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Biomimetic polymeric transcatheter heart valve leaflets with low calcification and good regenerative ability

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Supplementary Material

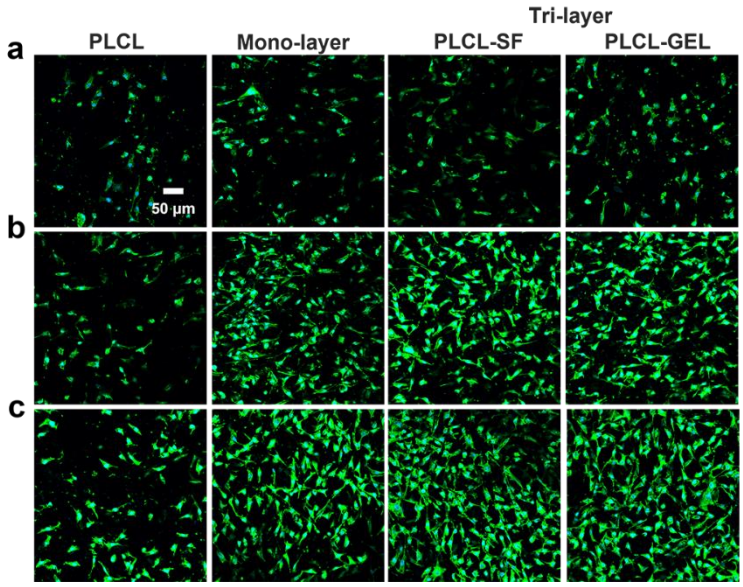


Fig. S1. Cell adhesion of HUVECs on PLCL, mono-layer and the biomimetic tri-layer valves. (a-c) CLSM images of actin/nuclei staining of adhered HUVECs (green: cytoskeleton; blue: nuclei) after (a) 1, (b) 3 and (c) 5 days of culture. Scale bars: 50 μm.

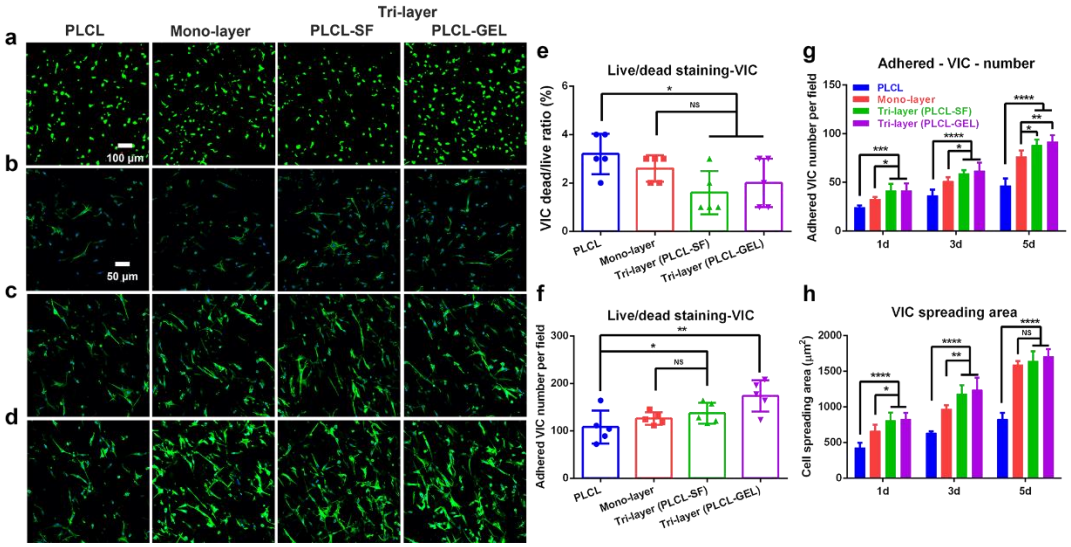


Fig. S2. Live/Dead staining and cell adhesion of VICs on PLCL, mono-layer and the biomimetic tri-layer valves. (a) CLSM images of VICs (green: live cells; red: dead cells) at 24 hours. Scale bars: 100 μm. (b-d) CLSM images of actin/nuclei staining of adhered VICs (green: cytoskeleton; blue: nuclei) at (b) day 1, (c) day 3 and (d) day 5. Scale bars: 50 μm. (e-f) Quantitative analysis of live/dead assay. (g) Adhered

VICs' number and (h) spreading area calculated from actin/nuclei staining.

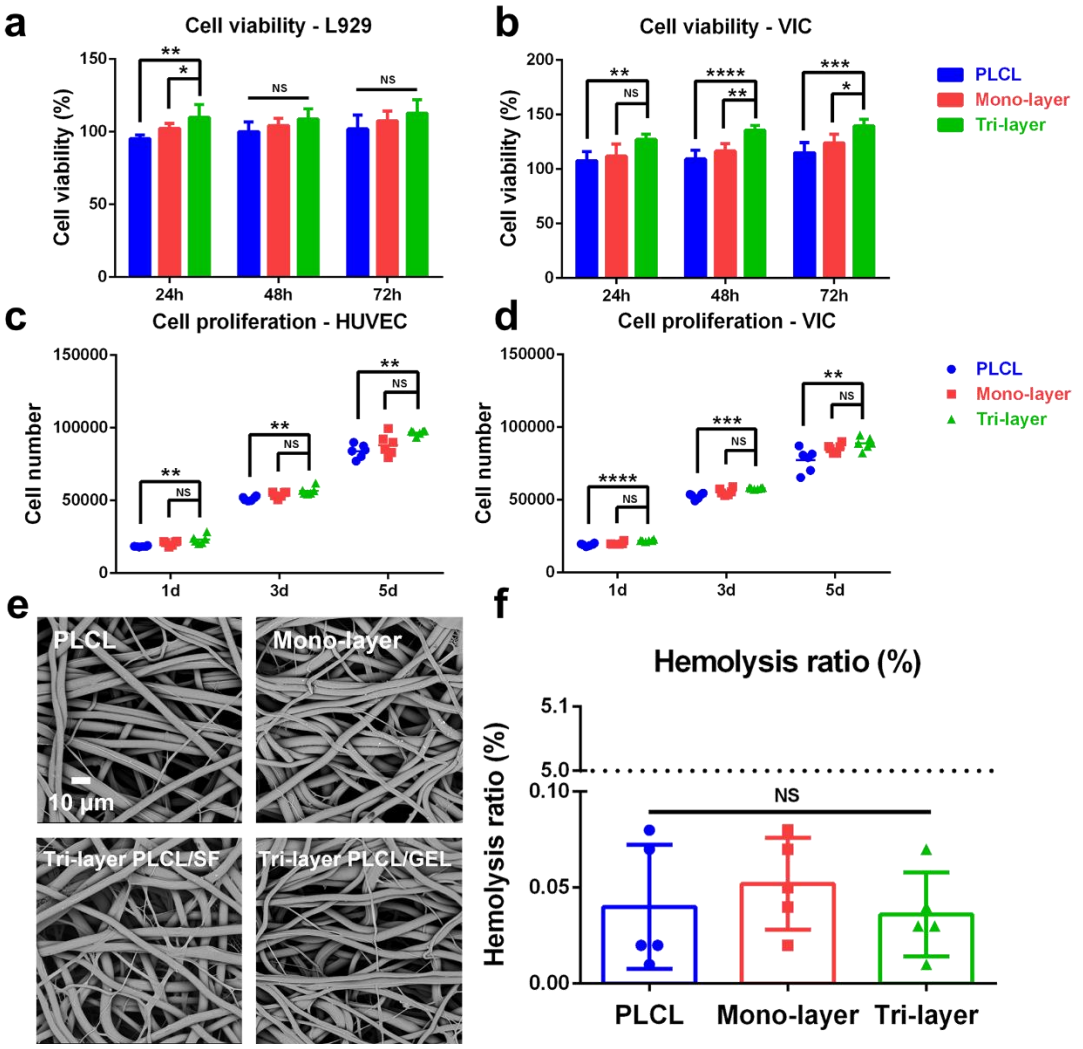


Fig. S3. Cell viability and proliferation grown on PLCL, mono-layer valve and the biomimetic tri-layer valve. The cell viability of (a) L929 and (b) VICs. The cell proliferation of (c) HUVECs and (d) VICs. (e-f) Blood compatibility test. (e) Platelet adhesion test and (f) Hemolysis ratio of PLCL, mono-layer valve and the biomimetic tri-layer valve. Tri-layer group was observed from both PLCL/SF and PLCL/GEL side. Scale bars: 10 μm.

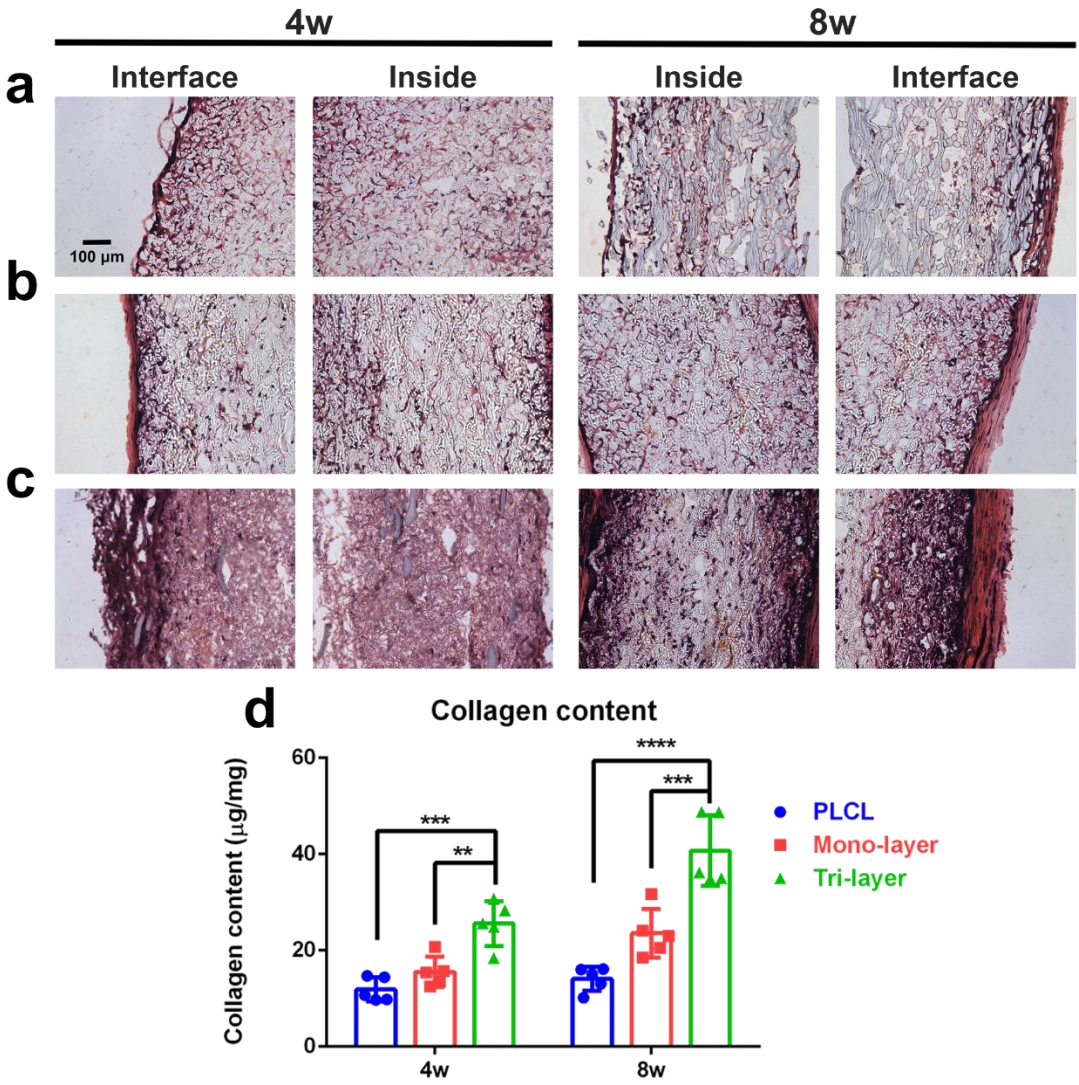
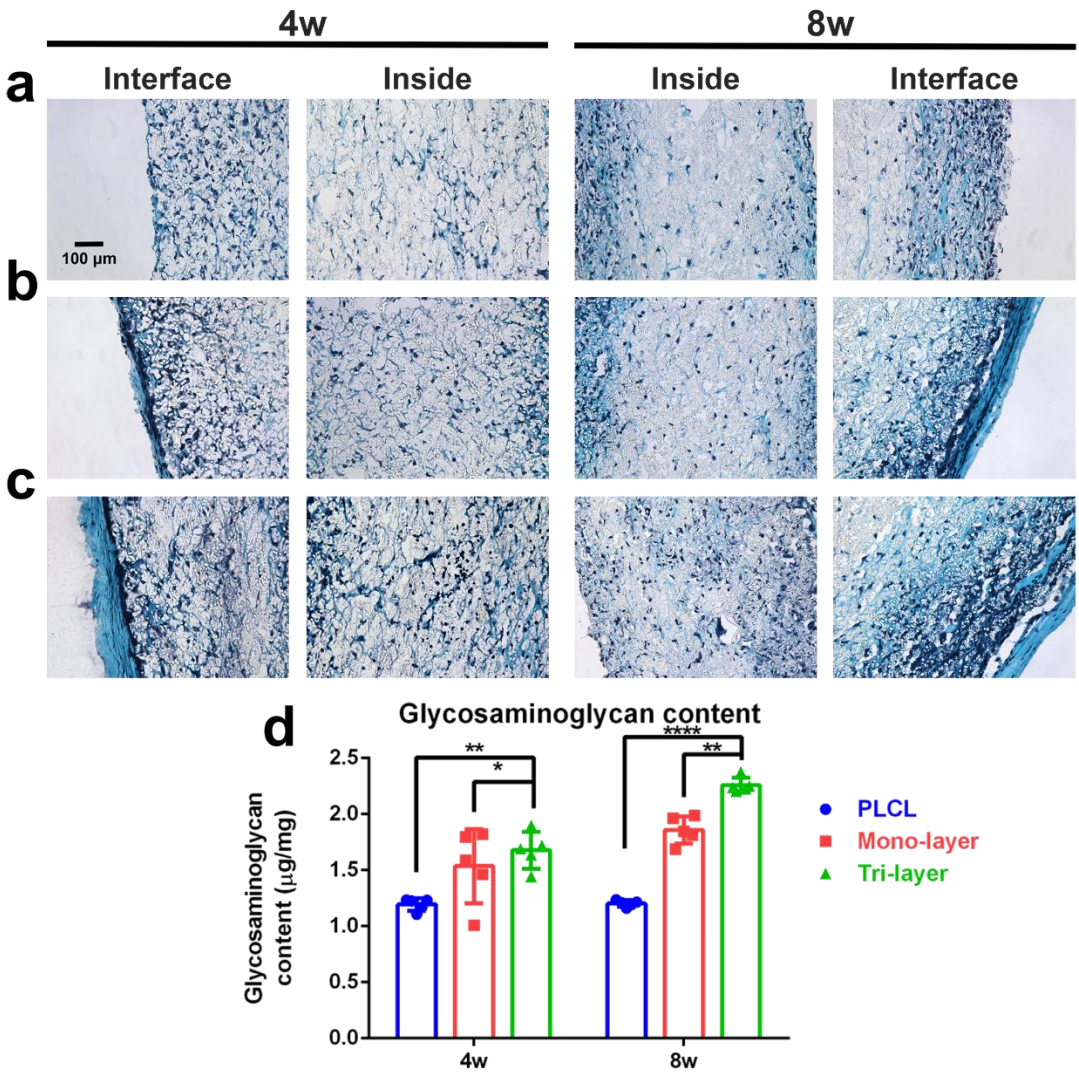


Fig. S4. Collagen stained by Masson's Trichrome of (a) PLCL, (b) mono-layer, and (c) the biomimetic tri-layer valves after subcutaneous implantation for 8 weeks. Scale bars: 100 μm. (d) Quantitative content of collagen.



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53 **Fig. S5.** Glycosaminoglycan stained by Safranin O Solid Green of (a) PLCL, (b) mono-layer, and (c) the

54 biomimetic tri-layer valves after subcutaneous implantation for 8 weeks. Scale bars: 100 μm. (d)

55 Quantitative content of glycosaminoglycan.

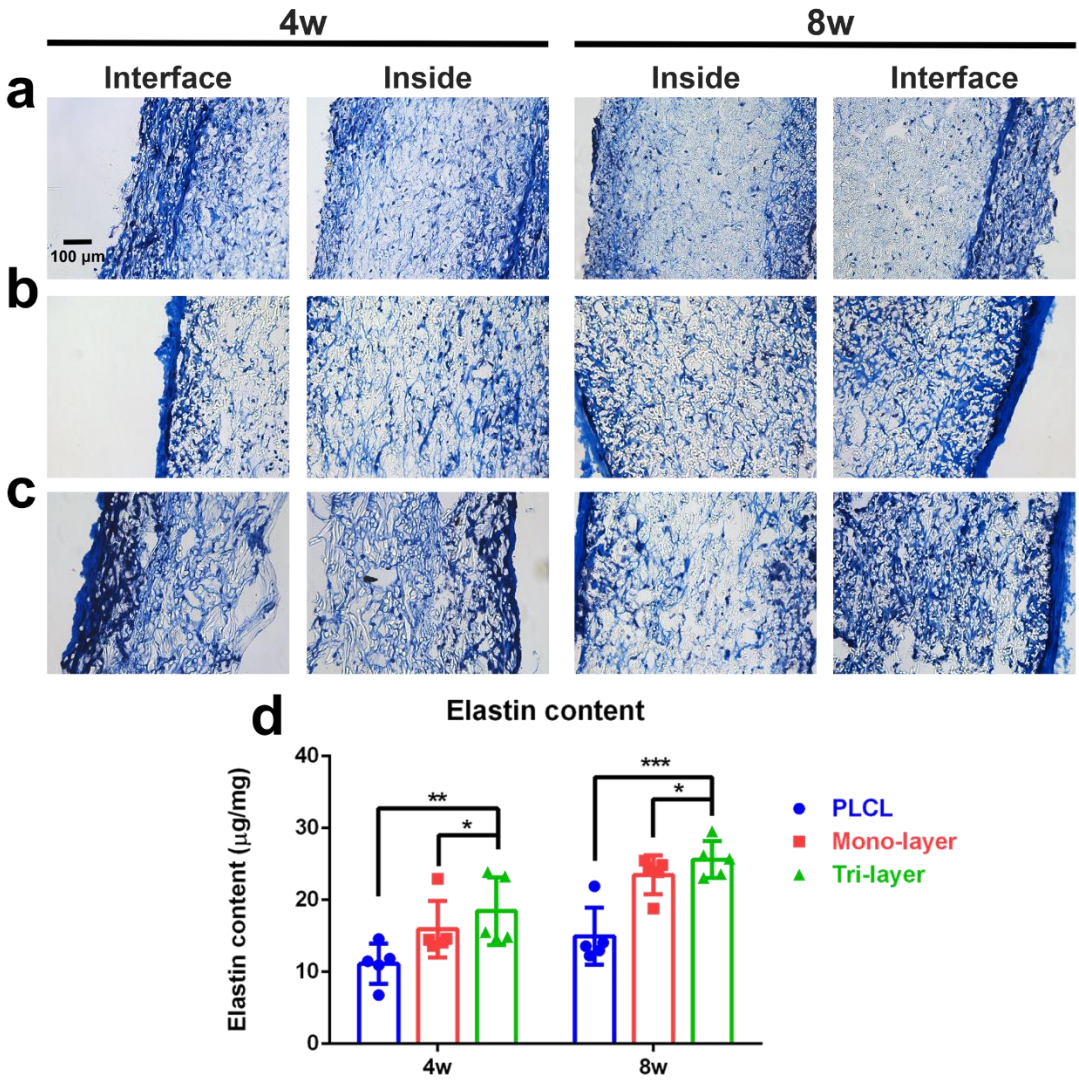

Fig. S6. Elastin stained by Verhoeff-Van Gieson of (a) PLCL, (b) mono-layer, and (c) the biomimetic tri-layer valves after subcutaneous implantation for 8 weeks. Scale bars: 100 μm. (d) Quantitative content of elastin.

Table S1. Testing parameters for Pulsating flow test

Abbreviations	Cardiac output (L/min)	Beat rate (cycles/min)	Average aortic pressure (mmHg)
5-70-100	5	70	100
5-120-80	5	120	80

5-120-100	5	120	100
5-120-120	5	120	120

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