Electronic Supplementary Material (ESI)

Gelatin methacryloyl granular hydrogel scaffolds for skin wound healing

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The Supporting Information includes proton nuclear magnetic resonance (^{1}H NMR) spectroscopy, GHS drying, SEM imaging details, and Figures **S1** to **S5**.

Proton nuclear magnetic resonance (^{1}H NMR) spectroscopy

The degree of methacrylate/methacryloyl substitution (DS) of GelMA was calculated using ^{1}H NMR (400 MHz Bruker NEO, MA, USA) at the NMR facilities of The Pennsylvania State University, University Park. Gelatin powder and lyophilized GelMA were separately dissolved in deuterium oxide (D_2O, MilliporeSigma, MA, USA) (final concentration = 4 % w/v), followed by performing the NMR spectroscopy. The area under the lysine methylene proton peak for DS calculation was integrated via TopSpin 4.0.7 software. The aromatic acid peaks with the chemical shift around 7-7.5 ppm were considered as a reference. The change in the area under the lysine methylene proton peak at ~ 3.0 ppm was calculated based on equation **S1**, yielding the DS.

$$DS(\%) = \left(1 - \frac{Area\ under\ lysine\ methylene\ proton\ peak\ in\ GelMA}{Area\ under\ lysine\ methylene\ proton\ peak\ in\ gelatin}\right) \times 100 \quad \textbf{(S1)}$$

GHS drying and scanning electron microscopy (SEM) imaging

Ethanol solutions with concentrations of 70%, 80%, and 90% v/v were prepared using ultrapure water. Disk-shape GHS (diameter = 10 mm and height = 3 mm) were placed in a Petri dish, containing 10 mL of 70% v/v ethanol, and incubated for 10 min at room temperature. The samples were then sequentially incubated in Petri dishes with increasing ethanol concentrations at 10 min intervals. After incubation in 90% v/v ethanol, the samples were finally immersed in 100% v/v ethanol for 60 min at room temperature. Once ethanol treatment was completed, the scaffolds were left to dehydrate overnight in a desiccator at room temperature. For SEM imaging, the samples were coated with a thin gold layer (~ 5 nm) using a low-vacuum sputter coater (DESK V, Denton Vacuum LLC, NJ, USA). The surface morphology of dehydrated GHS was observed using a Hitachi tabletop SEM operated at 10 kV, with magnifications between 100x and 800x.

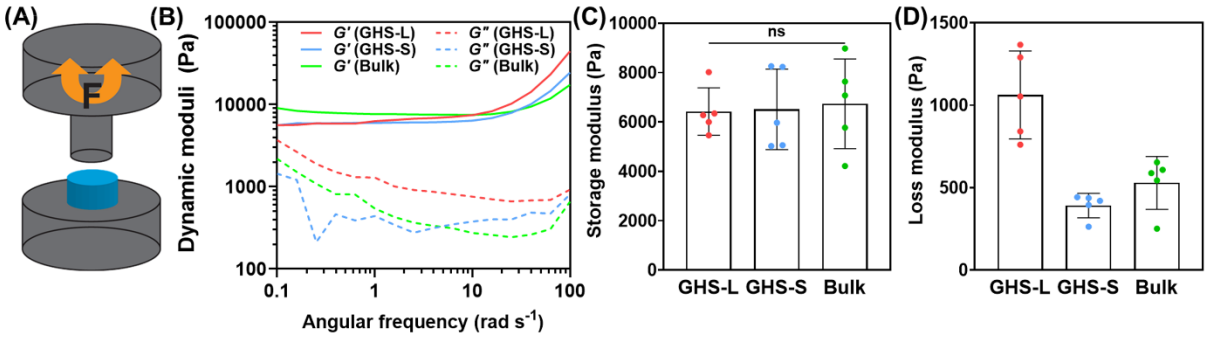

Figure S1. Rheological characterization of scaffolds. **(A)** Schematic of the oscillatory rheology experiment, conducted on the hydrogel scaffolds. **(B)** Representative storage (G') and loss (G'') moduli of scaffolds versus angular frequency at constant oscillatory strain (0.1%). The average **(C)** G' and **(D)** G'' of bulk scaffold, GHS-L, and GHS-S at an angular frequency of 1 rad s^{-1} and oscillatory strain of 0.1%.

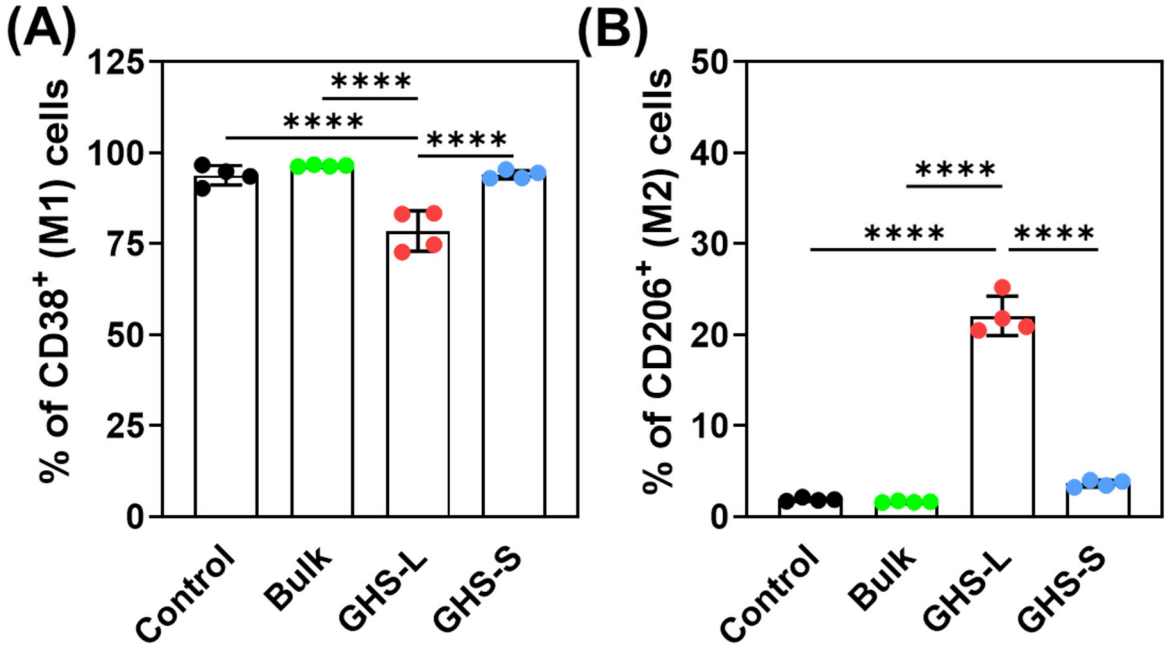

Figure S2. Flow cytometry quantification of BMDM polarization following culture. (A) Quantification of M1 macrophages ($CD38^+$ cells) and **(B)** M2 macrophages ($CD206^+$ cells). ****$p < 0.0001$.

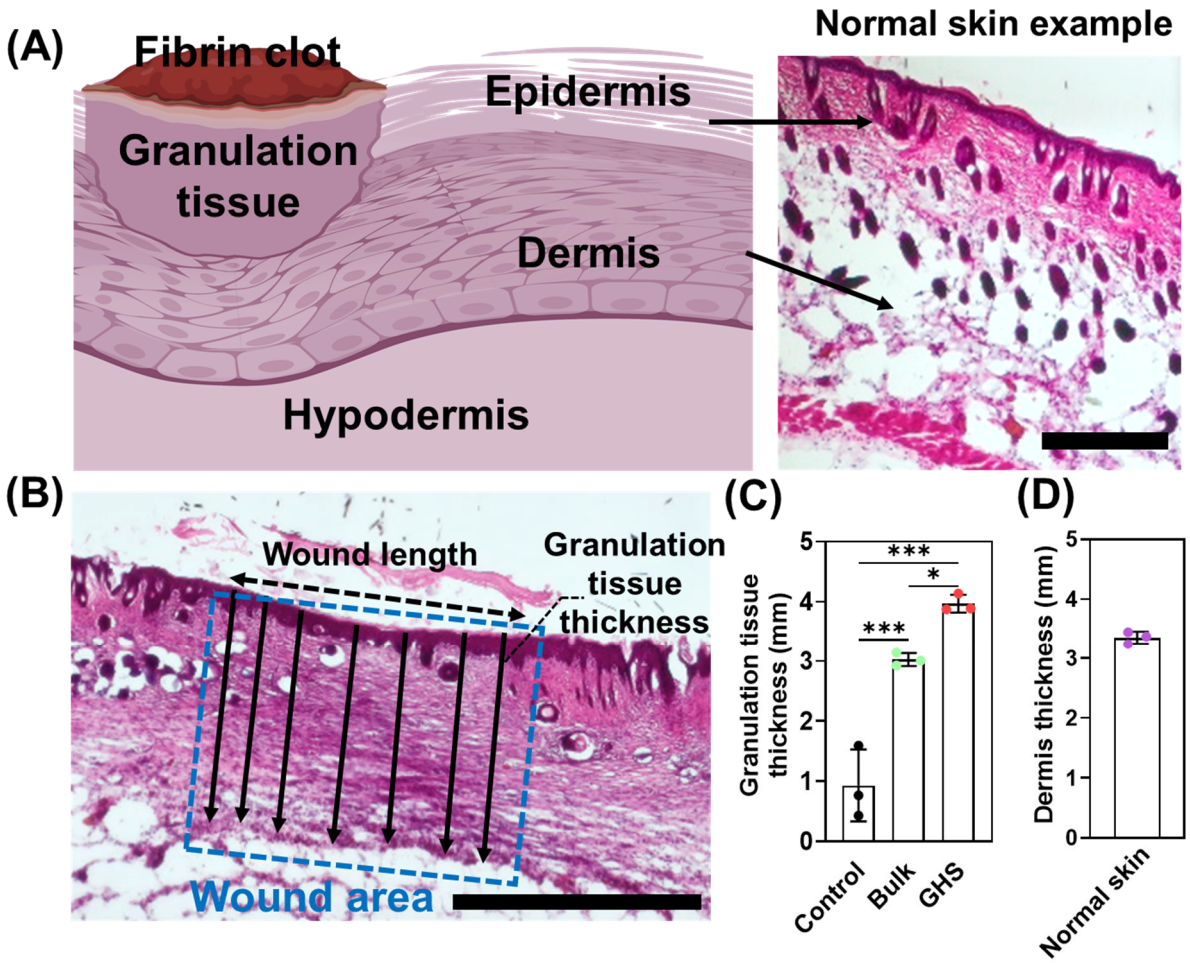

Figure S3. Wound closure area and granulation tissue thickness measurement. (A) The schematic of full thickness skin with granulation tissue, along with an H&E representative of normal skin tissue. (**B)** The H&E staining of collected skin tissue on day 11 after surgery, showing a magnified view of Figure 3D (main manuscript) for the GHS treatment. The wound area is shown with a blue dashed rectangle, the dashed double arrow shows the wound length, and the black arrows show the granulation tissue thickness. **(C)** Granulation tissue thickness, measured via dividing the wound area by wound length. **(D)** The dermis thickness of normal skin. Scale bars: (A) 500 μm and (B) 1 mm. *$p < 0.05$ and ***$p < 0.001$.

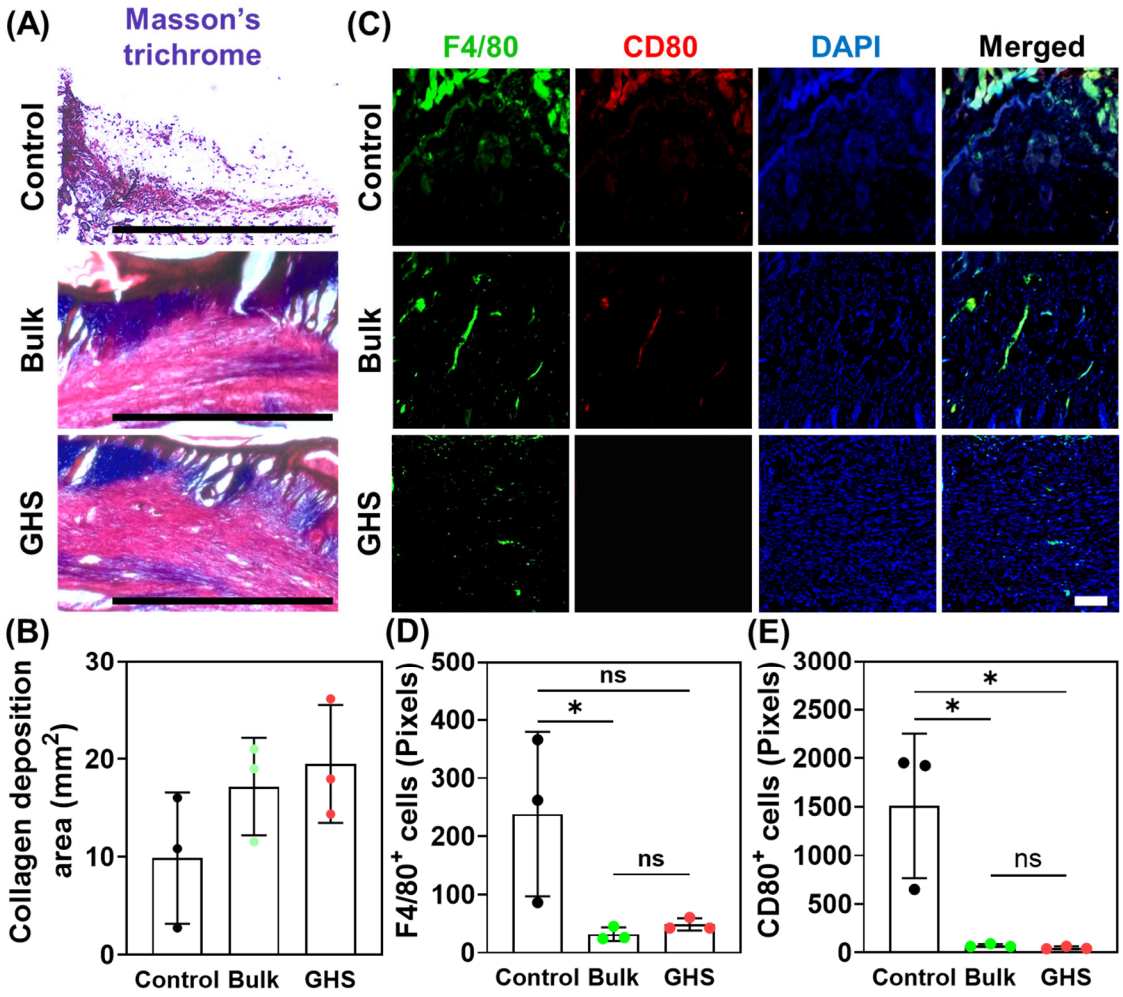

Figure S4. Histological characterizations of wound healing. (A) Masson's trichrome staining of tissues on day 11 after surgery to assess collagen deposition. **(B)** The quantification of collagen deposition area based on the Masson's trichrome staining. **(C)** The F4/80 and CD80 immunofluorescence staining of tissue samples on day 11 after surgery. **(D)** $F4/80^+$ cell quantification. **(E)** $CD80^+$ cell quantification. Scale bars: **(A)** 1 mm and **(C)** 100 μm. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, and $****p < 0.0001$.

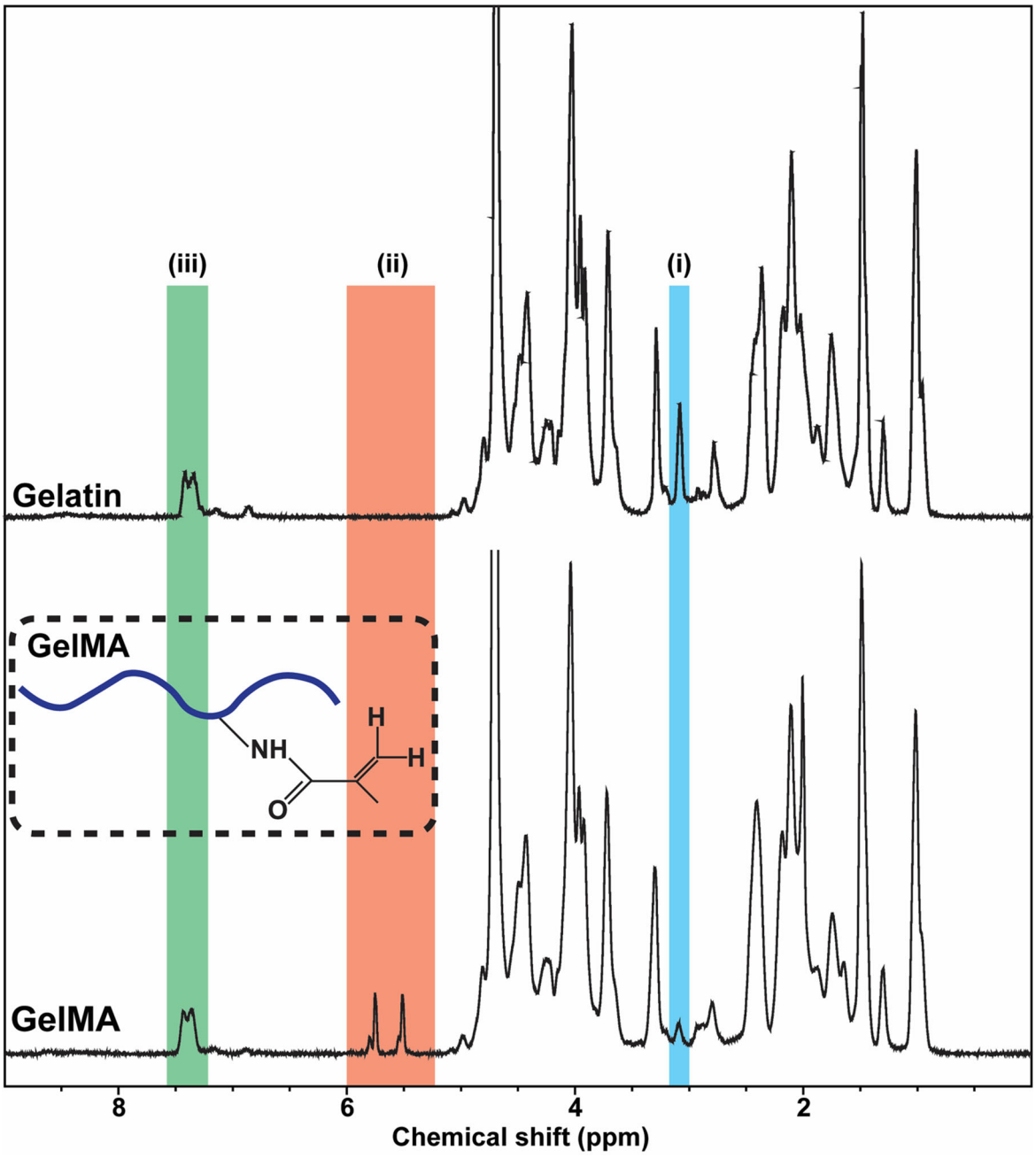

Figure S5. The ^{1}H NMR spectra of gelatin and GelMA (40 mg in 1 mL of D_2O), showing the MA modification of lysine groups in GelMA compared with the non-modified gelatin: (i) lysine proton peak, (ii) GelMA vinyl group proton peaks, and (iii) aromatic acid proton peaks in gelatin and GelMA, considered as a reference.