Adhesive Hydrogel Barriers Synergistically Promote Bone Regeneration by Self-Constructing Microstress and Mineralization Microenvironment

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Supplementary methods

1. In Vitro Degradation of PN-GEL@BP-PE Hydrogel Membrane

Enzymatic biodegradation of PN-GEL@BP-PE hydrogel membranes was evaluated using collagenase II, following established protocols.[1] Briefly, pre-weighed hydrogel specimens (8 cm × 2 cm × 0.5 cm) were immersed in a collagenase solution (2 μg/mL), corresponding to the enzyme concentration observed in vivo. The degradation assay was maintained at 37 °C, and the collagenase solution was refreshed daily to sustain continuous enzymatic activity. At predetermined time points (3, 6, 9, 12, 15, 18, and 21 days), samples were retrieved, blotted with filter paper to remove surface moisture, and re-weighed. The degree of in vitro degradation was expressed as the percentage of weight loss over time. Each condition was tested in triplicate to ensure reproducibility. In parallel, tensile tests were performed on hydrogel membranes at each time point to assess whether the collagenase-mediated degradation influenced their mechanical integrity and thus their ability to generate autonomous micro-stress during the degradation process.

2. Screening of BPN Concentration in GEL@BP hydrogel

Based on previously reported protocols, GEL@BP hydrogels were formulated with BPNs loadings of 0.01 ‰, 0.05 ‰ and 0.1 ‰ by weight, representing low, medium and high concentrations, respectively.[2] Each hydrogel formulation was photopolymerized and cast into 24-well plates, and BMSCs were seeded directly onto the hydrogel surfaces. After 24 h, cells were fixed and stained with rhodamine-phalloidin to visualize F-actin filaments and with DAPI to label nuclei. To assess cytocompatibility and proliferation, a Cell Counting Kit-8 (CCK-8; APExBIO, China) assay was performed. Briefly, BMSCs were plated in 96-well plates at 5×10^3 cells per well. After 24 h, the medium was replaced with hydrogel extracts (10 mg mL^{-1}) and cells were cultured for an additional 1,3 and 5 days, with PBS washes between treatments. At each time point, 100 μL α-MEM and 10 μL CCK-8 reagent were added per well; following a 2 h incubation at 37 °C, absorbance was measured at 450 nm. Finally, to evaluate early osteogenic differentiation, BMSCs were seeded onto each hydrogel in 24-well plates and cultured in osteogenic induction medium for 7 days. Cells were then fixed in 4 % paraformaldehyde for 30 min and ALP staining was visualized by NBT/BCIP staining (Beyotime, China).

Supplementary figures

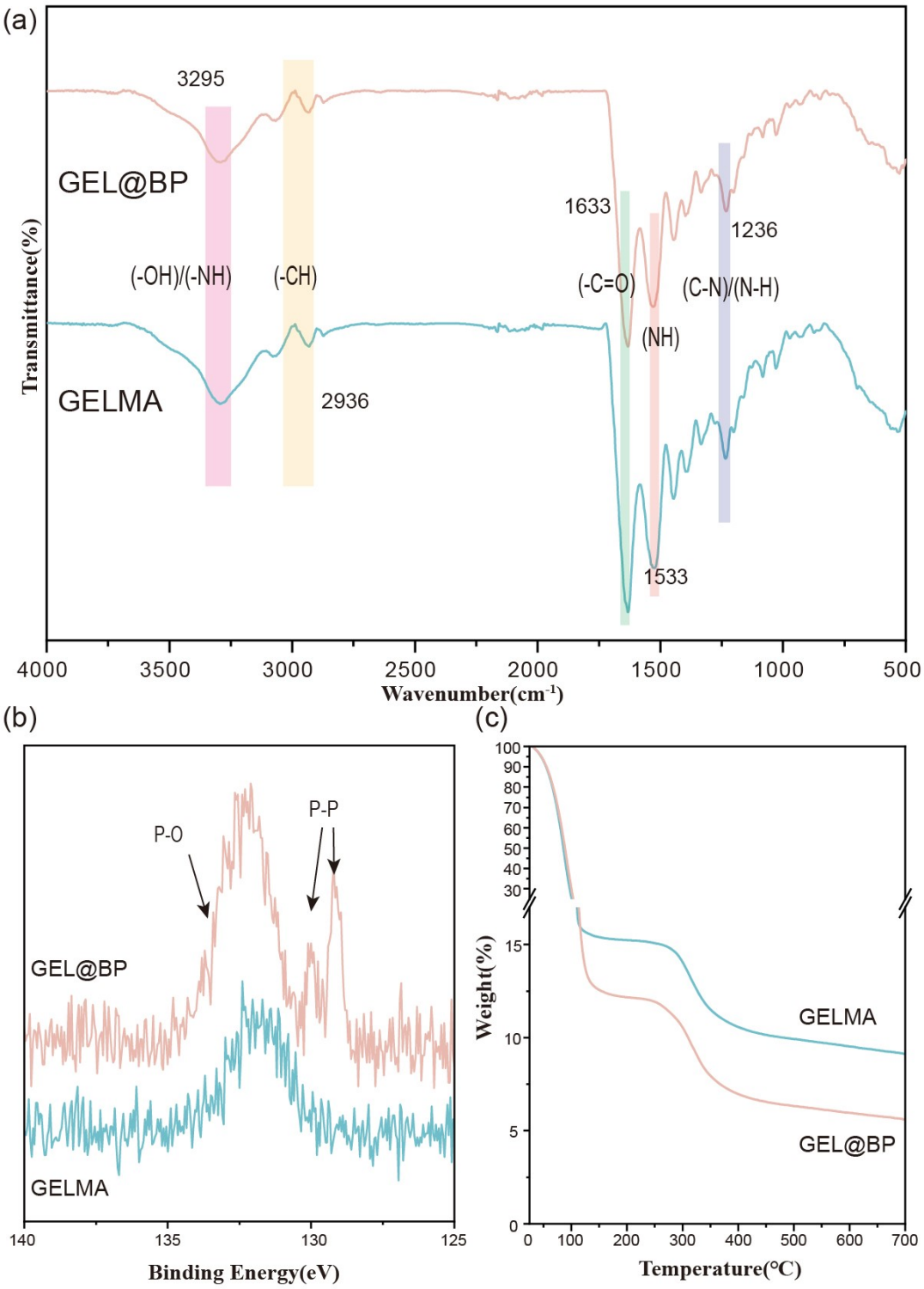


Fig.S1 (a)FTIR spectra of GELMA and GEL@BP. (b) XPS spectra of GELMA and GEL@BP. (c) TGA curves for GELMA and GEL@BP.

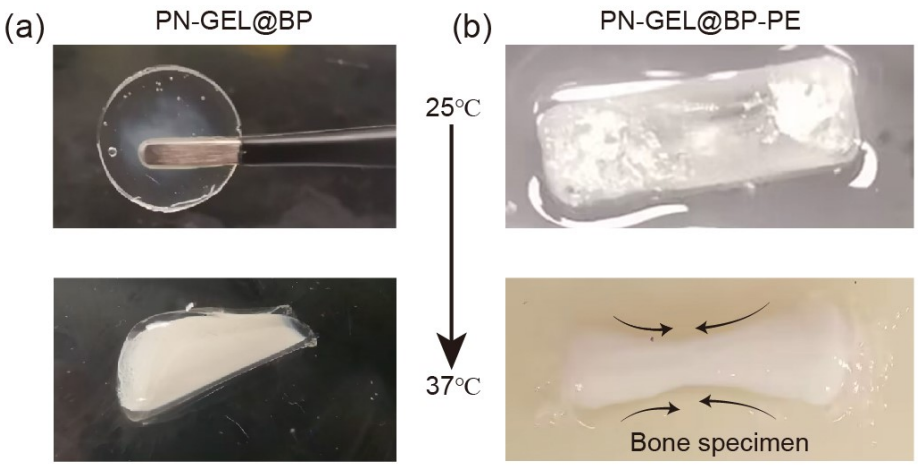
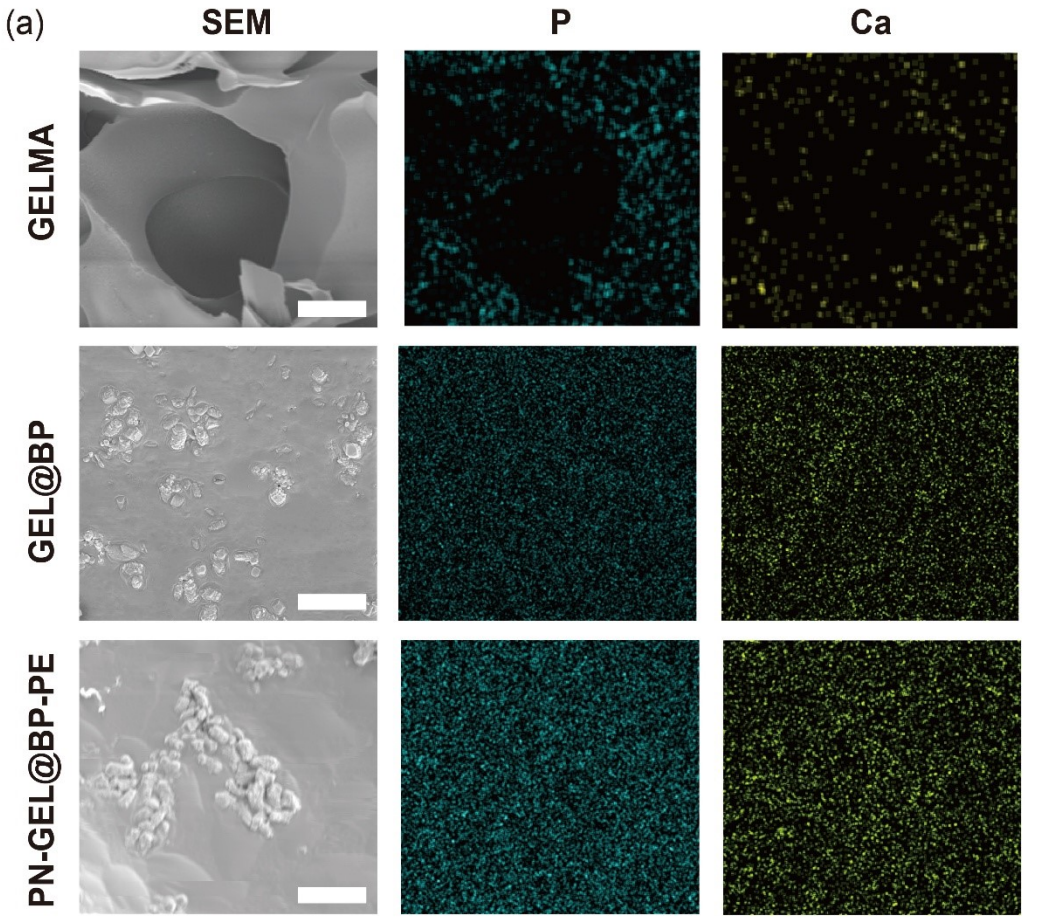


Fig.S2 (a) Images of the PN-GEL@BP hydrogel membrane to confirm the temperature-responsive volumetric deformation from 15℃ to 37℃. (b) Images of the PN-GEL@BP-PE hydrogel membrane to confirm that it can firmly adhere to the bone specimen while undergoing temperature-responsive volumetric deformation from 15℃ to 37℃.


(a)
SEM
P
Ca
GELMA
GEL@BP
PN-GEL@BP-PE

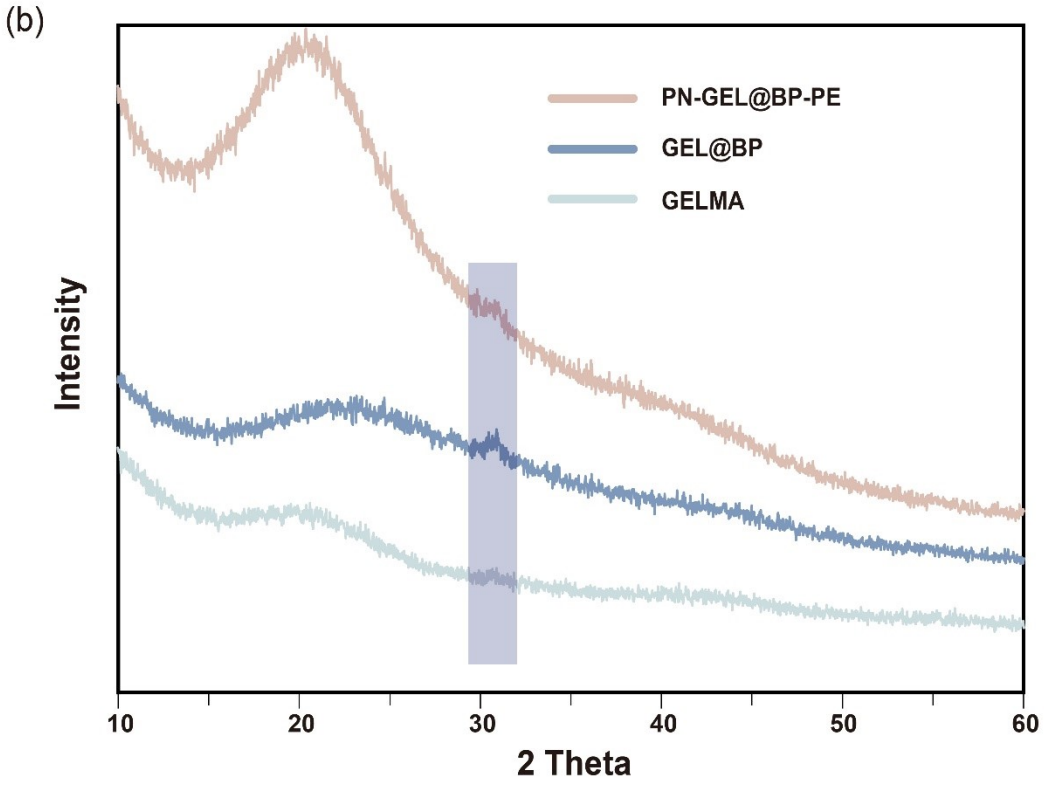

(b)
PN-GEL@BP-PE
GEL@BP
GELMA
Intensity
10
20
30
40
50
60
2 Theta

Fig.S3 (a) SEM and EDS images of GELMA, GEL@BP, and PN-GEL@BP-PE after in vitro mineralization (EDS elemental maps for Ca and P; scale bar = 10 μm). (b) XRD patterns of GELMA, GEL@BP, and PN-GEL@BP-PE following in vitro mineralization.

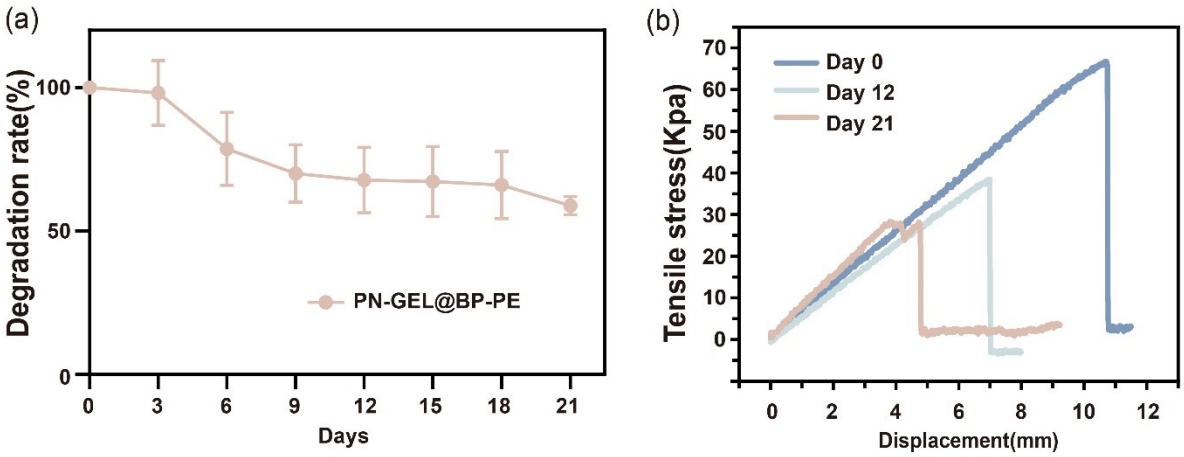


Fig.S4 (a) In vitro enzymatic degradation of PN-GEL@BP-PE. (b) Tensile stress–strain behavior of PN-GEL@BP-PE at days 0, 12, and 21 during the in vitro degradation assay.

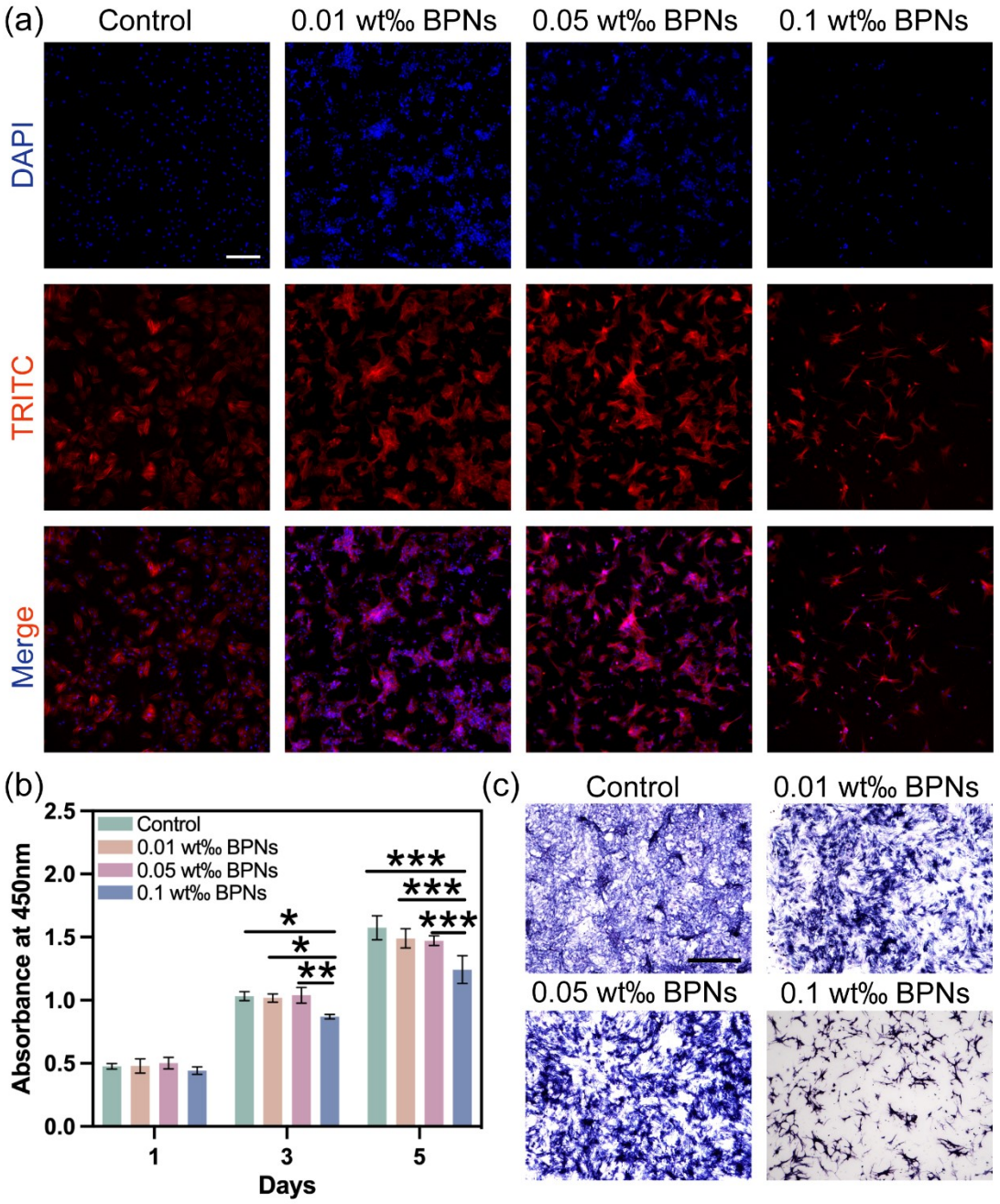


Fig.S5 (a) Cell immunofluorescence staining of BMSCs cultured for 24 hours on GEL@BP hydrogels containing 0.01 wt‰, 0.05 wt‰, and 0.1 wt‰ BPNs. Cell F-actin filaments were stained with rhodamine-phalloidin (red), and nuclei were stained with DAPI (blue) (bar=100 μm). (b) CCK8 assays performed at 1, 3, and 5 days to evaluate the proliferation of BMSCs cultured with GEL@BP hydrogel extracts (10 mg/mL) containing 0.01 wt‰, 0.05 wt‰, and 0.1 wt‰ BPNs. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (c) ALP staining of osteogenic induction of BMSCs cultured for 7 days on GEL@BP hydrogels containing 0.01 wt‰, 0.05 wt‰, and 0.1 wt‰ BPNs (bar=2mm). All data are Mean ± S.D. Statistics was calculated by one-way ANOVA followed by Tukey's post-test.

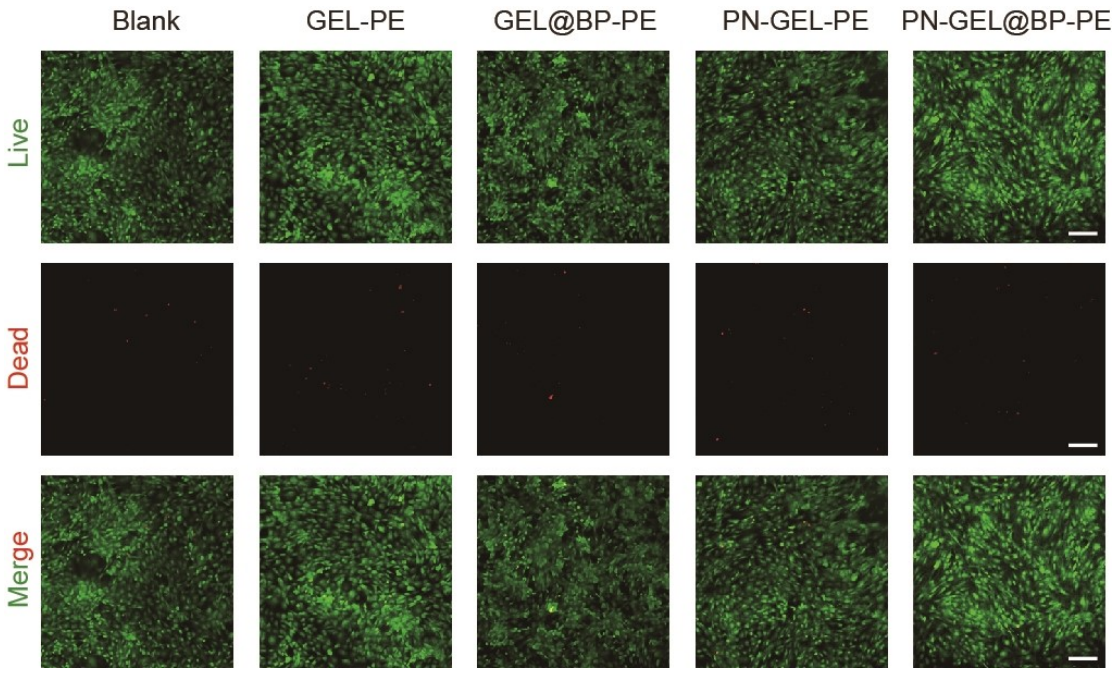


Fig.S6 Live/dead staining of the cells after incubating with the different hydrogel membrane extracts for 3 days (scale bar = 200 μm).

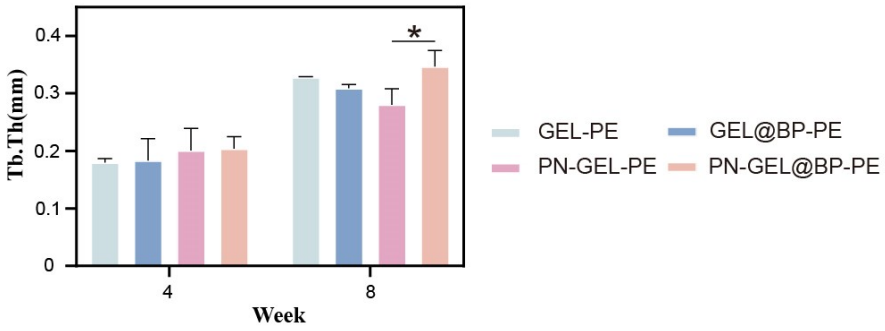


Fig.S7 Statistical analysis of Micro-CT (Tb.Th) at 4 and 8 weeks after implantation of different membranes. All of the data are presented as the mean ± SD(n=3). *$p < 0.05$. Statistics was calculated by one-way ANOVA followed by Tukey's post-test.

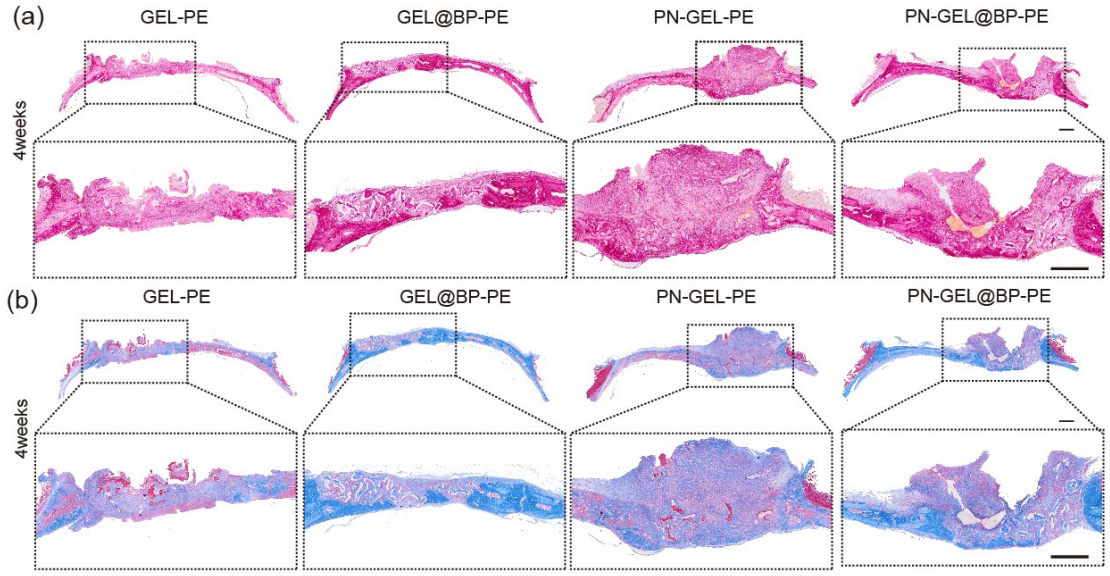


Fig.S8 (a) Sirius red staining and (b) masson staining at 4 weeks after implantation (Scale bar=1mm)

Video.1 Temperature-responsive deformation of PN-GEL@BP-PE

References

1. Y. Han, J. L. Yang, W. W. Zhao, H. M. Wang, Y. L. Sun, Y. J. Chen, J. Luo, L. F. Deng, X. Y. Xu, W. G. Cui and H. Y. Zhang, *Bioact. Mater*, 2021, **6**, 3596-3607.
2. K. Q. Huang, J. Wu and Z. P. Gu, *Acs Appl Mater Inter*, 2019, **11**, 2908-2916.