Protective coating for blood-contacting materials by the combination of passive antifouling and active nitric-oxide generation

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1 Experimental section

1.1 Coating catalytic stability test

To evaluate the resistance of the coating's catalytic activity to physiological conditions, PC-EGCG/Cu^{2+} coatings were prepared on PVC substrates and immersed in PBS (37 ℃) for 14 days. Samples were removed at 1, 3, 5, 7 and 14 days, rinsed with deionized water, and stored for analysis. Following a previously described method, the rate of NO release from the coating was determined using the Griess assay[1, 2]. Briefly, the coatings were cut into sheets with an area of 1 $cm^2$and placed in a 24-well plate. Test solution (2000 μL PBS containing 60 μM SNAP and 30 μM GSH) was added to each well. At each time point, 50 μL of the PBS solution was withdrawn for the Griess assay and absorbance was measured at 540 nm. Absorbance values were converted to nitrite concentrations using a standard curve, then to cumulative NO released and NO release rate.

2.1 Cell culture

Human umbilical vein endothelial cells (HUVECs) and mouse fibroblasts (L929) were purchased from Service-bio Technology Co., Ltd. (China). HUVECs and L929 were cultured in ECM and DMEM media, respectively, each supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin. Cells were incubated at 37 ℃ in a humidified atmosphere containing 5% CO_2 until they reached approximately 80% confluence. Cells were washed with PBS and detached using 0.25% (w/v) trypsin. Detached cells were collected by centrifugation and resuspended in their respective culture media for subsequent passaging or biocompatibility assays.

2.2 Cytotoxicity assay

Cytotoxicity of the coatings was evaluated by CCK-8 assay according to ISO 10993-5 using extract leachate samples. PC and PC-EGCG/Cu^{2+} samples were placed in well plates, with uncoated PVC serving as the control, all samples were sterilized under ultraviolet light for 2 h. Samples were rinsed with PBS, then immersed in complete ECM or DMEM medium at a ratio of 3 cm^2/ml and incubated at 37 ℃ for 24 h to obtain extracts. After extraction, the extracts were filtered through a 0.22 μm membrane filter before use. HUVECs and L929 cells were seeded in 96-well plates at a density of 4,000 cells per well and allowed to attach and grow for 24 h. The original culture medium was removed and replaced with 100 μL of the respective sample extract for treatment, the blank control wells received fresh complete medium. After 24h and 72 h, the medium was removed and replaced with CCK-8 working solution. After incubation, absorbance was measured at 450 nm using a microplate reader. All results were normalized to the blank control.

2 Supporting figure

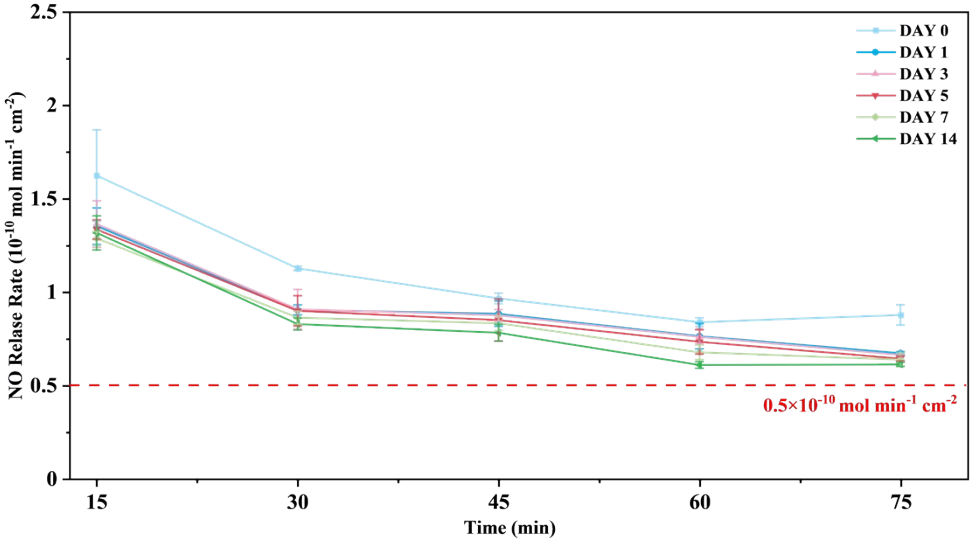

figure S1. NO catalytic release rates of coatings after different durations of PBS immersion.

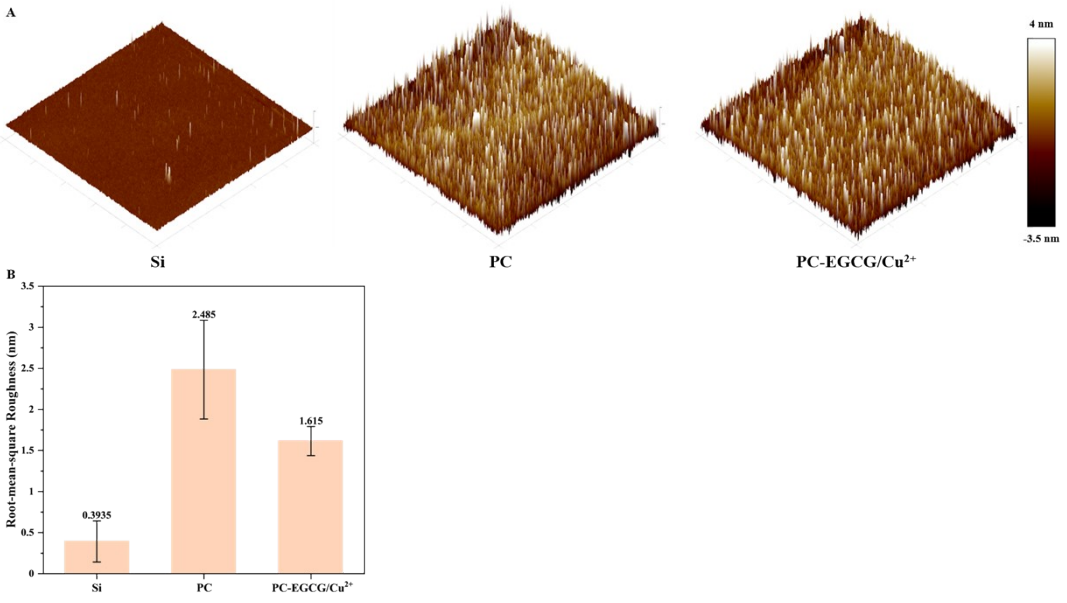

figure S2. (A) AFM results for the PC and PC-EGCG/Cu^{2+} coatings; (B) Root-mean-square roughness of difficult coatings. A silicon wafer was used as the control because PVC are relatively soft and prone to scratches, which could affect the measurements.

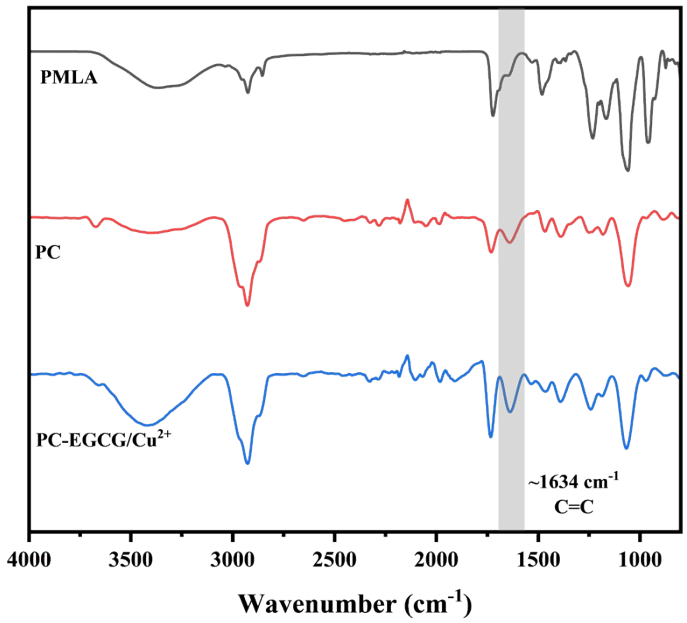

figure S3. The FTIR spectra of PMLA and PC, PC-EGCG/Cu^{2+} coating.

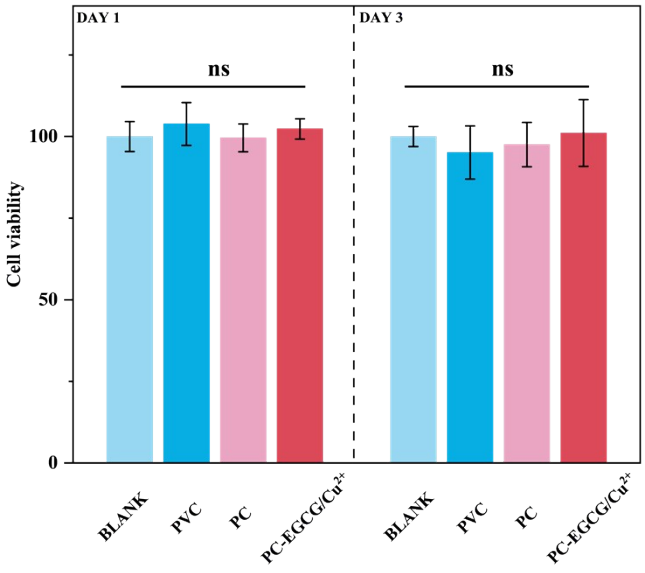

figure S4. Cell viability of HUVECs in different coatings(n=4).

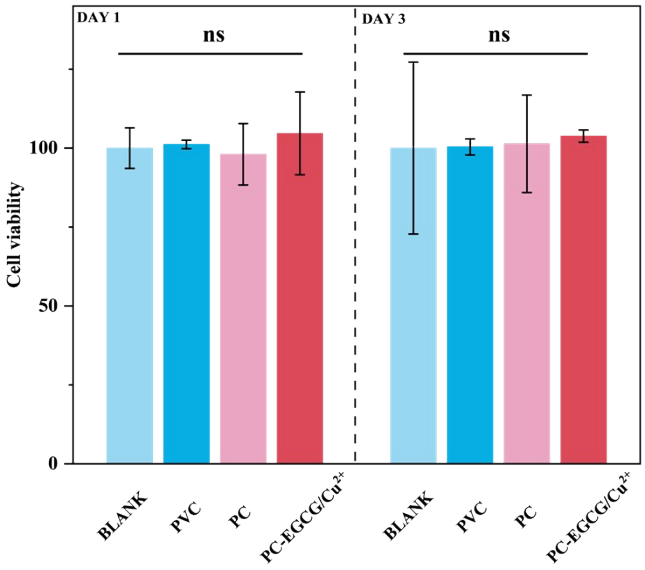

figure S5. Cell viability of L929 in different coatings(n=3).

table S1. Copolymer formulae and average molecular weight.

Sample	Copolymer formulae (molar ratio, %)			M_w *(g/mol)*
	MPC	LMA	APMA	
PMLA	1	3	1	270225

table S2. Semi-quantitative XPS elemental analysis of the coatings.

Sample	Atomic %				
	C 1s	O 1s	N 1s	P 2p	Cu 2p
PC	74.31	16.45	8.97	0.27	-
PC-EGCG/Cu^2	71.08	19.17	6.75	0.32	2.68

Reference:

1. H. Wu, Q. He, L. Li, L. Li, Z. Zhou, N. Chen, M. Yang, Q. Luo, B. Zhang, R. Luo, L. Yang and Y. Wang, *Chemical Engineering Journal*, 2022, **427**.
2. Z. Xiang, Y. Xiang, Y. Li, J. Zhang, C. Zhou, H. Yan, D. Fu and Y. Wang, *Advanced Functional Materials*, 2025, **35**.