Supplementary Information

Stealthy Nanoparticles Protecting Endothelium Barrier from Leakiness by Resisting the Absorption of VE-cadherin

Yuan Huang, [a] *Suxiao Wang,**[,a] *Jinzhi Zhang,* [a] *Hangxing Wang,* [a] *Qichao Zou,* [a] *and Limin Wu**[, b]

[a]Ministry of Education Key Laboratory for the Synthesis and Application of Organic Functional Molecules, Hubei Collaborative Innovation Centre for Advanced Organic Chemical Materials, College of Chemistry and Chemical Engineering, Hubei University, Wuhan, 430062, China

[b]Department of Materials Science and State Key Laboratory of Molecular Engineering of Polymers, Fudan University, Shanghai 200433, China

*Corresponding authors: wsx@hubu.edu.cn; lmw@fudan.edu.cn

Supplementary Figure S1

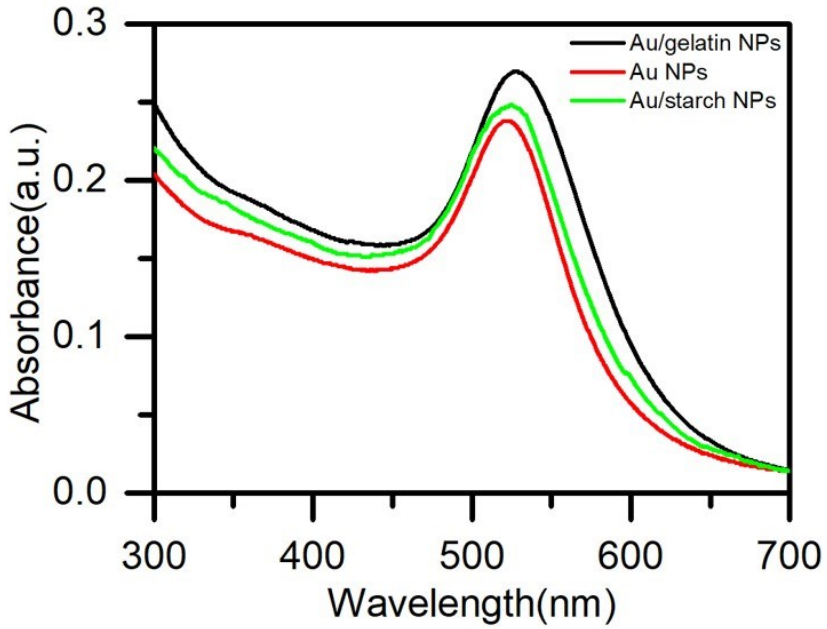


Figure S1. UV-Vis absorption spectra of Au/gelatin (black line), Au NPs (red line) and Au/starch NPs (green line) in aqueous solution.

Supplementary Figure S2

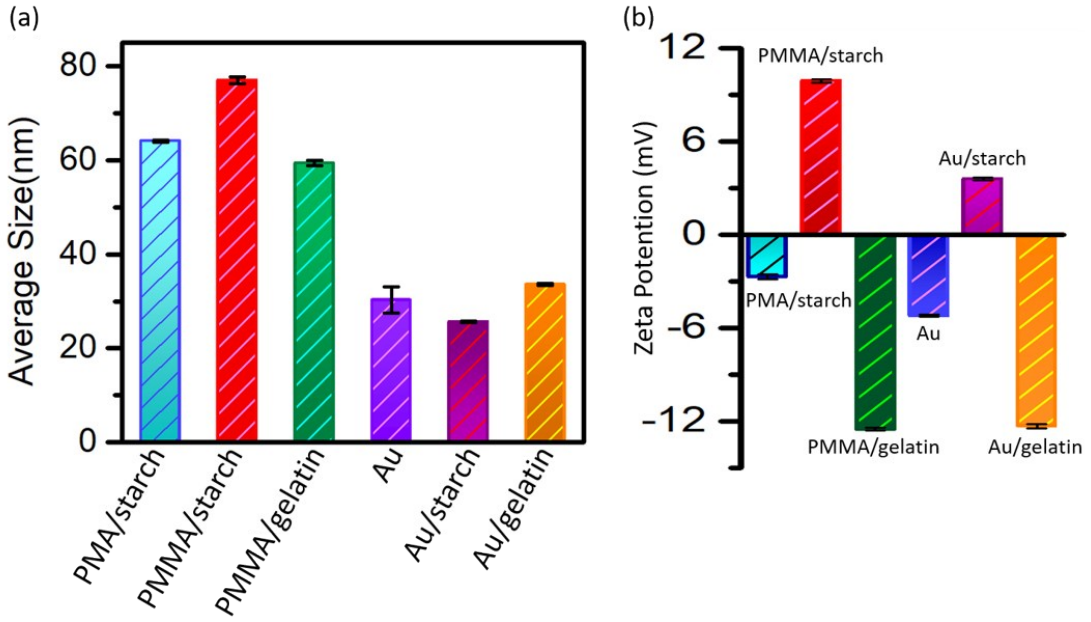
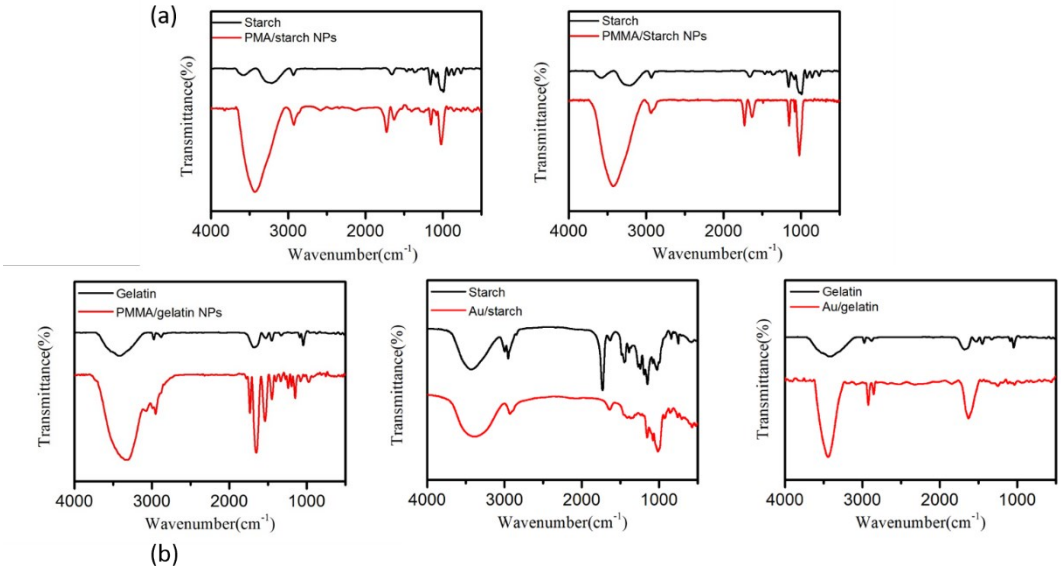


Figure S2. Dynamic light scattering (DLS) size (diameter, nm) and surface zeta-potential (ζ, mV) of PMA/starch, PMMA/starch, PMMA/gelatin, Au, Au/starch and Au/gelatin NPs (n = 3).

Supplementary Figure S3



(b)

PMM v(cm-1)	Starch v(cm-1)	PMMA/starch NPs v(cm-1)	PMA/starch NPs v(cm-1)	Gelatin v(cm-1)	PMMA/gelatin NPs v(cm-1)	Au NPs v(cm-1)	Au/starch NPs v(cm-1)	Au/gelatin NPs v(cm-1)	Peak Assignment
750	766	-	767	-	765	-	-	-	C-C stretching
841	860	848	865	-	-	-	-	-	CH_2 deformation
1010	1023	1024	1024	1050	-	1045	1040	1040	C-O-C stretching vibrations
1149	1159	1156	1156	-	1151	-	1156	-	C-O,C-H stretching
1243	1237	1240	1247	-	1243	-	-	1256	CH_2OH side chain related mode
1388	1368	-	1406	1453	1451	1394	-	1391	
-	-	-	1536	1532	1543	1591	-	-	C-H stretching of CH_3
-	1660	1630	1634	1646	1650	-	-	1634	
1731	-	1725	1732	-	1731	1735	1690	1738	-C=O groups
-	-	-	-	2879	-	-	2835	2849	C-H stretching of CH_3,CH_2
2951	2931	2946	2929	2974	2953	-	2916	2923	C-H stretching
3438	3276	3431	3431	3403	3350	3427	3482	3442	O-H stretching vibrations

Figure S3. (a) FTIR spectra of PMA/starch, PMMA/starch, PMMA/gelatin, Au, Au/starch and Au/gelatin NPs. (b) Corresponding peak position and assignment of FTIR spectra.

Supplementary Figure S4

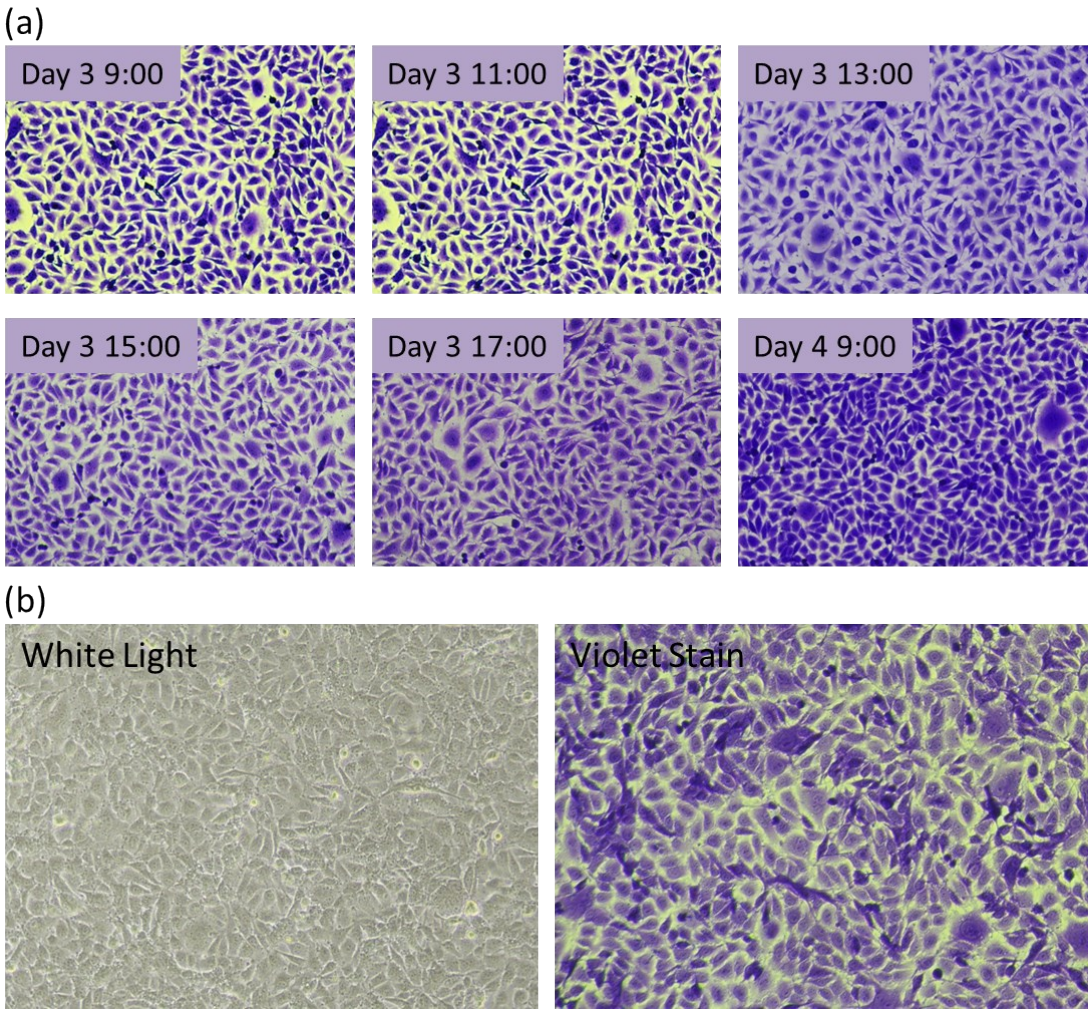


Figure S4. The optimal conditions of endothelial cell culture were investigated by crystal violet lactone staining to produce confluent endothelial monolayer. (a) The larger the color range, the denser the cells, and the darker the color, the more heavily the cells are stacked. We chose 17:00 on the third day as the best time for cell growth. (b) The imagines of confluent HMVEC monolayer under white light (left) and stained by crystal violet (right).

Supplementary Figure S5

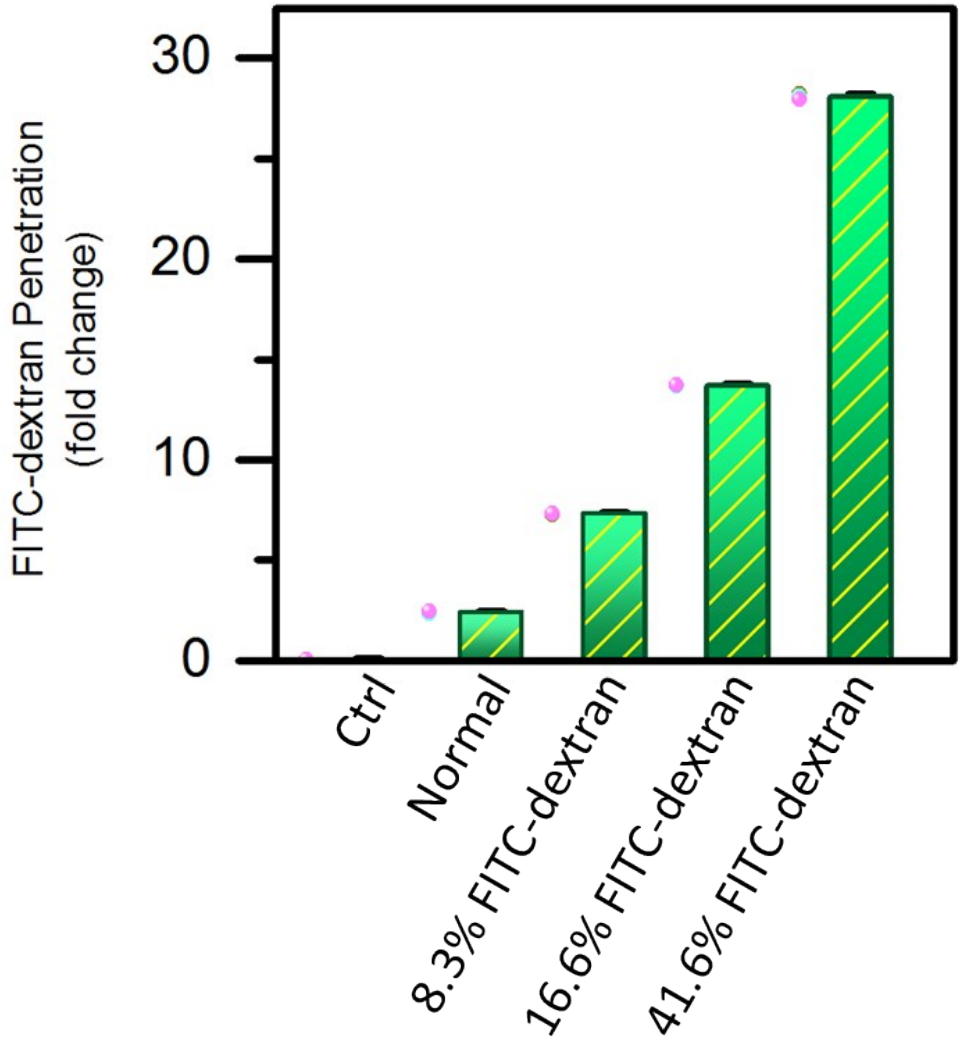


Figure S5. Bias due to biological duplication in each group itself. The fluorescence intensity of the normal group was between the blank control group and the reference group.

Supplementary Figure S6

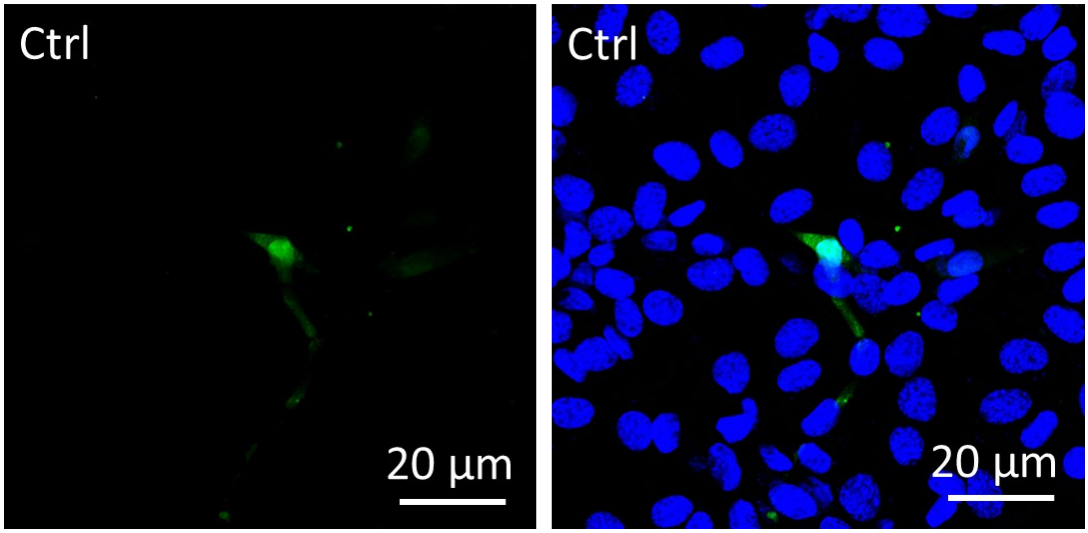


Figure S6. Confocal images of the control group of adhesion assay. The control group had a few sparsely attached MDA-MB-231 cells (green). The nuclei were stained blue with DAPI.

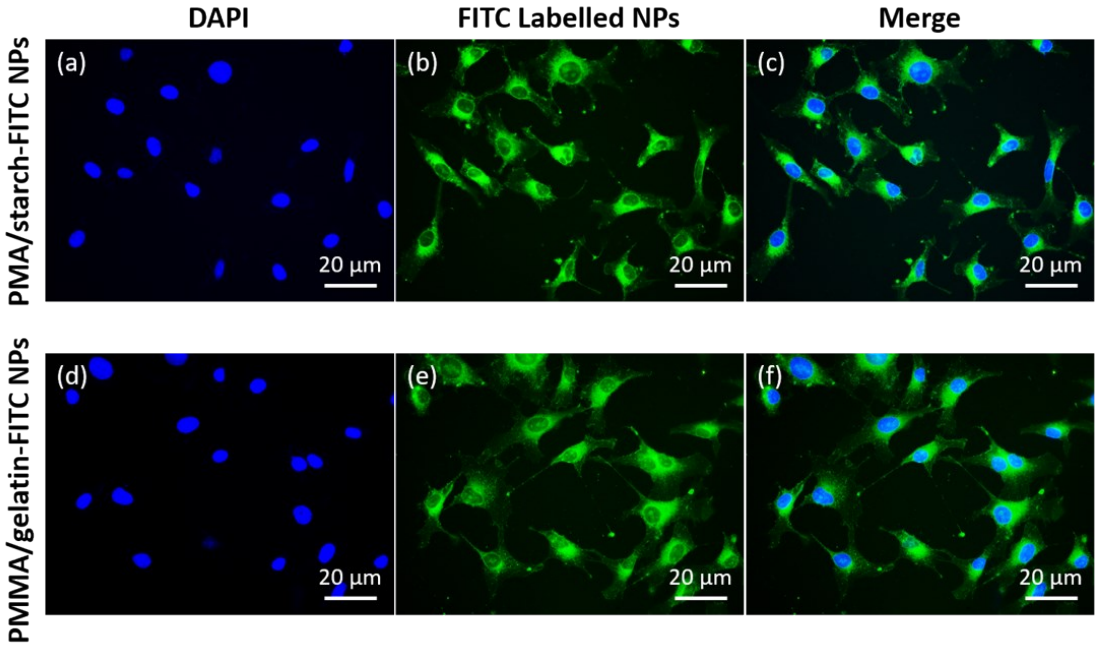


Figure S7. Confocal images of HMVEC cells incubated with FITC labelled (a-c) PMA/starch NPs and (d-f) PMMA/gelatin NPs. (a) and (d) Cell nuclear staining by DAPI under 361 nm excitation. (b) and (e) NPs internalization under 488 nm excitation. (c) and (f) merge images.

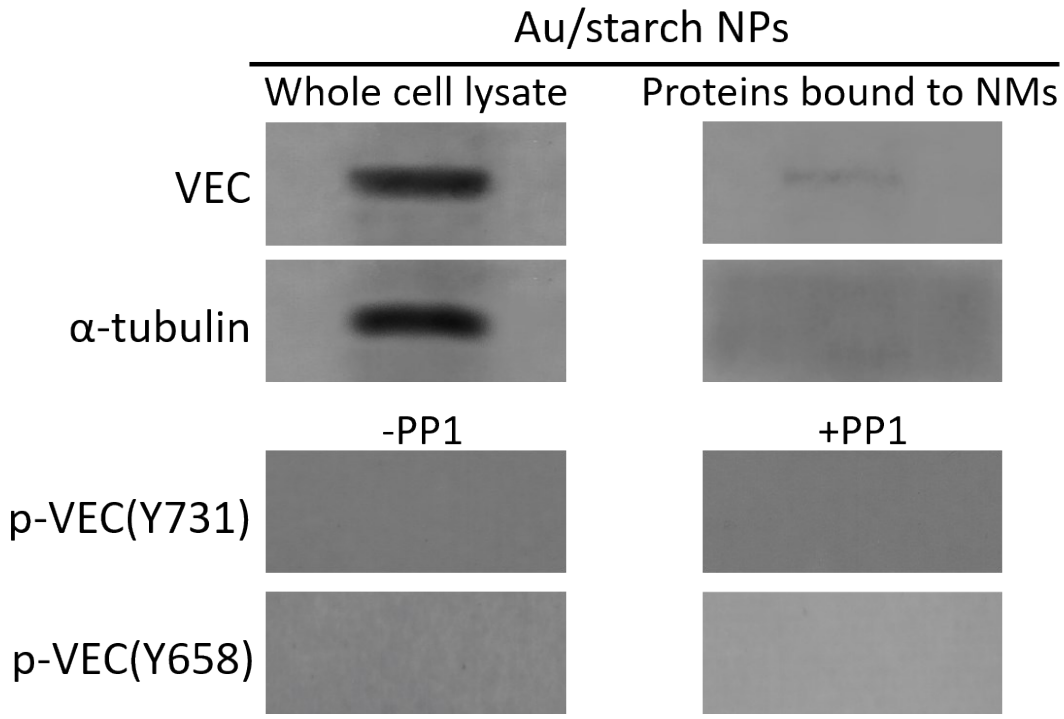


Figure S8. (a) Immunoblotting of VEC and α-Tublin from whole cell lysate and pulled down by Au/starch NPs. (b) Y658(P-VEC(Y658)) and Y731(P-VEC(Y731), from Au/starch NPs incubated whole cell lysate with/without Src kinase inhibitor, PP1.

Methods

Nanoparticle uptake assay. The fluorescein-labelled NPs were synthesised by dissolving 25.5 mg fluorescein into methyl methacrylate and use the same procedure as PMA/starch and PMMA/gelatinNPs. HMVEC cells were cultured at 37℃ in 5% CO_2/95% air and the cells in log phase were harvested by trypsin (Gibco). After centrifugation at 1200 rpm for 3 min, the cells were redispersed in medium at a concentration of 2×10^5 cells/mL. A total of 3 × 1 mL of cell suspension was separately loaded into a 12-well plate at 37°C and left overnight under 5% CO_2/95 % air conditions. Then, the medium was removed. Afterwards, 0.1 mg/mL PMA/starch-FITC NPs and PMA/gelatin-FITC NPs were separately mixed with serum-free medium at a total volume of 1 mL and added to the HMVEC incubated 12-well plate for 24 h. The cells were washed with PBS 3 times (3 min each time) and fixed at room temperature with 4% formalin for 15 min, followed by PBS washing 3 times. Triton X-100 (0.5% in PBS, Beyotime) was then added into the well for 20 min. To stain the cell nucleus, the samples were incubated for 5 min with 4′,6-diamidino2-phenylindole (DAPI, Beyotime), and extra DAPI was washed out by PBS / Tween-20 (0.002%) 4 times (5 min each time). The cells were observed using a Nikon C2+ confocal microscope.