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Postfunctionalization of biological valve leaflets with a polyphenol network and anticoagulant recombinant humanized type III collagen for improved anticoagulation and endothelialization

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Contents:

Method S1. Stability tests

The physical and biological stability of the biological valve leaflets modified with GLUT-PP, TA/Fe, and TA/Fe-rhCOLIII was investigated in the flowing system with PBS solution, using a peristaltic pump to simulate blood flow velocity. Briefly, the samples were stuck on the inner wall of the commercially bare tube (Φ5.3 mm × 130 mm) and assembled into an elastic hose of the peristaltic pump for completing the circuit. The device provided a stable flow rate of 20 mL/min and the PBS solution changed every day. After 3 d, 7 d, 15 d, and 30 d, respectively, the samples were taken out. Then the *ex vivo* arteriovenous shunt assay and LDH assay were conducted to investigate the antithrombotic properties of the modified valve leaflets. Besides, HUVECs adhesion and proliferation assay was applied to evaluate the cytocompatibility of the samples.

Method S2. In vivo evaluation of subcutaneous implantation

Male Sprague Dawley rats (SD rats) weighing approximately 300 g were applied to

evaluate the *in vivo* biocompatibility of the samples. Each group of samples was set up with 6 parallel samples at each time point. Briefly, tissue forceps were used to separate the skin of SD rat from the tissue after anesthetizing with 10% chloral hydrate (0.003 ml/g). Subsequently, the samples were placed in the subcutaneous. After 15 and 30 days of implantation, the neointima encapsulated with the sample was removed and fixed with 4% paraformaldehyde overnight. Part of the neointimal tissue surrounding the sample was histologically examined with hematoxylin and eosin (HE) staining to check biocompatibility of tissues *in vivo*, and the remaining neointimal tissue was utilized to label with mouse antirat CD68 antibody and CD3 antibody for further evaluation of the macrophage cells and T cells, respectively.

Method S3. In-vitro pulsatile flow test

The TA/Fe-rhCOLIII-coated valve was sewn onto the scaffold to obtain a BHV with a diameter of 29 mm. The hydrodynamics performance of the samples was evaluated with an *in-vitro* pulsatile flow instrument (Shanghai Heartpartner testing equipment Co., Ltd, China) by calculating the effective orifice area and regurgitation of the valves, and the physiologically equivalent aortic pressure and blood flows were replicated in accordance with ISO 5840 requirements. Pulsating flow test parameters: heart rate 45–120 /min, cardiac output 2–7 L/min, average pressure 80–160 mmHg, pressure difference 40 mmHg [1].

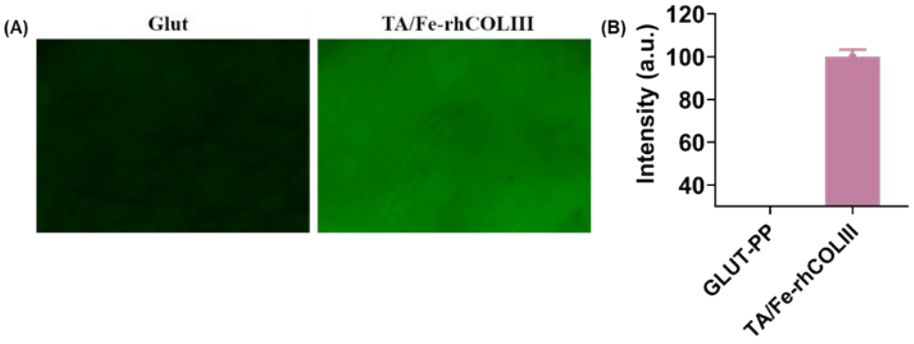

Figure S1. (A) Surface fluorescence image of GLUT and FITC-labeled TA/Fe-rhCOLIII. (B) Quantification of fluorescence intensity.

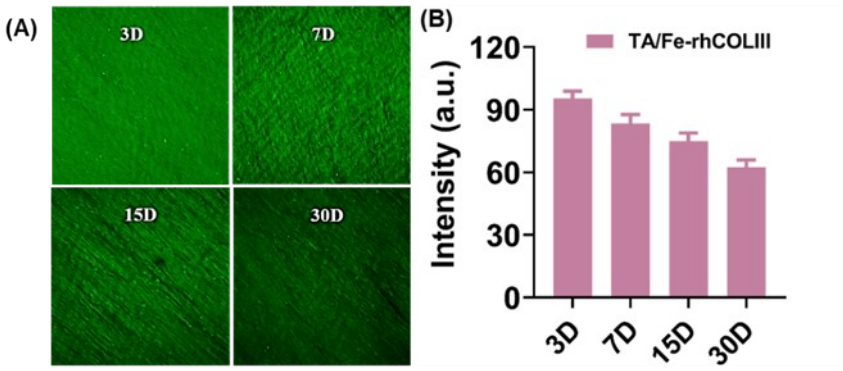

Figure S2. (A) Surface fluorescence image of FITC-labeled TA/Fe-rhCOLIII in the flowing system with PBS solution for 3 d, 7 d, 15 d, and 30 d, respectively. (B) Quantification of fluorescence intensity.

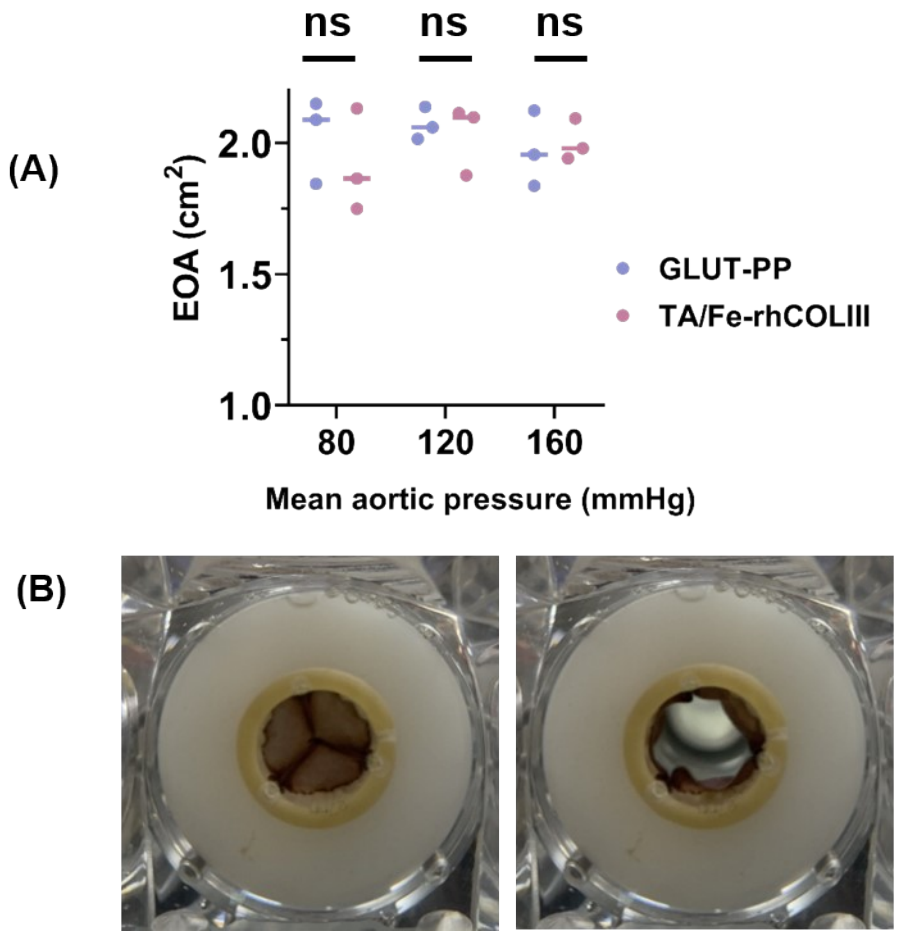

Figure S3. Hydrodynamic and fatigue performance of the biological valve leaflets. (A) EOA of the TA/Fe-rhCOLIII valve under different mean aortic pressures. (B) The photo of the closed and open status of BHV (modified with TA/Fe-rhCOLIII).

Reference

[1] T Yu, X Chen, W Zhuang, Y Tian, Z Liang, Q Kong, C Hu, G Li, Y Wang, Nonglutaraldehyde treated porcine pericardium with good biocompatibility, reduced calcification and improved Anti-coagulation for bioprosthetic heart valve applications, Chemical Engineering Journal 414 (2021) 128900.