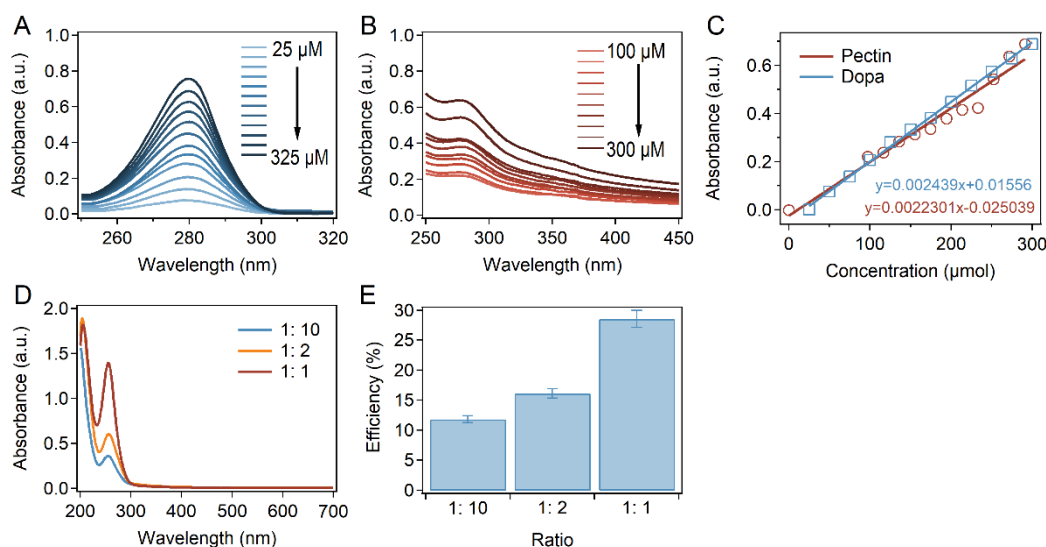


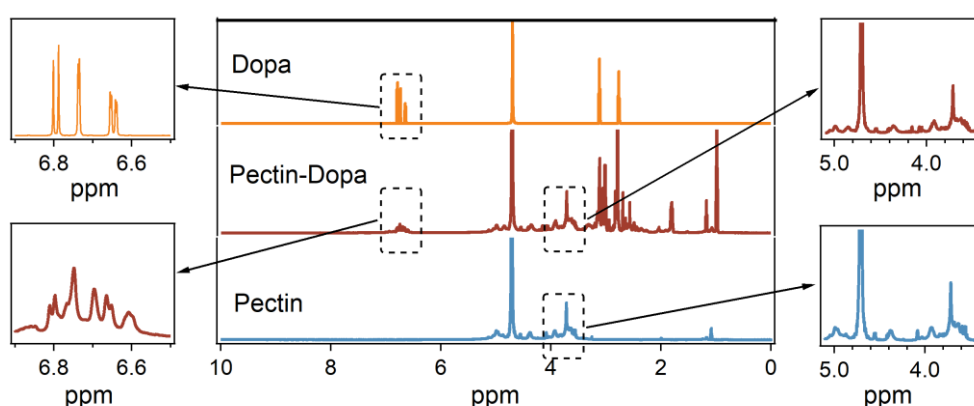
## Supplementary Information

# Engineering bio-adhesives based on protein-polysaccharide phase separation

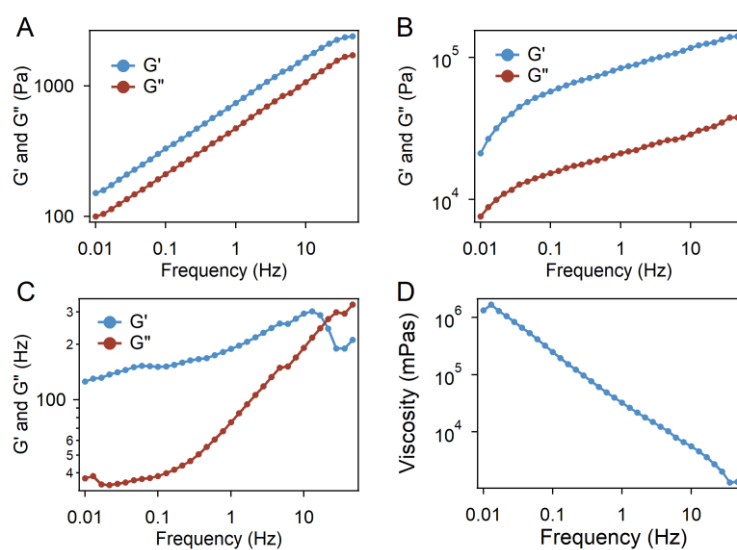
Zoobia Bashir<sup>†</sup>, Wenting Yu<sup>†</sup>, Zhengyu Xu, Yiran Li, Ying Li<sup>\*</sup>, Yi Cao and Bin Xue<sup>\*</sup>



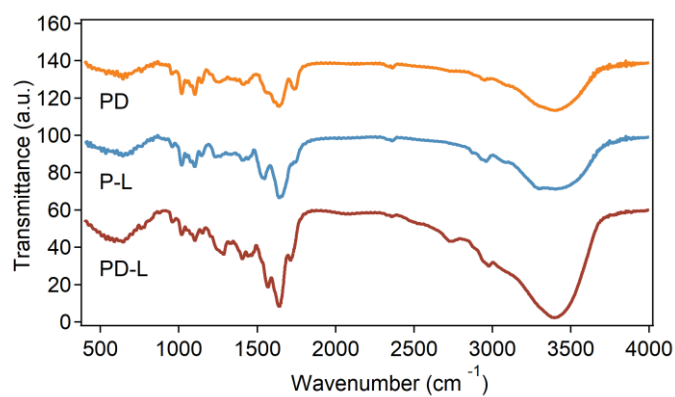
**Figure S1.** Determination of the reaction efficiency between dopa and pectin. (A) UV absorbance of Dopa at different concentrations (25  $\mu$ M - 325  $\mu$ M). (B) UV absorbance of pectin at different concentrations (100  $\mu$ M - 300  $\mu$ M). (C) Calibration curve of the dopa and pectin concentrations and OD<sub>212nm</sub>. (D) UV spectra of pectin-dopa at different mass ratios of dopa and pectin (1: 10, 1: 2 and 1: 1). (E) Reaction efficiency of dopa and pectin. Error bars represent SD.



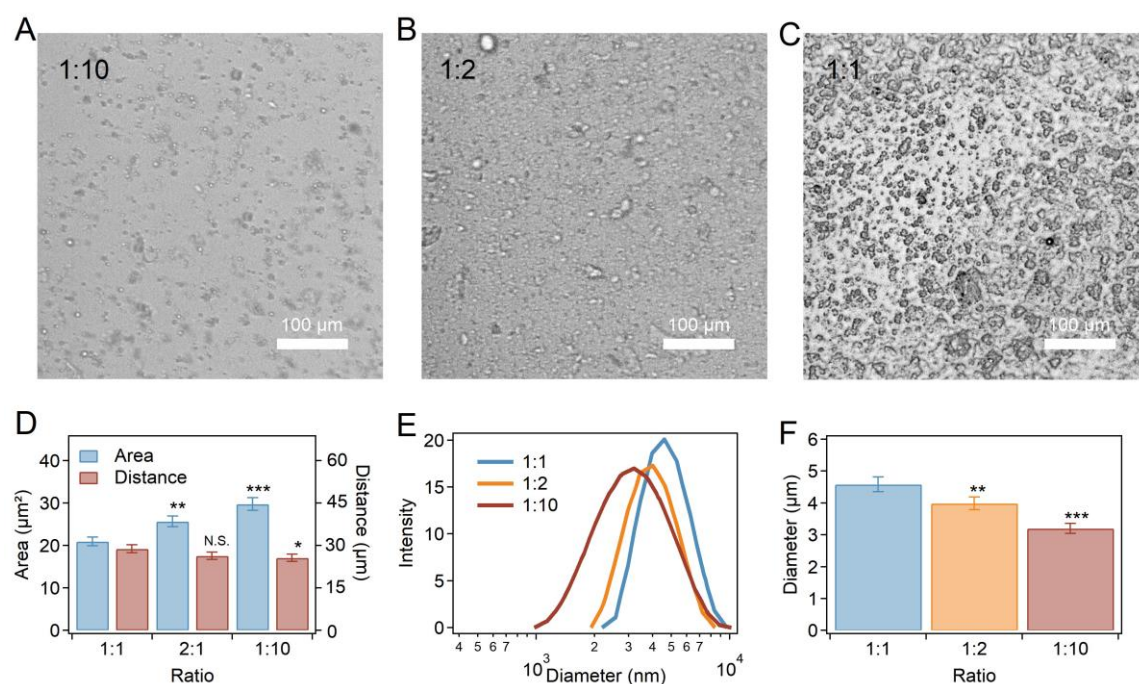
**Figure S2.** <sup>1</sup>H Nuclear Magnetic Resonance (<sup>1</sup>H-NMR) spectroscopy of dopa, pectin and pectin-dopa



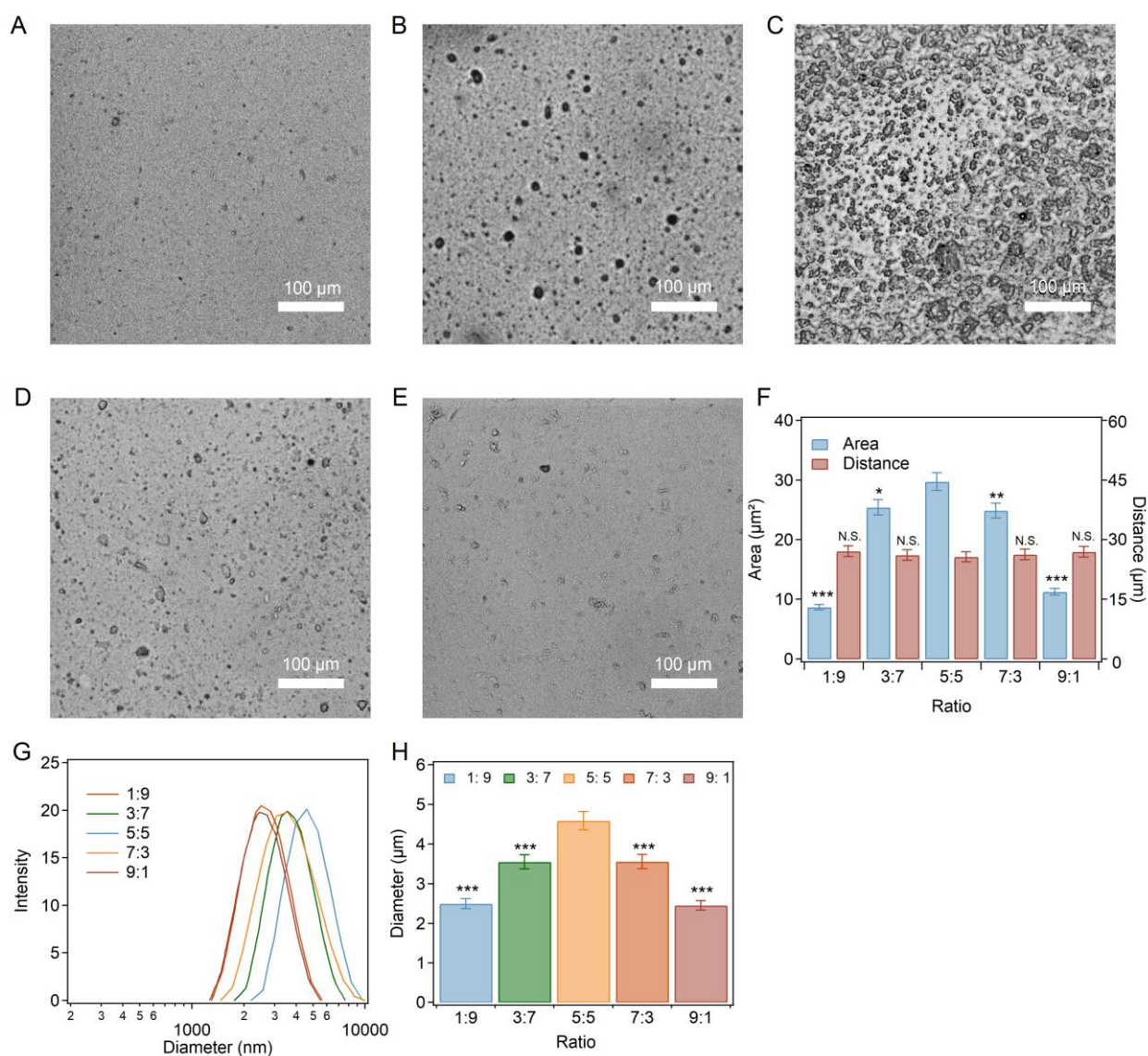
**Figure S3.** Rheological properties of the bio-glues. (A–C)  $G'$  and  $G''$  of pectin-dopa (A), P-L (B) and PD-L (C) bio-glue with ion measured in a frequency sweep experiment (from 0.01 to 90 Hz, 1% strain) at room temperature. (D) Viscosity measurement of PD-L bio-glue at 20 °C.



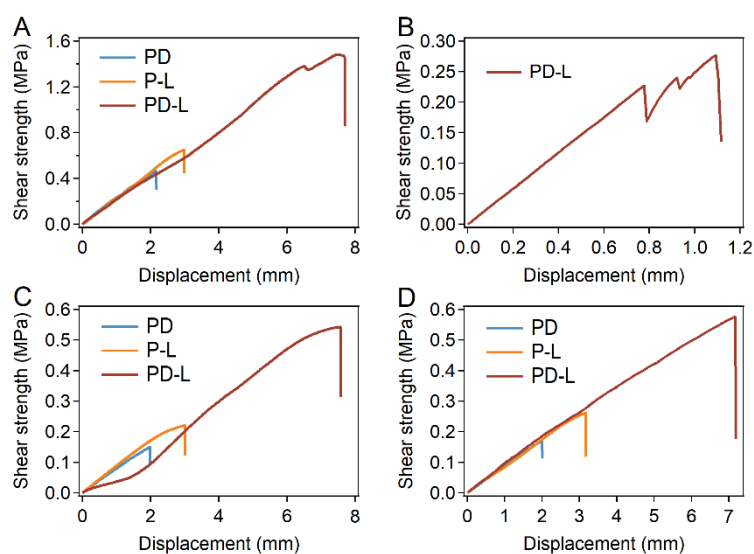
**Figure S4.** Fourier transform infrared (FT-IR) spectroscopy of the PD, P-L and PD-L bio-glues.



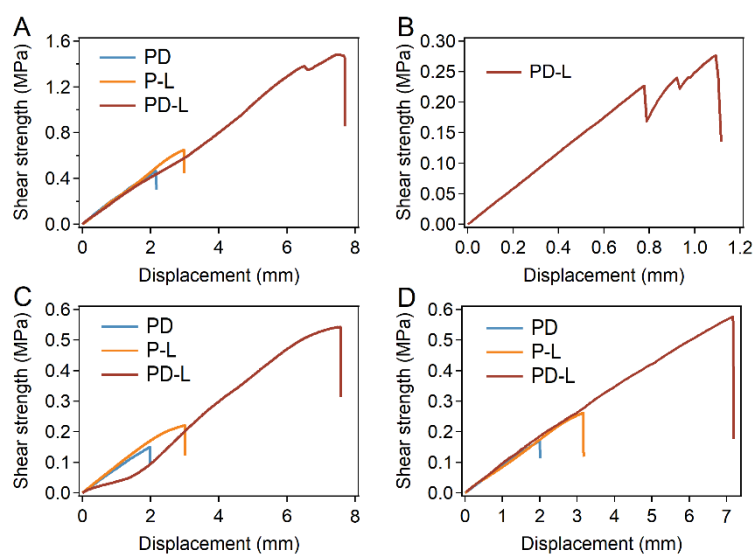
**Figure S5.** Phase separation of PD-L bio-glues at different synthesis ratios of dopa and pectin. (A–C) Micrographs of the mixture of PD-L bio-glue prepared using pectin-dopa synthesized at different mass ratios of dopa and pectin. A: 1:10; B: 1:2; C: 1:1. (D) Average distance between the adjacent coacervates and average area of the coacervates in PD-L bio-glue at different synthesis ratios of dopa and pectin. Error bars represent SD. (E–F) Diameter distribution (E) and average diameter of coacervates (F) in PD-L bio-glues at different synthesis ratios of dopa and pectin measured using DLS. Error bars represent SD. Asterisks denote statistical significance compared with bio-glue at a ratio of 1:1 ( $P > 0.05$ : N.S.;  $P < 0.05$ : \*;  $P < 0.01$ : \*\*;  $P < 0.001$ : \*\*\*).



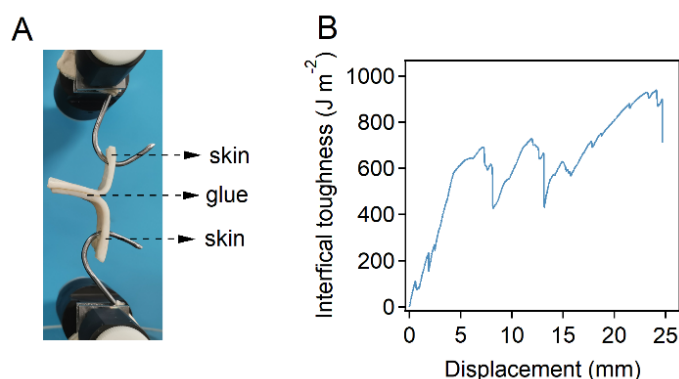
**Figure S6.** Phase separation of pectin-dopa and  $\beta$ -lactoglobulin at different mass ratios of pectin-dopa and  $\beta$ -lactoglobulin. (A-E) Micrographs of the mixture of pectin-dopa and  $\beta$ -lactoglobulin in PBS (10 mM, pH = 7.4) at different pectin-dopa:  $\beta$ -lactoglobulin ratios (1: 9, 3: 7, 5: 5, 7: 3 and 9: 1). (F) Average distance between the adjacent coacervates and average area of the coacervates at different pectin-dopa:  $\beta$ -lactoglobulin ratios (1: 9, 3: 7, 5: 5, 7: 3 and 9: 1). Error bars represent SD. (G) Coacervate size distribution (G) and summarized diameter of coacervates (H) in the mixture of pectin-dopa and  $\beta$ -lactoglobulin at different pectin-dopa:  $\beta$ -lactoglobulin ratios (1: 9, 3: 7, 5: 5, 7: 3 and 9: 1). Error bars represent SD. Asterisks denote statistical significance compared with bio-glue at a ratio of 5: 5 ( $P > 0.05$ : N.S.;  $P < 0.05$ : \*;  $P < 0.01$ : \*\*;  $P < 0.001$ : \*\*\*).



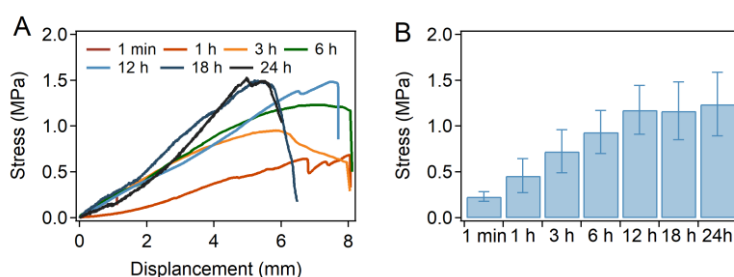
**Figure S7.** Typical force-displacement curves in shear strength measurements using different glues. (A-B) Typical force-displacement curves recorded in the shear strength measurements for long-term (A) and short-term (B) skin-skin adhesion using different glues. (C) Typical force-displacement curves recorded in the shear strength measurements for long-term glass-glass adhesion. (D) Typical force-displacement curves recorded in the shear strength measurements for long-term glass-skin adhesion.



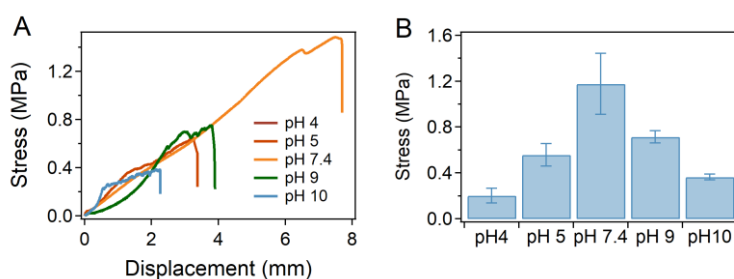
**Figure S8.** Typical force-displacement curves in tensile strength measurements using different glues. (A-B) Typical force-displacement curves recorded in the tensile strength measurements for long-term (A) and short-term (B) skin-skin adhesion. (C) Typical force-displacement curves recorded in the tensile strength measurements for long-term glass-glass adhesion. (D) Typical force-displacement curves recorded in the tensile strength measurements for long-term glass-skin adhesion.



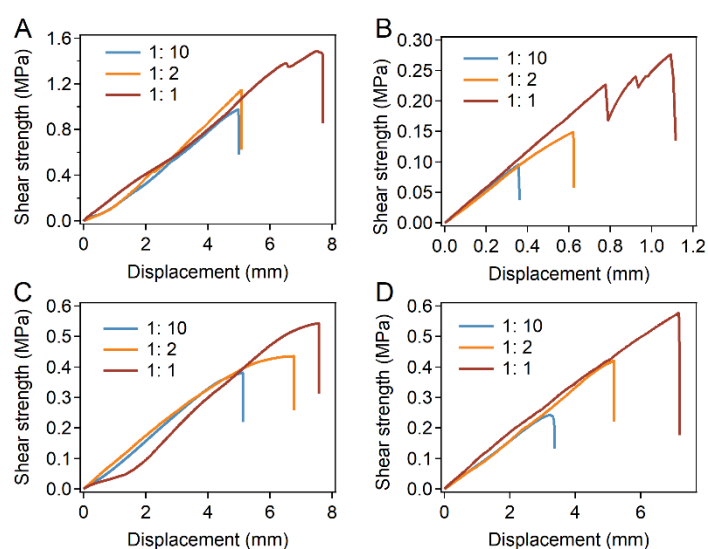
**Figure S9.** Interfacial toughness determination of the adhesion for porcine skin using PD-L glue. (A-B) Typical image (A) and force-displacement curve (B) recorded in the test of interfacial toughness.



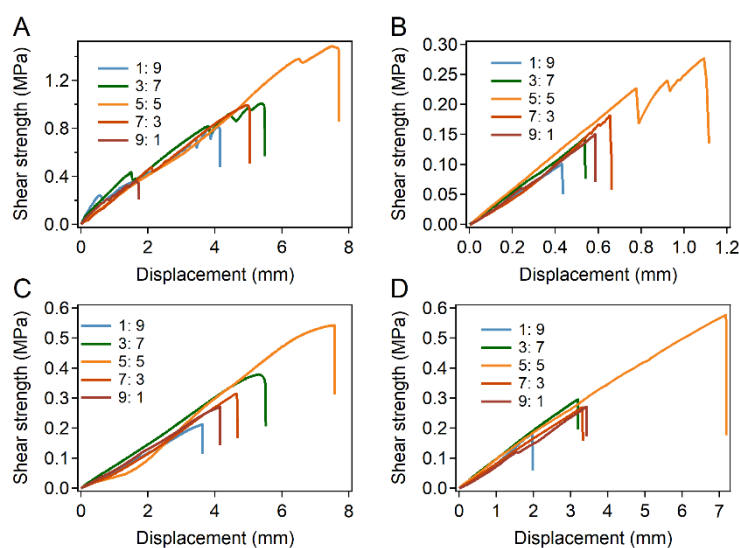
**Figure S10.** Adhesion strength of PD-L bio-glue at different oxidation times. (A) Typical force-displacement curves recorded in the lap shear experiment for the porcine skin adhesion using PD-L bio-glue after curing for different times. (B) Adhesion strength for the porcine skin using PD-L bio-glue after curing for different times. Error bars represent SD.



**Figure S11.** Adhesion strength of PD-L bio-glue at different pH. (A) Typical force-displacement curves recorded in the lap shear experiments for the long-term porcine skin adhesion using PD-L bio-glue prepared using PBS at different pH (4.0, 5.0, 7.4, 9.0 and 10.0). (B) Adhesion strength for the long-term porcine skin adhesion using PD-L bio-glue prepared using PBS at different pH. Error bars represent SD.

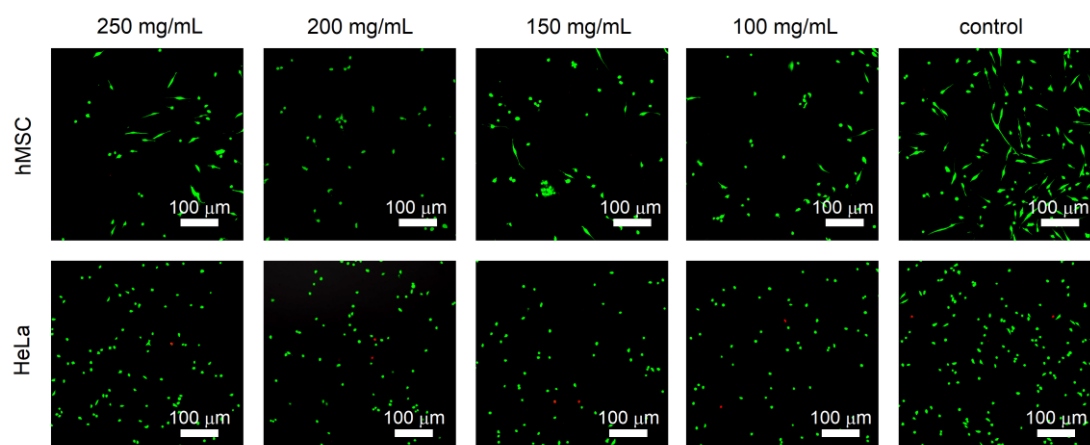


**Figure S12.** Adhesion performance of the bio-glue at different synthesis ratios of dopa and pectin (1: 10, 1: 2 and 1: 1). (A-B) Typical force-displacement curves recorded in the shear strength measurements for long-term (A) and short-term (B) skin-skin adhesion at different synthesis ratios of dopa and pectin. (C) Typical force-displacement curves recorded in the shear strength measurements for long-term glass-glass adhesion at different synthesis ratios of dopa and pectin. (D) Typical force-displacement curves recorded in the shear strength measurements for long-term skin-glass adhesion at different synthesis ratios of dopa and pectin.



**Figure S13.** Adhesion performance of bio-glue at different pectin-dopa:β-lactoglobulin ratios. (A-B) Typical force-displacement curves recorded in the shear strength measurements for long-term (A) and short-term (B) skin-skin adhesion at different pectin-dopa:β-lactoglobulin ratios (1: 9, 3: 7, 5: 5, 7: 3 and 9: 1). (C) Typical force-displacement curves recorded in the shear strength measurements for long-term glass-glass adhesion at different pectin-dopa:β-lactoglobulin ratios (1: 9, 3: 7, 5: 5, 7: 3 and 9: 1). (D) Typical force-displacement curves recorded in the shear strength measurements for long-term skin-glass adhesion at different pectin-dopa:β-lactoglobulin ratios (1: 9, 3: 7, 5: 5, 7: 3 and 9: 1).





**Figure S14.** Fluorescence microscopic images of HeLa (left) and hMSC (right) cells cultured in the presence of bio-glue (50, 100, 150, 200 and 250 mg mL<sup>-1</sup> and control group. The living and dead cells were stained with a live/dead assay (Calcein-AM/PI Double Staining Kit) after 24 h of culture.