-hexanediamine) (P(DA-co-HDA))²⁵ and fully cured. The initial weight of a hydrogel coating was determined by subtracting the weight of its underlying substrate from their gross weight. Thereafter, the hydrogel-coated foils were placed into plastic tubes (1.5 mL) containing PBS (1 mL) and then incubated at $37 \text{ }^{\circ}\text{C}$ in a shaker. The PBS in the tubes was refreshed every day. At the designated time points, the foils were taken out and the weights of the hydrogel coatings were determined in the same

way after they had been blotted dry. The water uptake (ΔW), which reflects the swelling behavior of a hydrogel, was defined and calculated as (Equation (1)):

$$\Delta W = \frac{m_t - m_0}{m_0} \times 100 \% \quad (1)$$

Where m_t is the weight of the hydrogel after incubation in PBS at 37 °C for time t , and m_0 is the initial weight of the hydrogel.

To investigate the swelling and degradation behaviors of the hybrid hydrogels in a fluidic environment, a simple mock circulatory system (Supplementary Fig. 13) was established using a peristaltic pump and a plastic catheter (D_{in} : 3 mm, D_{out} : 4 mm). The foils coated with A-D(49.1)/G_4/6 or A-M(9.3)/G_4/6 were dried, curled and then inserted into the catheter, after which they were constantly flushed by PBS (37 °C, $Q = 120 \text{ mL min}^{-1}$ or $v = 28.3 \text{ cm s}^{-1}$) for 1 week. Afterwards, the wet weight (m_w) and dry weight (m_d) of each hydrogel were determined before and after the test so that the swelling ratio (Q) and percent remaining solid (R) of it can be calculated as:

$$Q = \frac{m_w}{m_{w,0}} \times 100 \% \quad (2)$$

$$R = \frac{m_d}{m_{d,0}} \times 100 \% \quad (3)$$

Where $m_{w,0}$ and $m_{d,0}$ are the initial wet and dry weights of the hydrogel, while m_w and m_d are the wet and dry weights of it after the test.

Light absorbance and fluorescence of the hybrid hydrogels

A-D(49.1)/G_4/6 and A-M(9.3)/G_4/6 precursor solutions (150 mg mL⁻¹, 1 mL) were fully cured in cuvettes. Afterwards, the light absorption spectra of the hydrogels were measured using an ultraviolet–visible (UV-Vis) spectrophotometer (Amersham Biosciences Ultrospec 4300 Pro, GE healthcare). The scan was performed in the wavelength range of 400 to 800 nm at the interval of 1 nm. Similarly, the fluorescence spectra of them in the range of 508 to 800 nm (Ex: 488 nm; interval: 1 nm) were determined using the same equipment.

Culture of HUVECs and HUASMCs

Human umbilical vein endothelial cells (HUVECs, Cat. No.: C-12203) and human umbilical artery smooth muscle cells (HUASMCs, Cat. No.: C-12500) were purchased from Promocell (USA) and cultured in endothelial cell growth medium (Cat. No.: C-22010, Promocell) or smooth muscle cell growth medium (Cat. No.: C-22062, Promocell), respectively. Both media were supplemented with 1 v/v% penicillin/streptomycin solution (Cat. No.: 15140122, Thermo Fisher Scientific) and 1 v/v% amphotericin B solution (Cat. No.: LS15290026, Thermo Fisher Scientific). The cells were raised at 37 °C and under 5% CO₂ on tissue culture dishes (Cat. No.: 93100, TPP, Switzerland) until they reached about 80% confluence. To detach the cells, they were rinsed with PBS and then treated with trypsin (0.05 w/v%)/EDTA (0.53 mM) solution (Cat. No.: 15400054, Thermo Fisher Scientific). The floated cells were deposited after centrifugation and then resuspended in their own growth medium for subculture or biological tests.

Cytocompatibility of A-M versus A-D

The cytocompatibilities of A-M and A-D with HUVECs were assessed by live/dead staining and flow cytometry. For live/dead staining, the cells were seeded in 48-well plates (Cat. No.: 92048, TPP) at the density of 40,000 cells cm⁻². After 6 h, the cell growth medium was refreshed with that containing varying concentrations of A-M(9.3) or A-D(49.1) (10 µg mL⁻¹ to 10 mg mL⁻¹). The cells were further cultured in the biopolymer-containing media for 1 day or 1 week with the medium changed every other day. Thereafter, they were stained with fluorescein diacetate, propidium iodide and Hoechst 33342 (2 µg mL⁻¹ for all dyes) in the medium. After rinsing with PBS, they were photographed using an upright fluorescence microscope (Ni-U, Nikon, Japan). The cell viability was determined by dividing the number of living cells to the gross number of cells in the same region, and the cell density was calculated simultaneously.

For flow cytometry, the cells were raised on tissue culture dishes in the medium containing 1 mg

mL⁻¹ A-M(9.3) or A-D(49.1) for 1 day or 1 week. Subsequently, they were detached and deposited in the same way. The collected cells were fixed with paraformaldehyde (1 w/v% in PBS) for 15 min, permeabilized with Tween 20 solution (0.2 v/v% in PBS) for 15 min, and then incubated with Alexa Fluor[®] 488-conjugated rabbit anti-human Ki-67 monoclonal antibody (Cat. No.: ab197234, Abcam, USA, 1/50 dilution) and Cy5-labeled annexin V (Cat. No.: ab14150, Abcam, 1/50 dilution) diluted in PBS (containing 0.2 v/v% Tween 20 and 1 w/v% BSA) for 30 min. Finally, the cells were analyzed by a flow cytometer (FACSAria IIIu, BD Biosciences, USA) after being washed with PBS for twice.

Catalytic generation of NO from the EL dressings

The NO-generating capacity of an EL dressing was evaluated by measuring the release rate of NO catalyzed by it using a highly sensitive and selective analyzer (Sievers NOA 280i, GE Healthcare, USA). Briefly, 316L stainless foils (10 mm × 5 mm × 0.05 mm) were pre-deposited with P(DA-co-HDA) and then covered with a precursor solution (~10 µL) of the EL dressing. After full cure, the specimen was punched, hanged onto a stainless wire, and then plunged into PBS solution (5 mL) containing 10 µM GSNO and 30 µM GSH at 37 °C. The reaction solution was purged with N₂ gas and the generated NO was carried to the reaction chamber of the analyzer, wherein NO was oxidized by O₃ into NO₂ at its excited state (NO₂^{*}). Upon the relaxation of NO₂^{*}, a photon was emitted, which could be detected by the analyzer for the quantification of NO.

Sterilization of the specimens for *in vitro* and *in vivo* studies

The as-prepared specimens were sterilized with 75 v/v% alcohol. Subsequently, they were rinsed with 50 v/v%, 25 v/v% and 10 v/v% alcohols in sequence. Afterwards, they were bathed with PBS for immediate use, or washed with pure water and then dried for storage.

Competitive adhesion between HUVECs and HUASMCs

HUVECs and HUASMCs were pre-labeled with CellTracker™ Green CMFDA (Cat. No.: C2925, Thermo Fisher Scientific) and Orange CMTMR (Cat. No.: C2927, Thermo Fisher Scientific), respectively according to the protocols provided by the vendor. Subsequently, the cells were detached, deposited and then resuspended in their own growth medium supplemented with GSNO (10 μ M) and GSH (30 μ M). The two types of cells were diluted to the same density of 5,000 cells mL⁻¹, mixed at the ratio of 1:1, and then seeded onto the substrates placed in the 12-well plates at the density of 10,000 cells cm⁻². After incubation for 3 h, the cells were gently washed with PBS and then photographed using an inverted fluorescence microscope (CKX53, Olympus, Japan).

Proliferations of HUASMCs co-cultured with our coatings

HUASMCs were seeded in 24-well plates (Cat. No.: 92024, TPP) at the density of 1×10⁴ cells cm⁻². After incubation for overnight, they were co-cultured with the blank hydrogel or EL dressings (discs with a diameter of 6.4 mm, ~12 mg) in the medium (0.4 mL) supplemented with GSNO (10 mM) and GSH (30 mM). The medium was refreshed every day with the replenishment of GSNO and GSH at 1 h, 2 h, 3 h, 6 h, 12 h and then every 12 h since the co-culture. At day 1 and day 5 since the co-culture, the viabilities of the cells were measured using Cell Counting Kit-8 (CCK-8, Dojindo Molecular Technologies, Japan) following the protocol provided by the vendor and normalized to the value of blank control at day 1. As another control, the viabilities of HUASMCs raised in the medium containing GSNO and GSH were also determined.

Migration test of HUASMCs on the substrates

HUASMCs were seeded onto L shape-folded stainless steel foils (2 cm × 1 cm) coated by the blank hydrogel or EL dressing (1.0 mM SeCA) at the density of 5×10⁴ cells cm⁻² in 24-well plates. After incubation for overnight, the unseeded half parts were flipped down and submerged in the medium (2

mL) supplemented with GSNO and GSH. The cells were allowed to migrate freely for 24 h, during which, GSNO and GSH were replenished at 1 h, 2 h, 3 h, 6 h and 12 h since the test. Subsequently, the specimens were fixed with paraformaldehyde (4 w/v% in PBS) for 1 h, permeabilized with Triton X-100 solution (0.2 v/v% in PBS) for 1 h, blocked with BSA solution (5 w/v% in PBS) for 12 h, and stained with phalloidin-TRITC ($1 \mu\text{g mL}^{-1}$ in PBS) for 3 h sequentially. After washing with PBS for three times and cutting off the seeded half parts of the foils, the cells were imaged using a fluorescence microscope (IX51, Olympus, Japan). As controls, the migrations of HUASMCs on bare stainless steel foils in the presence or absence of GSNO and GSH were tested as well.

Adhesion and growth of HUVECs on the substrates

The blank hydrogel or EL dressings ($\sim 45 \mu\text{L}$) were formed on mirror polished stainless sheets ($15 \text{ mm} \times 15 \text{ mm} \times 0.5 \text{ mm}$) pre-deposited with P(DA-co-HDA). The as-prepared specimens were placed into 12-well plates (Cat. No.: 92012, TPP) and then plated with HUVECs at the density of $20,000 \text{ cells cm}^{-2}$. The cells were cultured in the medium supplemented with GSNO ($10 \mu\text{M}$) and GSH ($30 \mu\text{M}$) for different periods of time, during which the medium was renewed every day. At the designated time, they were fixed with paraformaldehyde solution for 1 h, permeabilized with Triton X-100 solution for 1 h and then blocked with BSA solution for 12 h. Subsequently, they were incubated with Alexa Fluor[®] 488-conjugated rabbit anti-human VE cadherin monoclonal antibody (Cat. No.: ab225443, Abcam, USA, 1/100 dilution in PBS) for 6 h, followed by staining with phalloidin-TRITC ($1 \mu\text{g mL}^{-1}$ in PBS) and DAPI ($5 \mu\text{g mL}^{-1}$ in PBS) for 6h. After extensive washing with PBS, the cells on the substrates were photographed using a confocal laser scanning microscope (CLSM, TCS SP5 II, Leica, Germany).

Transcriptome analysis of HUASMCs

HUASMCs were seeded in tissue culture dishes (Cat. No.: 93100, TPP) at the density of $2 \times 10^4 \text{ cells cm}^{-2}$. After incubation for overnight, they were co-cultured with the blank hydrogel or EL dressing

(discs with a diameter of 3.5 cm, ~400 mg) in the medium (10 mL) supplemented with GSNO (10 mM) and GSH (30 mM). The medium was replenished with GSNO and GSH at 1 h, 2 h, and 3 h since the co-culture. After incubation for 6 h, both the medium and coatings were removed. Subsequently, the total RNA of the cells was extracted by TRIzol[®] reagent (Thermo Fisher Scientific, USA) following the protocol provided by the vendor. As controls, the total RNA was also extracted for the cells at normal culture condition or incubated with GSNO and GSH. The transcriptome sequencing and analysis were conducted by OE biotech Co., Ltd. (Shanghai, China). Raw data were processed using Trimmomatic. The reads containing poly-N and low quality reads were removed to obtain clean reads, which were mapped to reference genome using HISAT2. FPKM value of each gene was calculated using Cufflinks, and the read count of each gene was obtained by HTSeq-Count. Differentially expressed genes (DEGs) were identified using DESeq (2012) R package functions estimateSizeFactors and nbioTest. The threshold of $|\log_2FC| > 1$ and $P < 0.05$ was set for significantly differential expression. Hierarchical cluster analysis of DEGs was performed to explore transcript expression patterns.

Preparation of the coatings on vascular stents

Bare BMSs (18 mm × 2.70 mm; 316L SS for leporine model and CrCo for swine model; Kossel Medtech Co., Ltd., China) pre-deposited with P(DA-co-HDA) were dipped into the precursor solutions of the blank hydrogel or EL dressing. The stents were carefully pulled out with tweezers and rolled on clean glass slides to remove excess liquid. Subsequently, the precursor solutions left on the stents were cured at 37 °C in a humidified atmosphere for 12 h. The dip-coating procedure was repeated for twice to increase the thickness of the coatings and they were allowed to cure for another two days.

Mechanical stability testing of the coatings

Two BMSs of 316L stainless steel were pre-deposited with P(DA-co-HDA) and then dip-coated with

the blank hydrogel or EL dressing. Afterwards, they were loaded onto balloon catheters and compressed until close contact. The stent encapsulated by the blank hydrogel was dilated in PBS at 37 °C by inflating the balloon until the pressure reached 8 MPa, which was maintained for one minute. The other stent encapsulated by the EL dressing was dilated in a catheter (D_{in} : 3 mm, D_{out} : 4 mm) and then constantly flushed by PBS (37 °C, $Q = 120 \text{ mL min}^{-1}$ or $v = 28.3 \text{ cm s}^{-1}$) for 1 week. Both stents were photographed using the inverted fluorescence microscope (CKX53) before and after testing.

Thrombogenicity test in extracorporeal circulation

A total of 8 male New Zealand white rabbits (~4 month old, 2.5~3.0 kg) were used in this test. Each rabbit was anesthetized by intravenous injection (through marginal ear vein) of pentobarbital sodium salt solution (30 mg mL⁻¹ in saline, 1 mL kg⁻¹). The left carotid artery and right external jugular vein of the rabbit were isolated immediately. Subsequently, the animal was intravenously injected with GSNO (10 mM in saline, 0.1 mL kg⁻¹) and GSH (30 mM in saline, 0.1 mL kg⁻¹) solutions. Afterwards, an arteriovenous extracorporeal circuit was established by cannulating the left carotid artery and the right jugular vein. The circuit had four parallel shunt catheters in the middle and each contained a curled 316L stainless steel foil (15 mm × 8 mm × 0.02 mm) that was either bare or had been covered with a coating (~24 μL). The blood flow through the circuit was started by removing the hemostatic clamps on the artery and vein. After 3 h, the blood flow was stopped using the clamps again, and the animal was euthanized by injecting excess pentobarbital sodium salt solution (3~5 mL) when the circuit had been removed. The catheter segments containing the specimens were cut with scissors, extensively flushed by heparin solution (50 U mL⁻¹) and then photographed. The thrombogenicity of a specimen was assessed by measuring the occlusion and patency of the host catheter segment as well as the weight of thrombus formed on the guest foil. After that, the specimens were fixed by glutaraldehyde (2.5 v/v% in saline), dehydrated by gradient alcohols (50 v/v%, 75 v/v%, 90 v/v%, 100 v/v%, each for 1 h), dried in air, sputter-coated with gold and then examined using a scanning electron

microscope (SEM, Quanta 200, FEI, USA).

Vascular stent deployment in rabbits

A total of 36 male New Zealand white rabbits (~3 months old, 2.0~2.5 kg) were used for this study, which were raised with high-fat diet. The vascular stent encapsulated by the EL dressing containing 1.0 mM SeCA was assumed as the experimental group and BMS of 316 L stainless steel as the control group. Two stents (one for each group) were implanted bilaterally into the left (experimental group) and right (control group) iliac arteries of each rabbit. The arteries were over dilated to induce injury by increasing the pressure of the balloon catheter to 10 atm and maintaining it for 1 min. The animals were intravenously injected with GSNO (10 mM in saline, 0.1 mL kg⁻¹) and GSH (30 mM in saline, 0.1 mL kg⁻¹), and intramuscularly with benzylpenicillin sodium salt solution (20,000 u mL⁻¹, 0.5 mL kg⁻¹) every day for 7 consecutive days since stent implantation. At 1 week, 1 month and 3 months post implantation, the stented arteries ($n = 12$ for each group at each time point) were explanted and the animals were euthanized with excess pentobarbital sodium salt solution.

Vascular stent deployment in pigs

Four types of vascular stents were compared in the preclinical trial in pigs. All of them were constructed based on the same kind of CoCr BMSs purchased from Kossel Medtech to avert the disturbance of stent design in the outcome. EL dressing and blank hydrogel-coated stents were prepared by ourselves as aforementioned, while DES and polymer-coated stent were produced by this company. Poly(vinylidene fluoride-co-hexafluoropropylene) (PVDF-HFP) was utilized as the polymer coating and everolimus was assumed as the anti-restenotic drug ($8.5 \pm 2.1 \mu\text{g mm}^{-1}$).

A total of 12 ($n = 6$ for each time point) male Bama miniature pigs (~ 6 months old, ~30 kg) were used for this study. The pigs had been fed with clopidogrel (75 mg) and aspirin (100 mg) every day since three days prior to stent deployment and was fasted overnight before it. Each animal was sedated

with Zoletil® 50 (50 mg mL⁻¹, 5 mg kg⁻¹; Virbac, France) and ketamine (50 mg mL⁻¹, 10 mg kg⁻¹). Afterwards, it was intubated and then connected to a ventilator (DM-6A, Superstar Medical Equipment, China). Anesthesia was maintained with isoflurane (2 v/v%) throughout the procedure. After administering heparin (200 U kg⁻¹) and antibiotic prophylaxis intravenously, the right femoral artery was exposed under sterile condition. Four vascular stents (one for each type) were randomly implanted into three or four coronary arteries through a 6 Fr catheter under the guidance of DSA (Innova IGS 530, GE Healthcare, USA). The stents were dilated till the stent-to-artery diameter ratio reaching ~1.1 and the pressure was maintained for 30 s. The animals were also intravenously injected with GSNO (10 mM in saline, 0.1 mL kg⁻¹) and GSH (30 mM in saline, 0.1 mL kg⁻¹) every day for 7 consecutive days since stent implantation. At 2 weeks and 3 months post implantation, the stented arteries were explanted after the patency of them had been checked with DSA. In the end, the animals were euthanized with excess potassium chloride solution at anesthesia.

Characterization of the stented arteries

The harvested stented arteries were extensively washed with heparin solution (50 U mL⁻¹) and then cut transversely into two segments. One half of the segments were fixed in paraformaldehyde solution while the other half of them were further cut lengthwise and then fixed in paraformaldehyde or glutaraldehyde solution.

The columnar segments in paraformaldehyde solution were dehydrated by gradient alcohols, dried in air and then cured in methyl methacrylate (with 2 w/v% benzoyl peroxide, 50 °C, 24 h). Afterwards, the solidified resins were sliced by a hard tissue microtome (BQ1600, Lan Ming Medical Treatment, China). The generated tissue sections were treated by a van Gieson staining kit (Cat. No.: abs9349, Absin Bioscience, China) following the protocol provided by the vendor, and then photographed using an optical microscope (CKX41, Olympus, Japan).

The semi-columnar segments in paraformaldehyde solution were further permeabilized with Triton

X-100 solution for 3 h and then blocked with BSA solution for 12 h. Subsequently, they were incubated with mouse anti-rabbit CD31 antibody (IgG, Cat. No.: NBP2-44342, Novus Biologicals, USA, 1/100 dilution in PBS) or mouse anti-porcine CD31 antibody (IgG, Cat. No.: NB100-65336, Novus Biologicals, USA, 1/100 dilution in PBS) for 6 h, and then Alexa Fluor® 488-conjugated goat anti-mouse IgG secondary antibody (Cat. No.: abs20013, Absin Bioscience, 1/100 dilution in PBS) for another 6 h. After that, they were simultaneously stained with phalloidin-TRITC and DAPI for 6h. Finally, the samples were photographed using a CLSM (A1 Plus, Nikon, Japan) after being extensively washed with PBS. The remaining semi-columnar segments in glutaraldehyde solution were dehydrated by gradient alcohols, dried in air, sputter-coated with gold and then examined by SEM.

Statistical analysis

All data were expressed as mean $\pm$ s.d. with the sample size (n) indicated in figure legends. One-way analysis of variance (ANOVA) with Tukey post-hoc test was performed for comparison among multiple groups. Independent two-tailed student's t -test was assumed to determine the difference between two groups in some cases.

Ethical issues on animal experiments

All procedures on the rabbits were approved by the Institutional Animal Care and Use Committee of Sun Yat-Sen University and by the Committee on Ethics in Medical Research of Southwest Jiaotong University.

Reporting summary

Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

The authors declare that all data supporting the findings of this study are available within this article and its Supplementary Information files. Additional data are available from the corresponding authors upon reasonable request.

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Figures

Figure 1

Development of a tough EL dressing for vascular stents using a de novo designed hydrogel. a, Schematic for the design of our EL dressing. b, Shear moduli (at 37 °C) of the hydrogels formulated with A-M of varying degrees of maleimidyl modification (DMM) and gelatin at different mass ratios. c, Comparison in shear modulus among the selected hydrogels at 25 °C and 37 °C (n = 6). d, Tensile testing of them at ambient temperature. The insets on the right exhibit the photographs of A-M(9.3)/G_4/6 hydrogel prior to

and during extension. (Scale bar: 1 cm) e, Radar charts showing their scores in shear modulus (at 37 °C) (A), fracture strength (B), fracture strain (C), toughness (D), and capacity for further modification (E). Dashed frameworks represent the average values. f, Photographs of a vascular stent coated with A-M(9.3)/G_4/6 hydrogel before (left) and after (right) balloon dilation in PBS at 37 °C. g, Fluorescence images of it before (left) and after (right) balloon dilation. The hydrogel coating was labeled with FITC.

Figure 2

Mechanism on the toughness of A-M(9.3)/G_4/6 hydrogel. a, Effects of different chemical modifications to gelatin on the molecular structure, melting point and tensile behavior of its hydrogel. b, Comparison in tensile behavior among A-M(9.3)/G_4/6, A-M(9.3)/GG_4/6 and A/G_4/6 hydrogels. c, Schematic illustration for the mechanism on the toughness of A-M(9.3)/G_4/6 hydrogel.

Figure 3

Catalytic generation of NO from the EL dressings. a, Mechanism on the catalytic generation of NO from NO donors (R'S-NO) by SeCA conjugated to alginate. b, Representative curves of NO generation from GSNO (10 μM) in PBS (pH 7.4) at 37 °C with the blank hydrogel or EL dressings conjugated with varying contents of SeCA (0.2 to 1.0 mM). c, Correlation between the fluxes and rates of NO generation catalyzed by the EL dressings. d, Summary on the release features of NO from the EL dressings conjugated with varying contents of SeCA (n = 6). e, Quantification of the immobilized SeCA on the EL dressings after catalytic generation of NO. f, Release rates of NO from GSNO catalyzed by the EL dressing conjugated with 1.0 mM SeCA after pre-incubation in PBS at 37 °C for different durations (n = 6). One-way analysis of variance (ANOVA) with Tukey post-hoc test was performed to determine the difference among various groups. (n.s., not significant; ####P < 0.0001 compared to other groups; **P < 0.01 between two groups)

Figure 4

Effects of the EL dressings on cellular behaviors in vitro. a, Fluorescence images exhibiting the competitive adhesion between HUVECs and HUASMCs on various substrates. The cell growth medium was supplemented with GSNO (10 μ M) and GSH (30 μ M). (Scale bar: 500 μ m) b, Quantitative analyses on the competitive adhesion between HUVECs and HUASMCs ($n = 6$). c, Confocal laser scanning microscopy (CLSM) images displaying the adhesion, spreading and proliferation of HUVECs seeded onto various substrates in the presence of GSNO. (Scale bar: 100 μ m) d-f, Summary of cell density, cell coverage and individual cell area on those substrates ($n = 6$). g, CLSM images showing the formation of adherens junctions (VE-cadherin) between HUVECs grown on the EL dressing containing 1.0 mM SeCA. (Scale bar: 20 μ m) h, Proliferation assay of HUASMCs co-cultured with the blank hydrogel or EL dressings containing varying contents of SeCA in the presence of GSNO. i, Migrations of HUASMCs on bare

stainless steel, the blank hydrogel and EL dressing containing 1.0 mM SeCA. One-way ANOVA with Tukey post-hoc test was performed to determine the difference among various substrates and two-tailed Student's t-test was assumed to determine the difference between the two types of cells on the same substrate. (####P < 0.0001 compared to other groups; *P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001).

Figure 5

Transcriptome analysis of HUASMCs. a, Principal component analysis (PCA) representing the general variations in gene expression of HUASMCs among different groups. The EL dressing containing 1.0 mM SeCA was selected as the delegate. b-d, Differential gene expression heat maps of HUASMCs after various treatments when compared to the blank control group. The gene expression levels for each set of comparison were normalized to the mean values within those two groups. e, Pie charts displaying the changes and numbers of significantly differential gene expression related to inflammation, proliferation, or apoptosis. The area of a pie represents the number of genes involved in. The gridded region represents the total number of anti-inflammatory, anti-proliferative, or anti-apoptotic alterations in gene expression. f,

Heat map showing the relative changes in expression level for selected genes when compared to the blank control group. g, Schematic illustration of NO signaling pathway.

Figure 6

Vascular stent deployment in rabbits. a, Schematic illustration for vascular stent deployment in rabbit iliac arteries. b, Optical images showing the cross-sections of stented arteries after van Gieson staining. (Scale bar: 500 μm) c, Quantitative analyses on the cross-sections (n = 12). d, CLSM images unveiling the endothelialization on the stents (outlined by the dashed lines). The right panels present the fluorescence intensities of different cell components along the line segments (OA, O'B) in the images. (Blue: cell nucleus, green: CD31, red: F-actin; scale bar: 50 μm). e, SEM images showing the luminal faces of stented arteries at 3 months post stent deployment. One-way ANOVA with Tukey post-hoc test was performed to determine the difference among various groups and Student's t-test was assumed to determine the difference between two groups. (n.s., not significant; *P < 0.05 and ****P < 0.0001)

Figure 7

Vascular stent deployment in pigs. **a**, Schematic illustration for vascular stent deployment in swine coronary arteries. **b**, Digital subtraction angiography prior to the harvest of stented arteries. The white arrows indicate the sites of implanted stents. The yellow arrow refers to severe restenosis occurring in a polymer-coated stent. (Scale bar: 1 cm) **c**, Photographs displaying the luminal faces of stented coronary arteries at 2 weeks and 3 months post stent deployment. (left to right: EL dressing-coated stent, blank hydrogel-coated stent, DES and polymer-coated stent; scale bar: 5 mm) **d**, Optical images showing the cross-sections of stented arteries after van Gieson staining. (Scale bar: 500 μ m) **e**, Quantitative analyses on the cross-sections (n = 6). **f**, CLSM images unveiling the endothelialization on the stents (outlined by the dashed lines). (Blue: cell nucleus, green: CD31, red: F-actin). **g**, SEM images showing the luminal faces of stented arteries at 2 weeks and 3 months post stent deployment. Student's t-test was performed to determine the difference. (*P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001 between two groups; ####P < 0.0001 compared to other groups)

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