Supplementary Information

A novel recyclable hemoperfusion adsorbent based on TiO$_2$ nanotube arrays for selective removal of β$_2$-microglobulin

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1. Materials

In the study, the β$_2$-microglobulin (β$_2$m) was homemade in the laboratory. Human plasma from healthy donors was provided by Shiyan Renmin Hospital in Hubei, China. Human β$_2$m ELISA Kit, human Alb ELISA Kit, and human TP ELISA Kit were purchased from Ningbo MedicalSystem Biotechnology Co., Ltd. in China. 2% rabbit red blood cells and potassium oxalate anticoagulant rabbit blood were purchased from Nanjing SenBeiJia Biological Technology Co., Ltd. in China. Mouse embryonic fibroblasts cell line (NIH3T3) was purchased from iCell Bioscience Inc. in China. Fetal bovine serum (FBS) and Dulbecco's modified eagle medium (DMEM) were purchased from Thermo Fisher Scientific. Cell Counting Kit-8 (CCK-8) was purchased from Wuhan Servicebio Technology Co., Ltd. in China.

2. Experiments

2.1 Analysis of the fluoride ion content of TNTAs after calcination

To determine the F^- releasing from the TNTAs-coated Ti microwires in DI water, we designed a device as shown in Fig. S3, where the microwires were put into a syringe with a catheter attached to each end of the syringe. DI water was circulated under the action of a peristaltic pump. After circulating for a few hours, the water sample was taken for Ion Chromatograph (IC) testing. DI water (without material) was tested as a control.

2.2 Adsorption experiments

2.2.1 Adsorption of β_2m in phosphate buffered saline (PBS)

The adsorption experiment included adding 60 mg and 75 mg of TNTAs-coated Ti microwires to a certain volume of β_2m solution (0.2 mg mL^{-1} and 0.3 mg mL^{-1} in PBS, pH 7.4) at 37 °C for 3 hours under oscillation of 200 rpm. After adsorption, the adsorbent was separated from the solution by centrifugation. The supernatant was collected. The concentration of the supernatant samples was measured using a UV-Vis spectrophotometer (Nanodrop 2000/2000C, Thermo Scientific, USA). The adsorption efficiency (%) of β_2m was calculated using the following equation:

$$Adsorption\ efficiency = \frac{C_0 - C_1}{C_0} \times 100\%$$

(1)

where C_0 (mg L^{-1}) and C_1 (mg L^{-1}) are adsorption concentrations of β_2m before and after, respectively.

2.2.2 Adsorption in human plasma

In the column adsorption experiment, 150 mg of Ti microwires and TNTAs-coated microwires were added in a certain volume of human plasma (50 mg L^{-1} β_2m) and circulated through a packed column at a flow rate of 1 mL min^{-1} using a peristaltic pump (LSP01-1A, China) at 37 °C. After adsorption for 15, 30, 45, 60, 180, and 360 minutes, plasma samples were taken to test the concentration of β_2m, albumin (Alb), and total protein (TP) using human β_2m ELISA Kit, human Alb ELISA Kit, and human TP ELISA Kit, respectively. The protein concentrations in human plasma were detected using an Automatic Analyzer (HITACHI 3100, Japan). The adsorption efficiency (%)

was calculated using equation (1), and the adsorption capacity (mg g^{-1}) was calculated using the following equation:

$$Adsorption\ capacity = \frac{(C_0 - C_1) \times V}{m}$$

$$(2)$$

where C_0 (mg L^{-1}) and C_1 (mg L^{-1}) are initial concentration and concentration after adsorption, respectively. V (mL) is the volume of human plasma, and m (g) is the weight of TNTAs-coated microwires.

2.2.3 Adsorption kinetics and isotherms of TNTAs-coated Ti microwires

To evaluate the adsorption kinetics of TNTAs towards β_2m, 150 mg of TNTAs-coated Ti microwires were added to a certain volume of human plasma (50 mg L^{-1} β_2m) and circulated through a packed column at a flow rate of 1 mL min^{-1} using a peristaltic pump for 15, 30, 45, 60, 180, and 360 minutes at 37 °C. The concentration of β_2m in the human plasma samples was determined using a human β_2m ELISA Kit. The β_2m uptake at time t (q_t) was calculated using the following equation:

$$q_t = \frac{(C_0 - C_t) \times V}{m}$$

$$(3)$$

where q_t is the adsorption capacity of 11.5 nm TNTAs at time t; C_0 (mg L^{-1}) and C_t (mg L^{-1}) are the initial β_2m concentration and the residual β_2m concentration at time t, respectively; V (mL) is the volume of human plasma, and m (g) is the weight of TNTAs-coated Ti microwires. The results were fitted by two widely used adsorption kinetic models, pseudo-first-order, and pseudo-second-order models, expressed as following equations:

$$ln(q_e - q_t) = ln q_{eq,cal,1} - K_1 t$$

$$(4)$$

$$\frac{t}{q_t} = \frac{1}{K_2 q_{eq,cal,2}^2} + \frac{t}{q_{e,cal,2}}$$

$$(5)$$

where q_e (mg g^{-1}) is the β_2m uptake at equilibrium; q_t (mg g^{-1}) is the β_2m uptake at time t (min); $q_{eq,cal,1}$ (mg g^{-1}) and $q_{eq,cal,2}$ (mg g^{-1}) are the calculated β_2m uptakes at equilibrium according to the pseudo-first-order and pseudo-second-order models, respectively; K_1 (min^{-1}) and K_2 (g mg^{-1} min^{-1}) are the rate constant of pseudo-first-order

and pseudo-second-order models, respectively; h = $K_2 q_{eq,cal,2}{}^2$ is the initial adsorption rate (mg g^{-1} min^{-1}) calculated by the pseudo-second-order model.

To evaluate the adsorption isotherms of TNTAs towards β$_2$m, 150 mg of TNTAs-coated Ti microwires were added to a certain volume of human plasma with initial β$_2$m concentrations of 5, 10, 25, 50, 100, and 200 mg L^{-1}. The mixture was then circulated through a packed column at a flow rate of 1 mL min^{-1} using a peristaltic pump for 3 hours at 37 °C. The concentration of β$_2$m in the human plasma samples was measured as described above. The results were fitted by two widely used adsorption isotherm models, namely the Langmuir and Freundlich models. Their linear and nonlinear equations are expressed as:

Linear Langmuir Model:

$$\frac{1}{q_e} = \frac{1}{K_L q_m C_e} + \frac{1}{q_m} \tag{6}$$

Nonlinear Langmuir Model:

$$q_e = \frac{q_m K_L C_e}{1 + K_L C_e} \tag{7}$$

Linear Freundlich Model:

$$ln(q_e) = \frac{1}{n}ln(C_e) + ln(K_F) \tag{8}$$

Nonlinear Freundlich Model:

$$q_e = K_F C_e^{\frac{1}{n}} \tag{9}$$

where q$_e$ (mg g^{-1}) is the β$_2$m uptake at equilibrium; C$_e$ (mg L^{-1}) is the equilibrium β$_2$m concentration; q$_m$ (mg g^{-1}) is the maximum adsorption capacity calculated by the Langmuir model; K$_L$ (L/mg) is the Langmuir constant indicating adsorption energy and affinity; K$_F$ ((mg/g)(L/mg)$^{1/n}$) is the Freundlich constant related to maximum adsorption capacity; 1/n is the Freundlich exponent related to surface heterogeneity and adsorption intensity.

2.3 *In vitro* biocompatibility

2.3.1 Hemolysis assay

In the hemolysis assay, 20 μL of 2% red blood cells (RBCs) were mixed with (a) 980 μL of DI water as a positive control; (b) 980 μL of PBS as a negative control; (c) 980 μL of Ti microwires/PBS; 11.5 nm TNTAs/PBS; 61.3 nm TNTAs/PBS; 96.5 nm TNTAs/PBS and reused 11.5 nm TNTAs/PBS suspensions as samples. The reused 11.5 nm TNTAs are samples that have been adsorbed once and then photocatalyzed. The weight of the Ti microwires and TNTAs-coated Ti microwires in the solution was 150 mg. The mixtures were incubated at 37 °C for 180 min. After incubation, the mixtures were centrifuged at 5000 rpm for 5 min and were photographed. The absorbance of the supernatant was evaluated at 541 nm, and the hemolysis ratio (HR) was calculated according to the following equation:

$$HR(\%) = \frac{(A_S - A_n)}{(A_p - A_n)} \times 100\%$$

(10)

where As, An, and Ap are the absorbance of the samples, negative control, and positive control, respectively.

2.3.2 Coagulation assay

In the blood coagulation assay, rabbit blood samples were first centrifuged at 4000 rpm for 15 minutes to obtain platelet-poor plasma (PPP). Then, 2 mL of PPP was incubated with materials at 37 °C for 60 minutes. The weight of the Ti microwires and TNTAs-coated Ti microwires (pore sizes: 11.5 nm, 61.3 nm, and 96.5 nm) in the solution was 150 mg. Pure PPP without samples was used as a control. After incubation, the mixtures were centrifuged at 4600 g for 5 minutes. The blood coagulation was assessed using four clinical assays: fibrinogen (FIB), prothrombin time (PT), thrombin time (TT), and activated partial thromboplastin time (APTT) test. These measurements were performed using an automatic blood coagulation analyzer (Coapresta 2000, Japan) at Huazhong University of Science and Technology Hospital (Hubei, China).

2.3.3 Blood routine assay

To study the influence of TNTAs-coated Ti microwires on blood cells, fresh anticoagulant blood from healthy rabbits (2 mL for each sample) was mixed with different adsorbents (150 mg for each sample). The mixture was then incubated at 37 °C for 60 minutes. Blood routine assays were determined using an Automatic Blood

Cell Analyzer (XT1800i, Sysmex, Japan).

2.3.4 Cell viability assay

The conditioned media were prepared by incubation of various Ti microwires into cell culture media containing DMEM, 10% FBS, and 1% penicillin/streptomycin at 37 °C. NIH3T3 cells were seeded in 96-well plates at an appropriate density of 3×10^3 cells per well and then incubated in an incubator at 37 °C and 5% CO_2 for 24 hours. Then cell culture media was replaced with conditioned media to evaluate the cytotoxicity of the adsorbents. After incubation for 24 hours, the media were replaced with fresh media containing CCK-8 solution. After incubation for 3 hours, the absorbance was measured by a microplate spectrophotometer (Eon, BioTek, Winooski, VT, USA) at a wavelength of 450 nm.
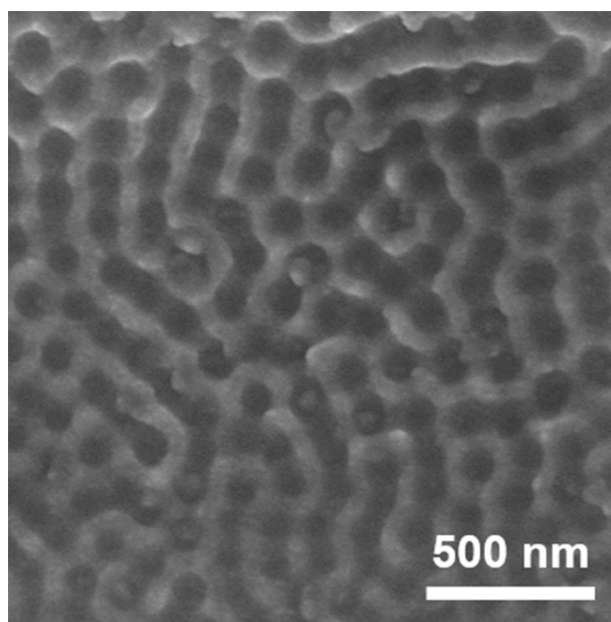
3. Results and discussions

Fig. S1. Neat and orderly textured Ti substrate obtained after ultrasonication.
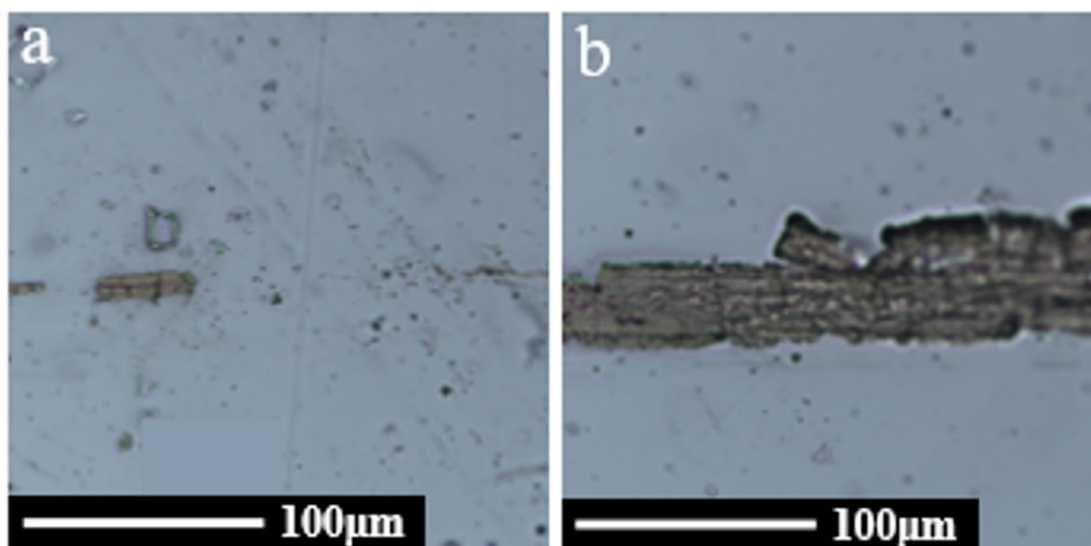
Fig. S2. Optical microscope images of TNTAs-coated Ti microwires without (a) and with (b) an additional compact layer during the scratch test.
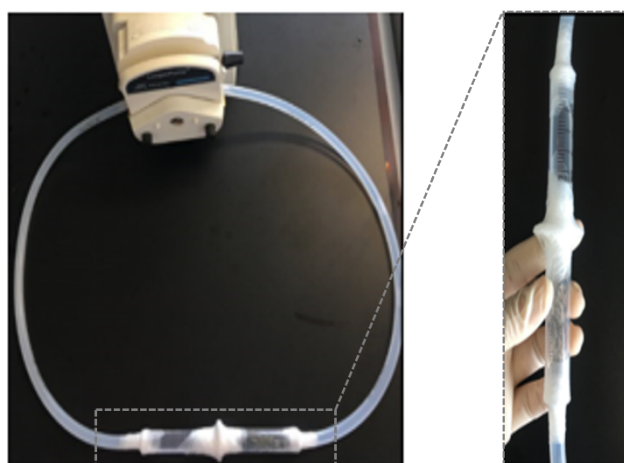

Fig. S3. Schematic representation of the circulation of calcined TNTAs-coated Ti microwires in DI water.

Table S1. Determination of F^- concentration by IC.

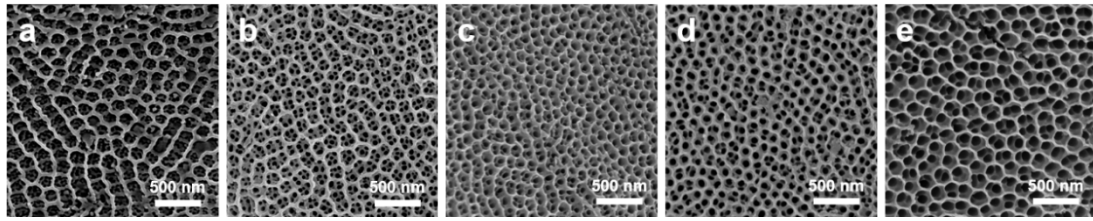| Sample	Height (μS/cm)	Rotention time (min)	Area ((μS/cm)×min)	F^- concentration (mg/L)
DI water (Control)	0.023	7.017	0.0050	0.067
Calcined TNTAs-coated Ti microwires releasing in DI water	0.143	7.162	0.0335	0.1461

Fig. S4. SEM images of TNTAs prepared at different anodization voltages. (a) 20 V, NH_4F content 0.25 wt%, anodization time 30 min. (b) 30 V, NH_4F content 0.25 wt%, anodization time 30 min. (c) 40 V, NH_4F content 0.25 wt%, anodization time 30 min. (d) 50 V, NH_4F content 0.25 wt%, anodization time 30 min. (e) 60 V, NH_4F content 0.25 wt%, anodization time 30 min.
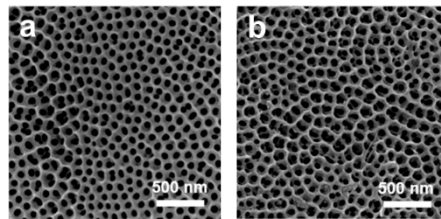

Fig. S5. SEM images of TNTAs prepared at different anodization time. (a) 60 min, voltage 60 V, NH_4F content 0.25 wt%. (b) 90 min, voltage 60 V, NH_4F content 0.25 wt%.
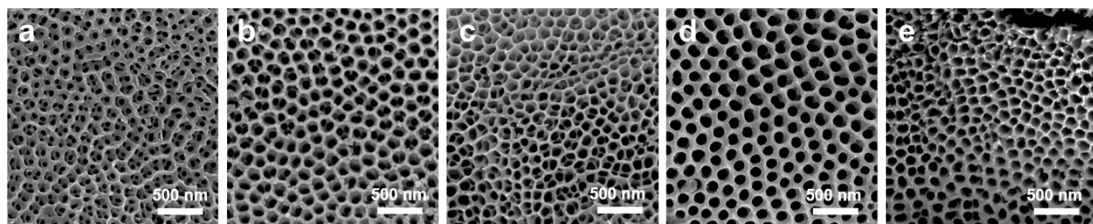

Fig. S6. SEM images of TNTAs prepared by anodization in electrolytes containing different NH_4F contents. (a) 0.15 wt%, voltage 60 V, anodization time 30 min. (b) 0.2 wt%, voltage 60 V, anodization time 30 min. (c) 0.25 wt%, voltage 60 V, anodization time 30 min. (d) 0.3 wt%, voltage 60 V, anodization time 30 min. (e) 0.35 wt%, voltage 60 V, anodization time 30 min.
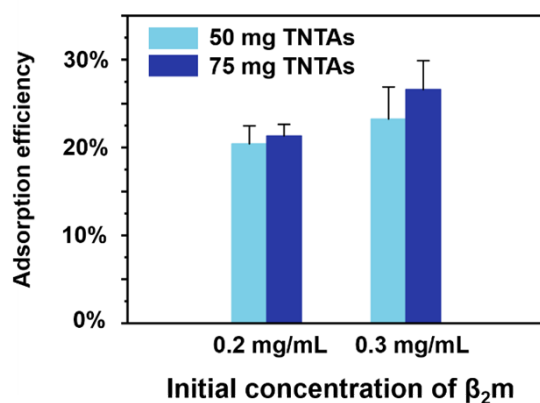
Fig. S7. The effect of different amount of the TNTAs-coated Ti microwires and different initial concentration of the β_2m solution on their adsorption efficiency.

Table S2. Adsorption kinetic parameters of TNTAs-coated Ti microwires towards β_2m in human plasma.

Sample	t_{eq} (min)	$q_{e, exp}$ (mg g^{-1})	Pseudo-first-order model			Pseudo-second-order model			
			$q_{eq, cal, 1}$ (mg g^{-1})	K_1 (min^{-1})	R^2	$q_{eq, cal, 2}$ (mg g^{-1})	K_2 (g mg^{-1} min^{-1})	h (mg g^{-1} min^{-1})	R^2
11.5 nm TNTAs	180	14.96	1.82	0.0059	0.8706	14.51	0.0263	5.5401	0.9992

Table S3. Adsorption isotherm parameters of TNTAs-coated Ti microwires towards β_2m in human plasma.

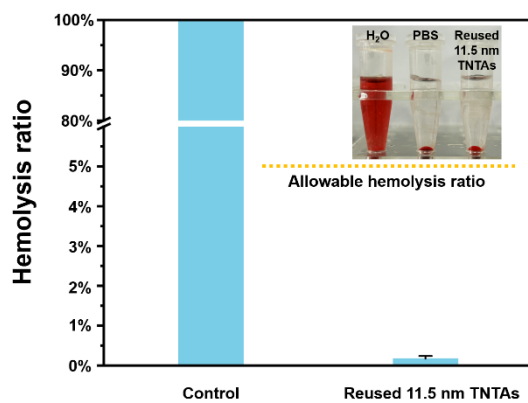| Sample	Fitting method	Langmuir model			Freundlich model		
		q_m (mg g^{-1})	K_L (L/mg)	R^2	K_F ((mg/g)(L/mg)$^{1/n}$)	$1/n$	R^2
11.5 nm TNTAs	Linear	40.40	0.0060	0.9943	0.5738	0.6489	0.9203
	Non-linear	17.30	0.0853	0.9843	4.4896	0.2481	0.9133

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Fig. S8. Hemolysis ratio of positive control group and reused 11.5 nm TNTAs-coated Ti microwires. Inset is a digital photograph for the hemolysis assay of reused 11.5 nm TNTAs-coated Ti microwires.