Supporting Information

Highly swellable and biocompatible graphene/heparin-analogue hydrogels for implantable drug and protein delivery

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1. Preparation and characterization of graphene oxide (GO)

Graphene oxide (GO) was prepared from natural graphite flakes by a modified Hummers method.[1] Briefly, 5 g graphite and 3.75 g $NaNO_3$ were placed in a flask. Then, 150 mL H_2SO_4 was added with stirring in an ice-water bath, and then 20 g $KMnO_4$ was slowly added for over 1 h. The mixture was stirred in an ice water bath for 2 h, followed by a vigorously stirring for 3 days at room temperature. Then, the mixture was diluted with DI water (500 mL) slowly, and the excessive $KMnO_4$ was decomposed by H_2O_2 (30 wt.%, 15 mL). The insoluble precipitations were removed by centrifugation. The resulted GO solution was filtered and washed with HCl (10 wt.%, 1 L) and DI water for several times to remove the metal ions. The pristine brown GO solution was dialyzed with DI water for 1 week before use to remove any residual salts and acids.

The prepared GO was further characterized with several methods. A Multimode Nanoscope V scanning probe microscopy (SPM) system (Bruker, USA) was used to obtain atomic force microscopy (AFM) images. The samples were prior prepared by dropping and drying on silicon wafers for the AFM analysis. Morphological observation was carried out with a transmission electron microscope (TEM) (JEM-1200EX, JEOL, Japan). Two to three drops of the solution (1 mg/mL) were spread on a 3 mm copper grid and dried at ambient temperature to prepare the TEM sample. FTIR spectrum was acquired on a FTIR spectrometer (Nicolet 560, USA) between 500 and 4000 cm^{-1}, using the KBr disk method. A Q500 Thermogravimetric analyzer

(TA instruments, USA) was used to get the thermogravimetric analysis (TGA) curves of the hydrogels under a dry nitrogen atmosphere, and the temperature ranged from 50 ºC to 700 ºC with a heating rate of 10 ºC/min.

2. Preparation of the hydrogels

Table S1. The synthesis conditions for the polymeric hydrogels.

Sample	AA	SSNa	GO	MBA	APS	DI water
	mg	mg	mg	mg	mg	mL
HH-M1	450	300	-	7.5	7.5	5
GHH1-M1	450	300	0.75	7.5	7.5	5
GHH5-M1	450	300	3.75	7.5	7.5	5
GHH15-M1	450	300	11.25	7.5	7.5	5
GHH25-M1	450	300	18.75	7.5	7.5	5
GHH25-M0.1	450	300	18.75	0.75	7.5	5
GHH25-M0.5	450	300	18.75	3.75	7.5	5
GHH25-M2	450	300	18.75	15	7.5	5

3. Characterization of the chemical structure and stability of the hydrogels

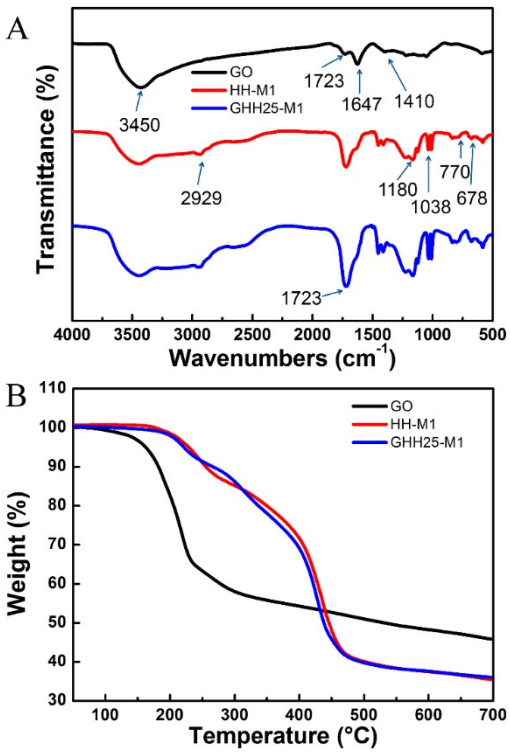

Figure S1. (A) FTIR spectra for GO, HH-M1 and GHH-M1 hydrogels. (B) The TGA curves for GO, HH-M1 and GHH-M1 hydrogels.

4. Compressive test of the hydrogels

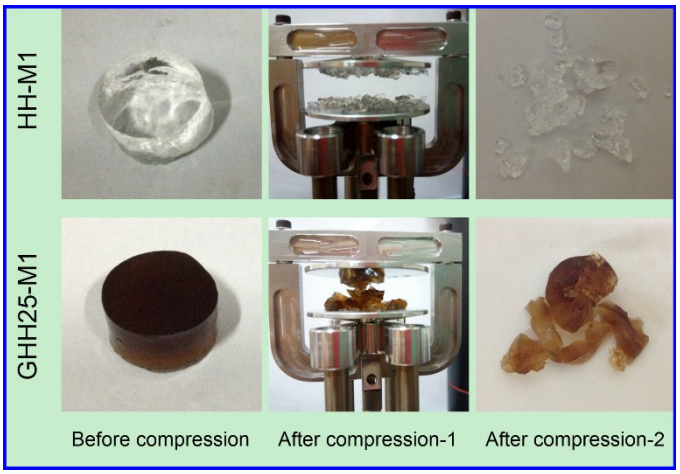

Figure S2. Photographs of the swollen hydrogels (HH-M1 and GHH25-M1) before and after compression.

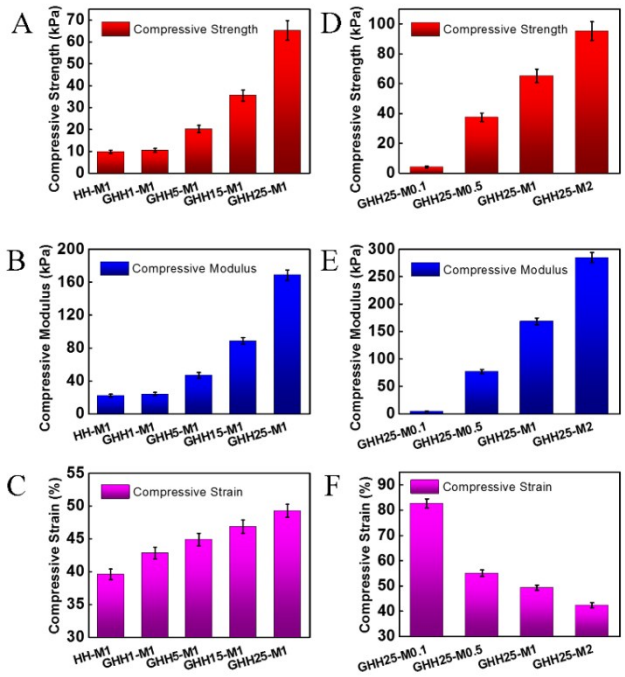

Figure S3. (A) Compressive strength, (B) Compressive modulus and (C) Compressive strain of the swollen hydrogels with various amounts of GO. (D)

Compressive strength, (E) Compressive modulus and (F) Compressive strain of the swollen hydrogels with various amounts of cross-linker MBA. Values are expressed as means ± SD (n = 3).

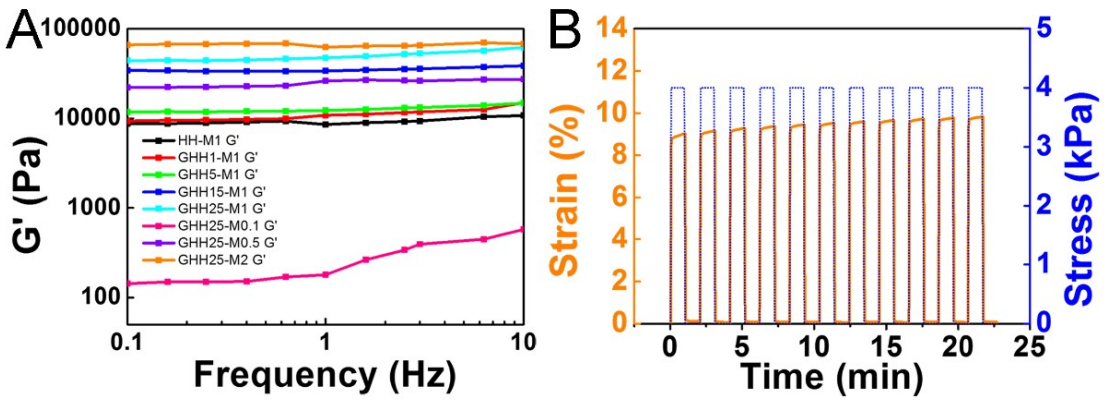

Figure S4. (A) Storage modulus (G′) of the hydrogels in equilibrium swollen state vs. the frequency. (B) The anti-fatigue performance of the swollen GHH25-M1 hydrogel by a constant stress loading and unloading, DMA creep mode with 4 kPa loading stress for 10 cycles.

5. Hemocompatibility

5.1. Red blood cell morphology

For the red blood cell (RBC) morphology observation, the red cells incubated with hydrogel suspensions (5000 μg/mL) for 3 h were immediately fixed with 1 mL 2.5% (wt.%) glutaraldehyde in PBS at 4 °C for 12 h.[2] And then, the red cell contained precipitates were diluted and carefully deposited onto clean silicon wafer by a pipette, washed twice with PBS, and subjected to a drying process by passing them through a series of graded alcohol–PBS solutions (30, 50, 70, 80, 90, 95 and 100%, 10 minutes

for each time). The critical point drying of the specimens was done with liquid CO_2. The specimens were sputter-coated with a gold layer and examined by a scanning electron microscope (JSM-7500F, JEOL).

5.2. Platelet morphology

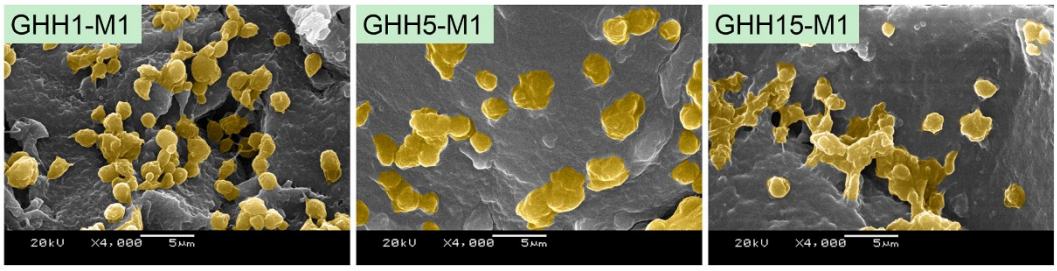

Figure S5. SEM images for the platelet adhered hydrogels. The scale bars: 5 μm.

5.3. ELISA

Contact activation and complement activation are important parameters to evaluate the reaction or interaction between blood and materials; they are also direct methods to evaluate the blood compatibility of material. Commercial enzyme-linked immunosorbent assays (ELISA) were used to evaluate the contact activation, including platelet activation (Platelet Factor 4 (PF4), Boatman Biotech Co., Ltd, China) and coagulation cascade activation (thrombin–antithrombin III complex (TAT), Enzygnost TAT micro, Assay Pro, USA), as well as the complement activation (C3a and C5a, BD Opt EIA™, BD Co., Ltd, US) for the prepared hydrogels. The whole blood incubated with the hydrogel for 2 h was centrifuged for 15 min at 1000 g (4 °C) centrifugal force to obtain the testing plasma. Then, the detections were carried out according to the respective instruction manuals.[3]

5.4. Anti-coagulant activity

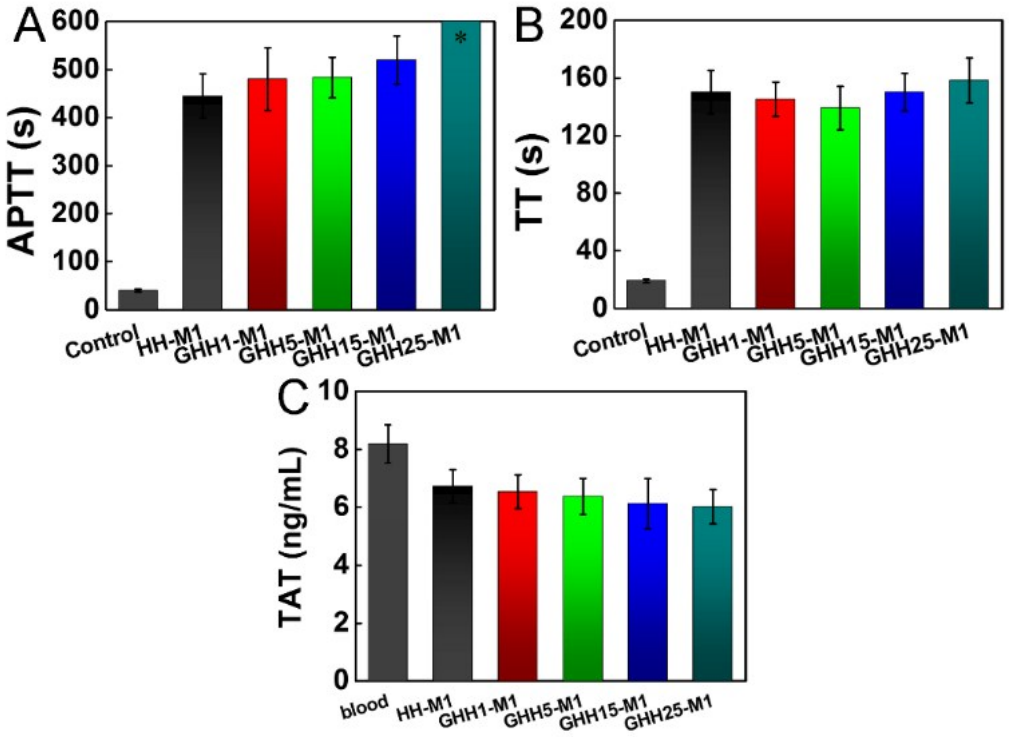

Figure S6. (A and B) APTT and TT values for the hydrogels. For the control group, 5 μL PBS was added instead. (C) Thrombin–antithrombin (TAT) concentrations for the samples. Values are expressed as means ± SD (n = 3). The marks (*) meant incoagulable.

6. Cytocompatibility

Human umbilical vein endothelial cells (HUVECs) were grown in R1640 medium supplemented with 10% fetal bovine serum (Hyclone, USA), 2 mmol L-glutamine and 1 vol.% antibiotics mixture (10,000 U penicillin and 10 mg streptomycin). Cultures were maintained in a humidified atmosphere of 5% CO_2 at 37 °C (Queue Incubator, France). Confluent cells were detached from the culture flask with

sterilized PBS and 0.05% trypsin/EDTA solution. The culture medium was changed every day.[4]

For fluorescence staining (fluorescein diacetate/propidium iodide (FDA/PI)), after cultured for 48 h, the culture polystyrene plate was washed three times by PBS solution, then 400 μL FDA (10 μg/mL, PBS) was added to each well, and the cell culture plate was placed into an incubator (37 °C, 5% CO_2). After incubating for 5 min, 200 μL PI (20 μg/mL, PBS) was added, and then the cell culture plate was washed three times by PBS solution. The whole experimentation was manipulated free from light. Then the cell culture plate was enwrapped in silver paper, and a fluorescence microscope (Olympus IX53, Japan) was used to observe the fluorescence images of the samples.

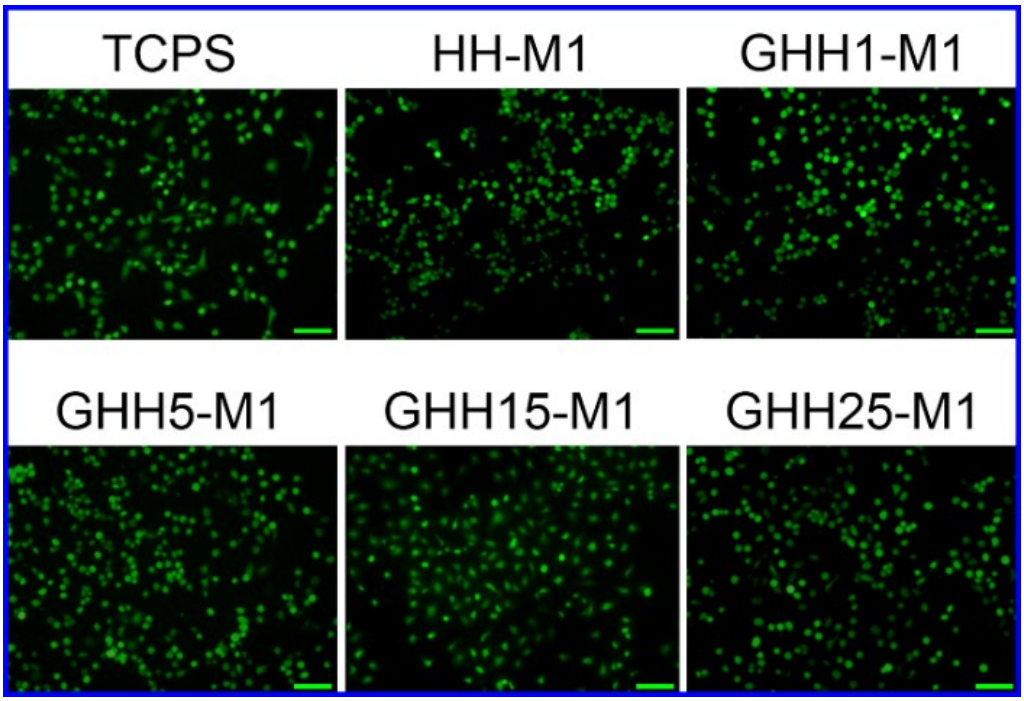

Figure S7. FDA/PI staining for the cells cultured with the hydrogels, the scale bars: 50 μm.

For confocal laser scanning microscopy observation, after culturing for 48 h, the cells were immediately rinsed with 37 °C PBS and fixed in 4 wt.% paraformaldehyde for 10 min. Then the cells were washed with PBS and further treated with 0.5% Triton X-100 in PBS for 5 min. The cellular actins were stained with Rhodamine-phalloidin (100 nM) in the dark for 30 min. The nuclei were stained with 4′, 6-diamidino-2-phenylindole (DAPI, 5 μg/mL) for 5 min. Subsequently, the stained cells were imaged with a confocal laser scanning microscope (CLSM, Leica).

The viability of the cells was determined by MTT assay after cell culture for different times (24, 48 and 72 h). The cells cultured in wells without hydrogel served as control in this study. For MTT assay, 50 μL of MTT solution (1 mg/mL in PBS) was added to each well and incubated for 4 h at 37 °C. Mitochondrial dehydrogenases of viable cells selectively cleave the tetrazolium ring, yielding blue/purple formazan crystals. Then, 400 μL of ethanol was added to dissolve the formazan crystals. Thus, the quantity of the formazan dissolved in the ethanol reflects the level of cell metabolism. The solution was shaken homogeneously for 15 min. The sample solution was aspirated into microtiter plates, and the optical density was read in a Microplate reader (model 550, Bio-Rad) at 492 nm. The results were expressed as the viable percentage of cells after various treatments relative to the control cells without any treatment. Cell viability was calculated by the following formula:[5]

$$Cell\ viability\ (\%) = \frac{Absorbance\ of\ test\ group}{Absorbance\ of\ control\ group} \times 100 \quad \text{(S1)}$$

The difference among individual groups was evaluated using the Student's t-test, and the level of significance was chosen as $P < 0.05$.

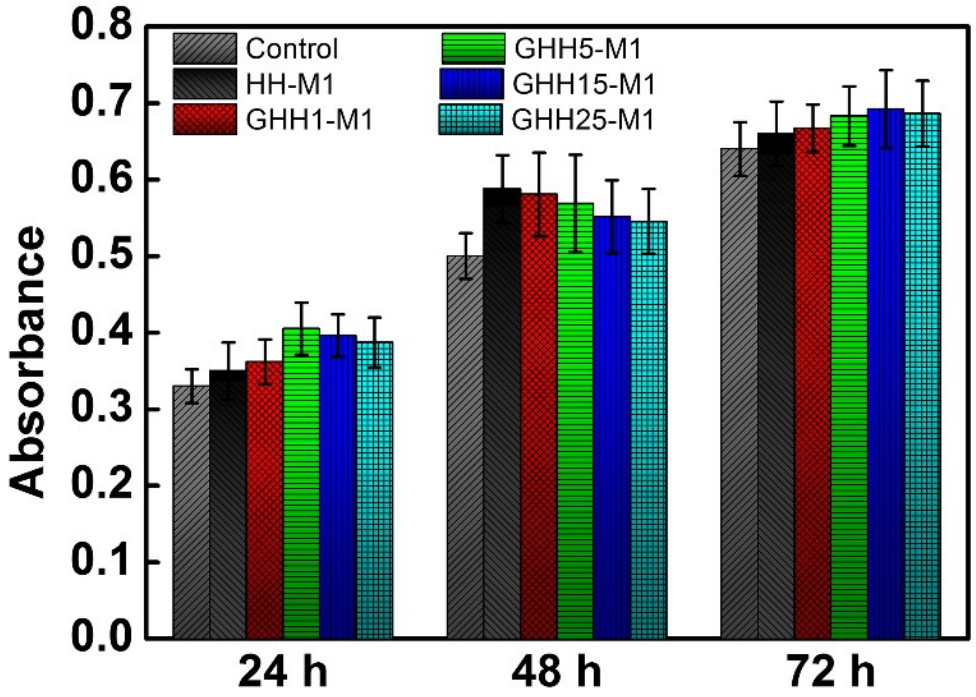

Figure S8. Absorbance of HUVECs determined from MTT assay after culturing for 24 h, 48 h and 72 h. Values are expressed as means ± SD (n = 3).

References

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5. W. Wu, W. Yao, X. Wang, C. Xie, J. Zhang and X. Jiang, *Biomaterials*, 2015, **39**, 260-268.