Supplementary Materials

Polydopamine-mediated long-term elution of the direct thrombin inhibitor bivalirudin from TiO_2 nanotubes for improved vascular biocompatibility

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1 Supporting experimental

1.1 Clotting time

Platelet poor plasma (PPP) was prepared by centrifuging (3000 rpm, 15 min) citrate anticoagulated fresh human whole blood. For the activated partial thromboplastin time (APTT) measurement, samples were placed in a 24-well culture plate. Fresh human platelet poor plasma (PPP, 200 μL) and actin-activated cephaloplastin reagent (200 μL) were added, followed by the addition of 0.03 M $CaCl_2$ solution (200 μL) after incubation for 30 min at 37 °C. The clotting time of the plasma solution was measured by a coagulometer (ACL 200, Instrumentation Laboratory Co. USA). In the case of the prothrombin time (PT) measurements, 200 μL PPP was dropped onto the surface of the samples. After incubation for 30 min at 37 °C, the PT measurements were performed by adding 200 μL incubated PPP solution into 400 μL PT reagent in a test tube, and then was measured in the coagulometer. In the case of the thrombin time (TT) measurements, 200 μL PPP was dropped onto the surface of the samples. After incubation for 30 min at 37 °C, the TT measurements were performed by adding 200 μL incubated PPP solution into 200 μL TT reagent in a test tube, and then was measured using the coagulometer.

1.2 Fibrinogen activation

Human fresh platelet poor plasma (PPP, 50 μL) was placed on the samples and incubated at 37 °C for 120 min. After washing with PBS, 20 μL mouse anti human g-fibrinogen monoclonal antibody (primary antibody, Product No: NYB4-2xl-f, ACCURATE CHEMICAL & SCIENTIFIC Corp) was added to each sample and incubated for 60 min at 37 °C. After washing with PBS, the samples were incubated with 20 μL of HRP sheep anti mouse polyclonal antibody (second antibody) solution for 60 min at 37 °C and were washed by PBS. Then they were reacted with 70 μL of chromogenic substrate TMB solution for 10 min. The color reaction was stopped by adding 50 μL of 1 M H_2SO4, and the optical density was examined using microplate reader (μQuant, Bio-tek instruments Inc.).

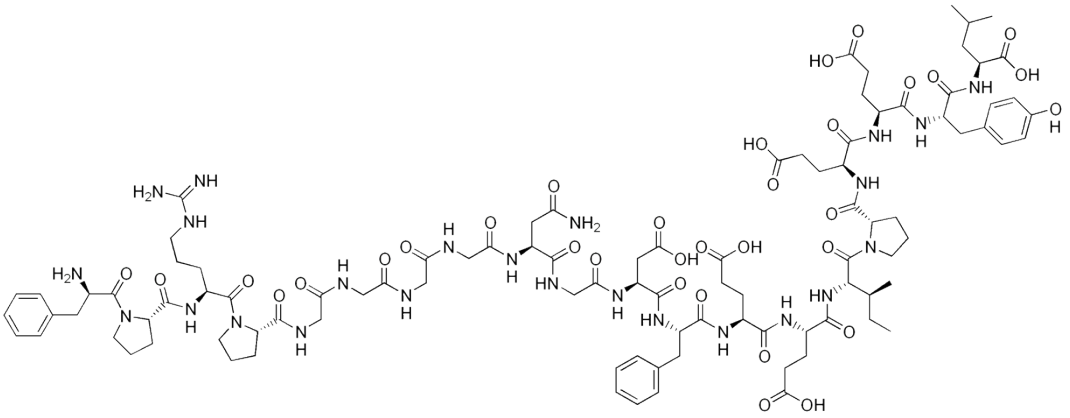

Fig. S1 Structure of D-Phe-Pro-Arg-Pro-Gly-Gly-Gly-Gly-Asn-Gly-Asp-Phe-Glu-Glu-Ile-Pro-Glu-Glu-Tyr-Leu-OH (Bivalirudin). Bivalirudin is a synthetic 20-amino acid peptide which was also designed on basis of hirudin structure. It contains two functional domains, N-terminal region (D-Phe-Pro-Arg-Pro), which binds with high affinity to the catalytic site of thrombin, and the C-terminal dodecapeptide (Asn-Gly-Asp-Phe-Glu-Glu-Ile-Pro-Glu-Glu-Tyr-Leu) that binds to the fibrinogen-binding region of thrombin. Those two functional domains are linked by a (Gly) 4 sequence.

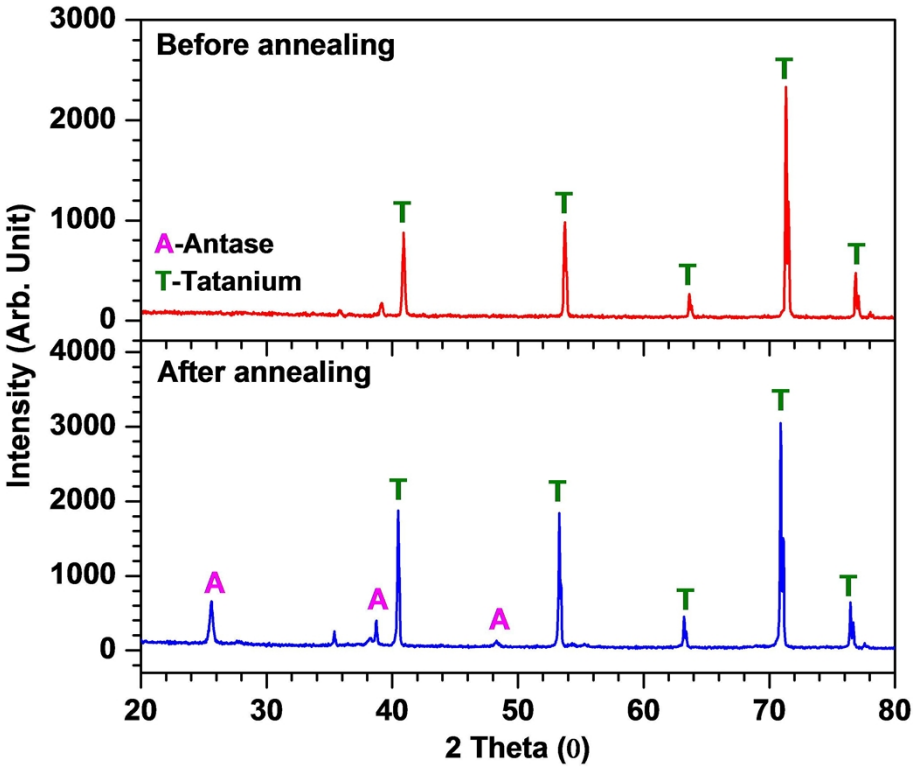

Fig. S2 XRD patterns of NTs before and after annealing at 450 °C.

Table S1 Chemical compositions of NTs, PDAM/NTs, BVLD-NTs and BVLD-PDAM/NTs obtained by XPS (n=3)

Sample	C (%)	N (%)	O (%)	Ti (%)
NTs	26.8±0.2	—	45.3±0.3	28.0±0.3
PDAM/NTs	64.2±0.3	8.8±0.1	21.7±0.2	5.3±0.2
BVLD-NTs	37.1±0.3	4.4±0.4	37.0±0.1	21.5±0.2
BVLD-PDAM/NTs	63.6±0.1	10.7±0.3	21.0±0.2	4.7±0.3

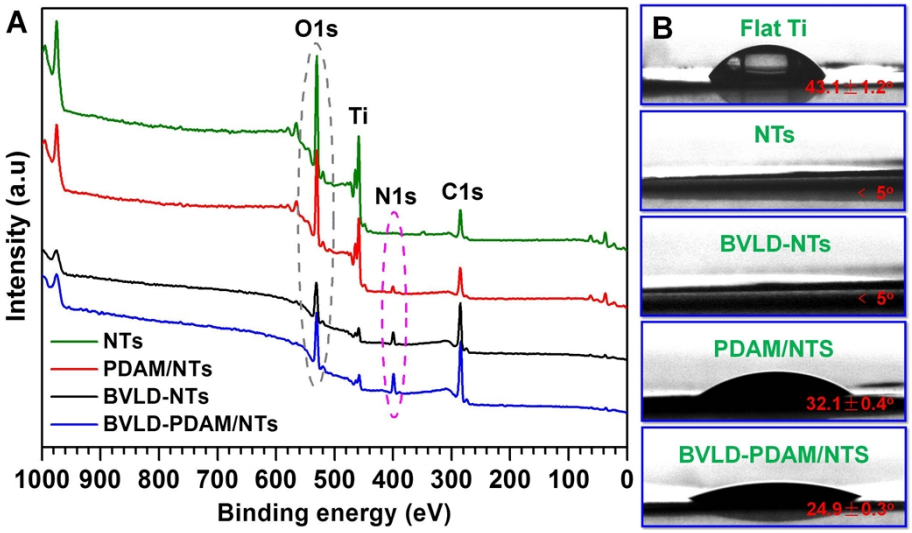

Fig. S3 (A) XPS wide scans and water contact angles of Flat Ti, NTs, BVLD-NTs, PDAM/NTs and BVLD-PDAM/NTs.

Table S2 Total amounts of BVLD eluted from Flat Ti, NTs and PDAM/NTs (n=8)

Sample	Amount of BVLD (μg/cm^2)
Flat Ti	22.3 ± 5.7
NTs	105.4 ± 7.1
PDAM/NTs	276.6 ± 9.8

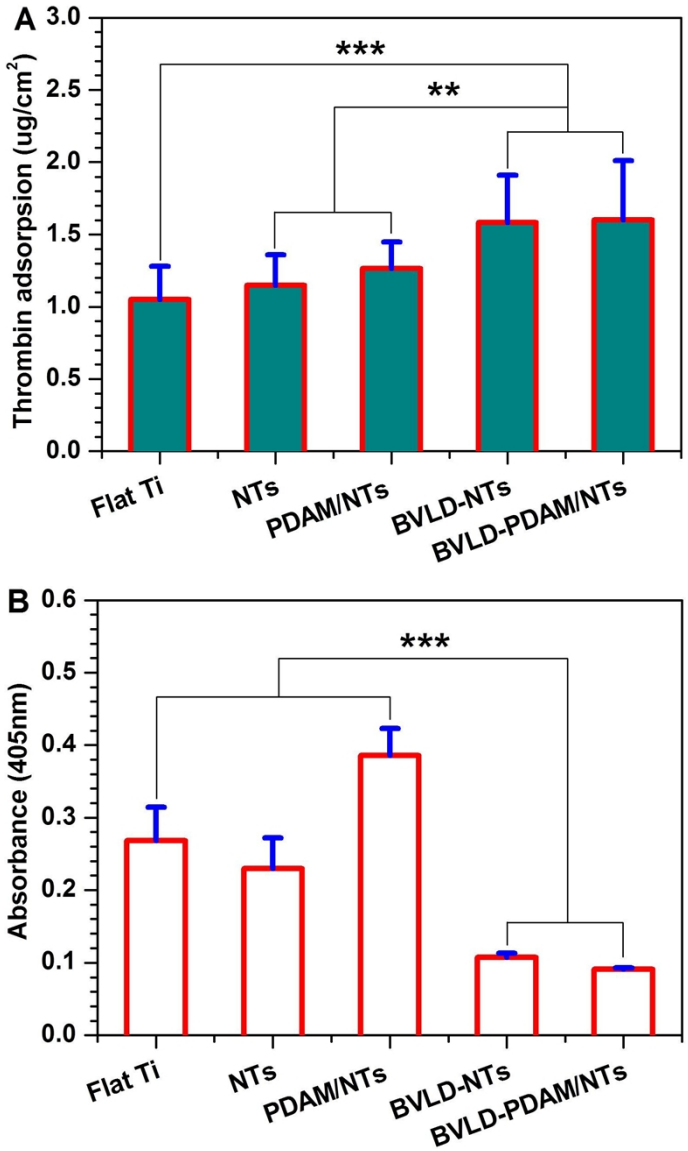

Fig. S4 (A) The amount of thrombin adsorbed on the Flat Ti, NTs, PDAM/NTs, BVLD-NTs and BVLD-PDAM/NTs that was calculated based on the equilibrium value of absorbance (405 nm) of S-2238 solution of kinetic chromogenic assay each sample; (B) The absorbance (405 nm) of S-2238 solution after 120 min in contact with different surfaces pre-immersed in thrombin solution.

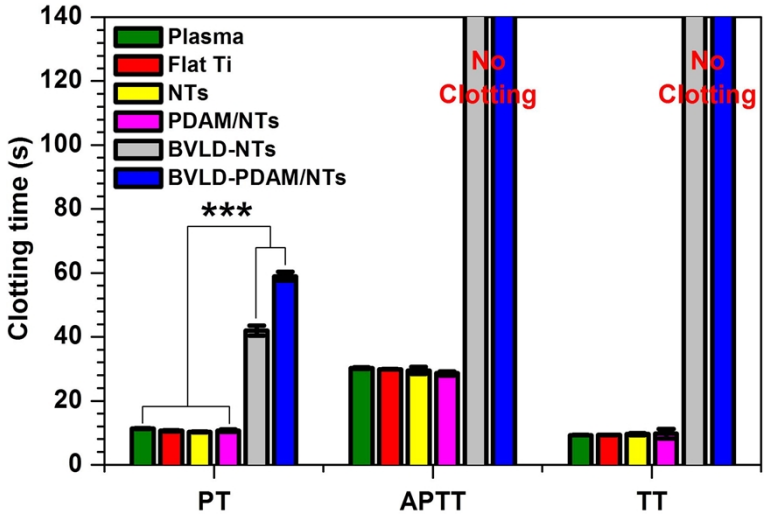

Fig. S5 Clotting times of control plasma, Flat Ti, NTs, PDAM/NTs, BVLD-NTs and BVLD-PDAM/NTs. Data presented as mean ± SD and analyzed using a one–way ANOVA, ***$p < 0.001$.

The APTT is a simple and highly reliable measurement of the capacity of blood to coagulate through the intrinsic coagulation mechanism and the effect of the biomaterial on possible delay of the process. The PT and TT tests were used to evaluate the activation of coagulation factors. When a foreign material is immersed into PPP, some coagulation factors are activated. Under the combined action of tissue thromboplastin and Ca^{2+}, factor X activates and transforms to factor Xa. Factor Xa activates prothrombin (factor II) to thrombin (factor IIa). Under the function of thrombin, fibrinogen transforms to fibrin, which forms the blood clot; especially origination from thrombin, the system has several activating feedback loops. The PT reveals the activity of the extrinsic coagulation factors and can be used to evaluate the antithrombogencity of biomaterials in vitro. The TT is the interval of activation of fibrinogen to fibrin. The longer the PT and TT, the lower is the activity of the coagulation factors.

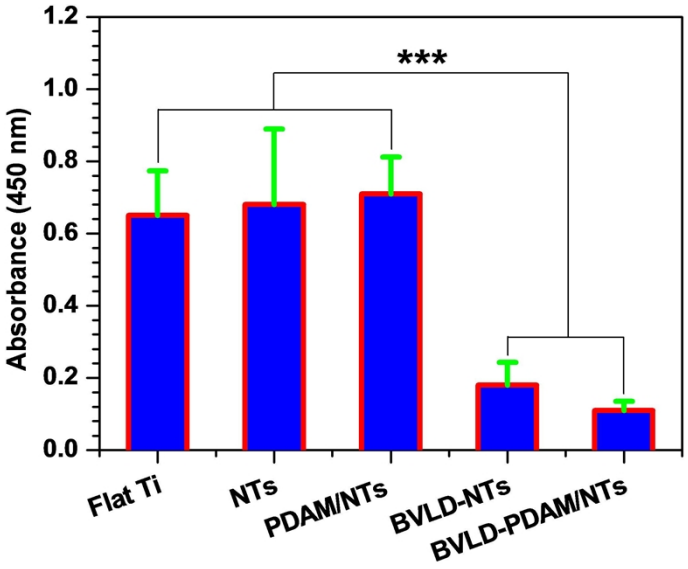

Fig. S6 Relative quantification activated fibrinogen adsorbed on different samples after incubation in PPP for 120 min. Data presented as mean ± SD and analyzed using a one-way ANOVA, ***$p < 0.001$.

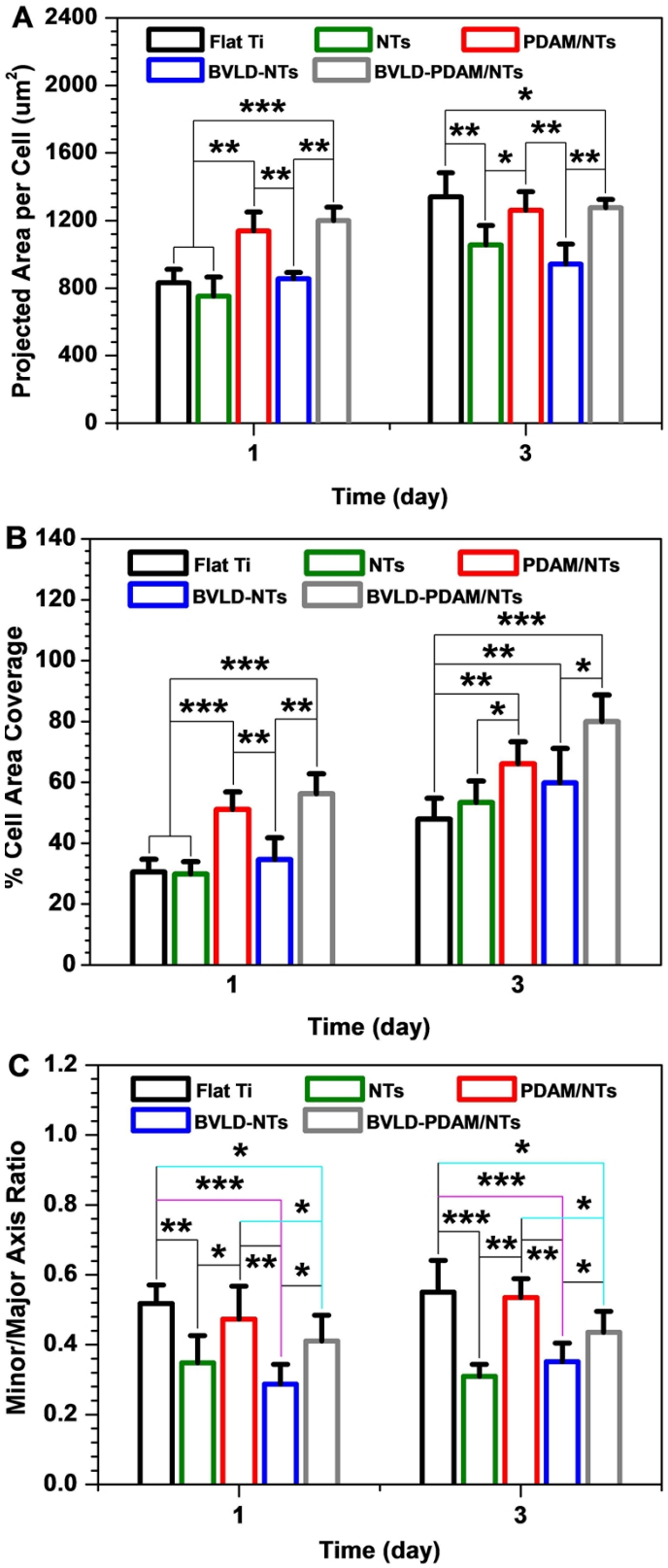

Fig. S7 (A) Projected area per cell; (B) Cover rate of cells and (C) Minor/major axis ratio are calculated from at least 100 cells from six different fields of each sample. Data presented as mean ± SD and analyzed using a one-way ANOVA, *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$.