Electronic Supplementary Material (ESI) for Nanoscale Advances.

Supplementary Information

PTFEP-Al_2O_3 Hybrid Nanowires Reducing Thrombosis and Biofouling

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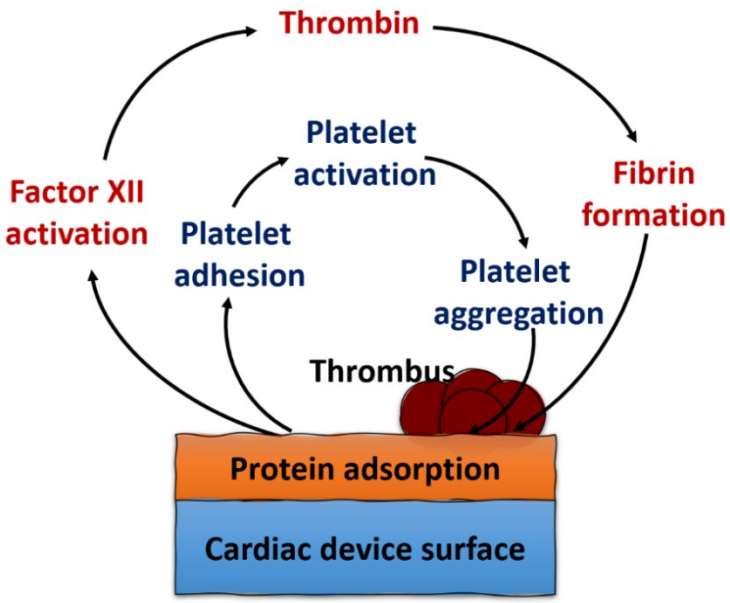

Figure S1. Schematic illustration for blood-contacting medical (cardiac) device-associated thrombosis. (Adapted from [1]).

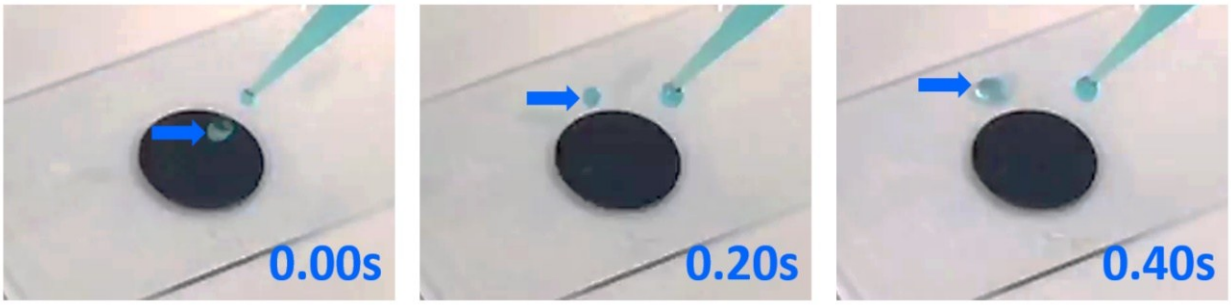

Figure S2. Sliding of the colored protein plasma fraction (PPF) solution drop on PTFEP-Al_2O_3 NWs (video-captured images from **Video S1**).

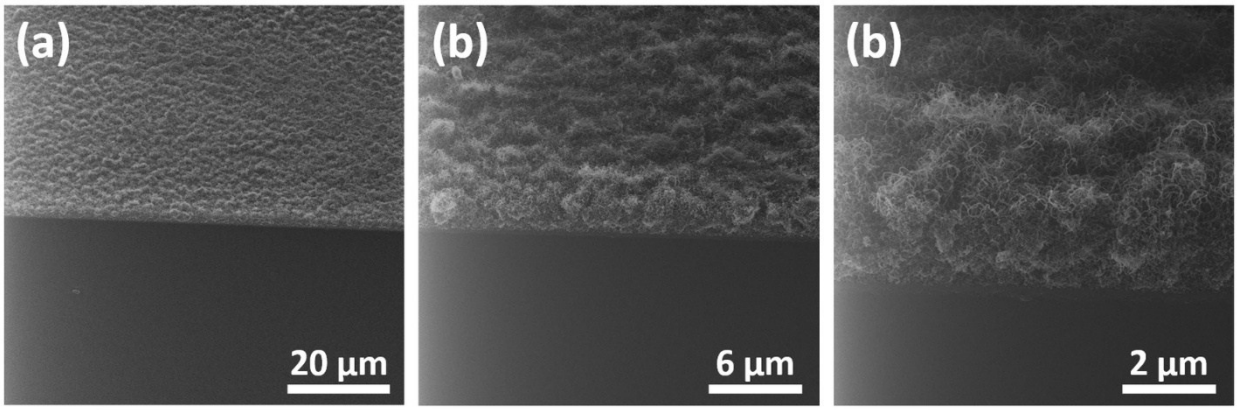

Figure S3. (a-c) Low magnification of Helium ion microscopy (HIM) images for Al_2O_3 NWs.

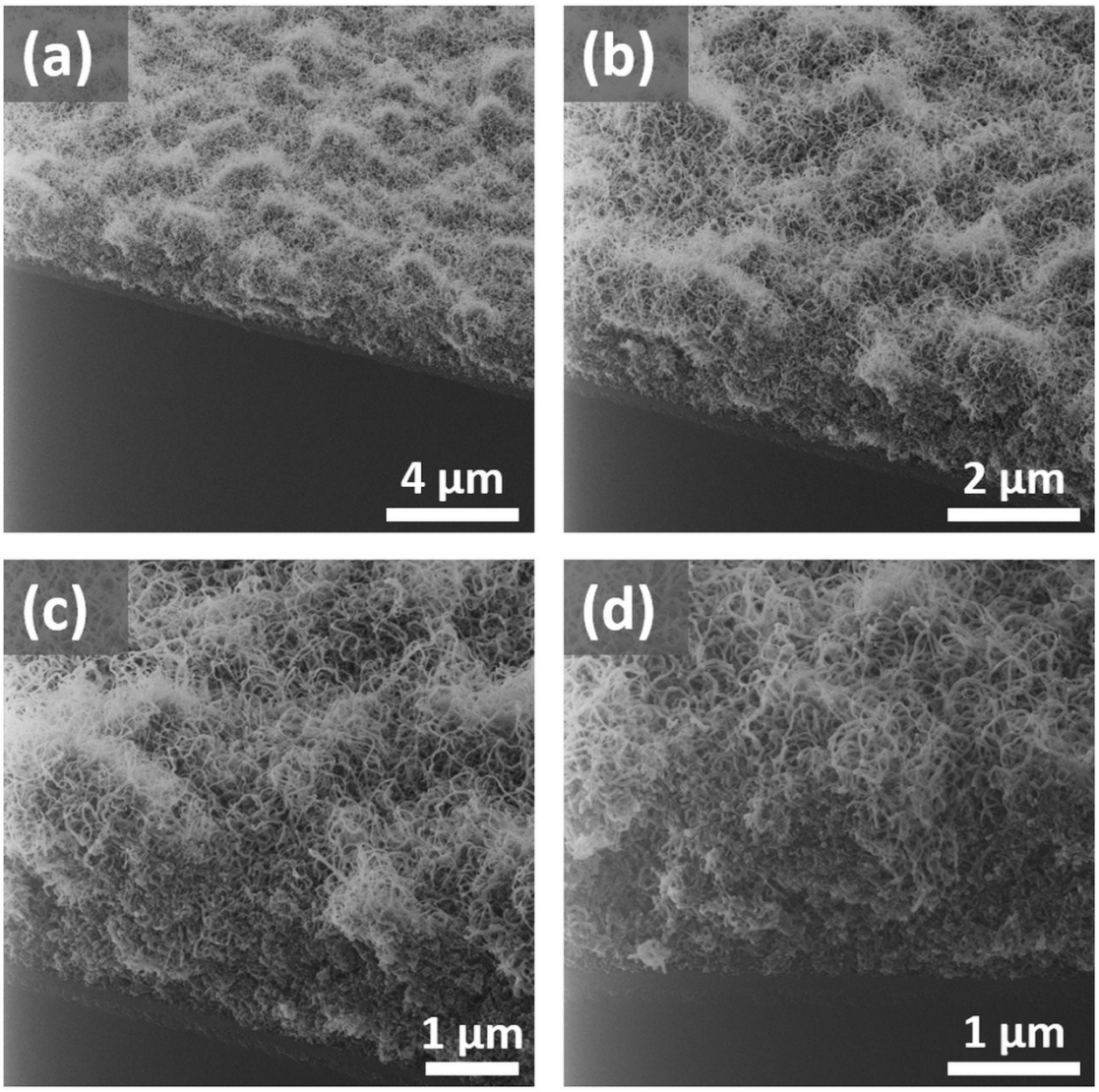

Figure S4. (a-d) High magnification of Helium ion microscopy (HIM) images for Al_2O_3 NWs.

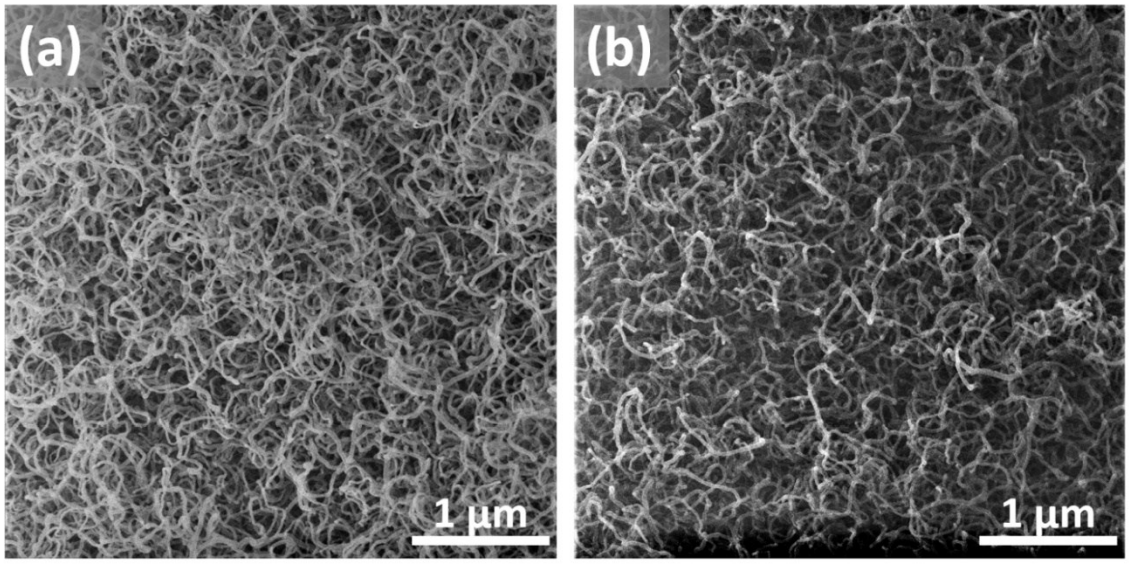

Figure S5. Helium ion microscopy (HIM) images of **(a)** Al_2O_3 NWs and **(b)** PTFEP-Al_2O_3 NWs.

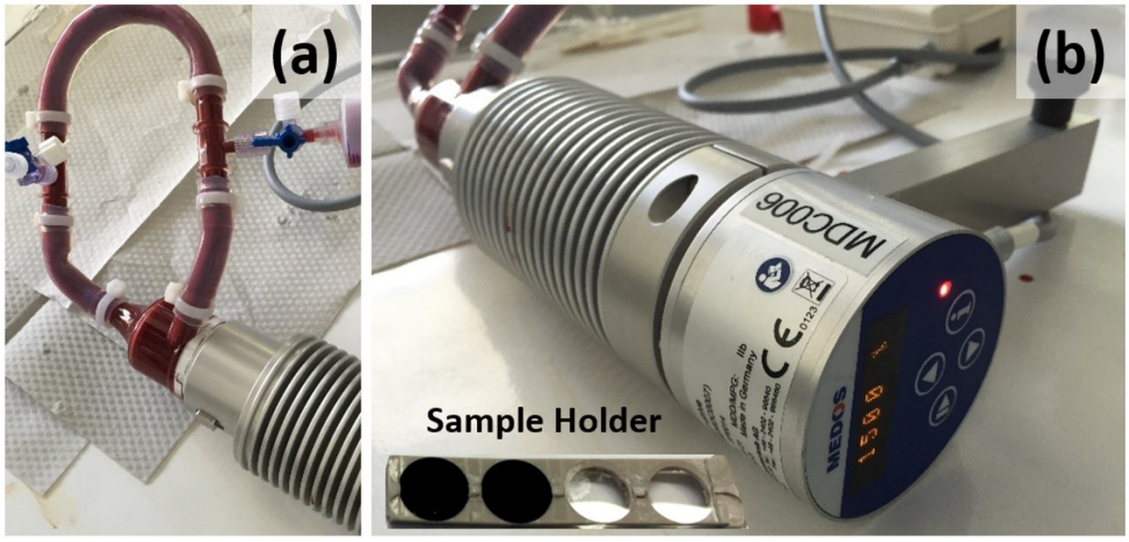

Figure S6. (a) and **(b)** Images for closed perfusion system using MEDOS MDC006 pump. (The sample holder is inserted in the ¾ inch silicon tube and perfused with 16 ml complete blood at 1500 rpm for 10 min.)

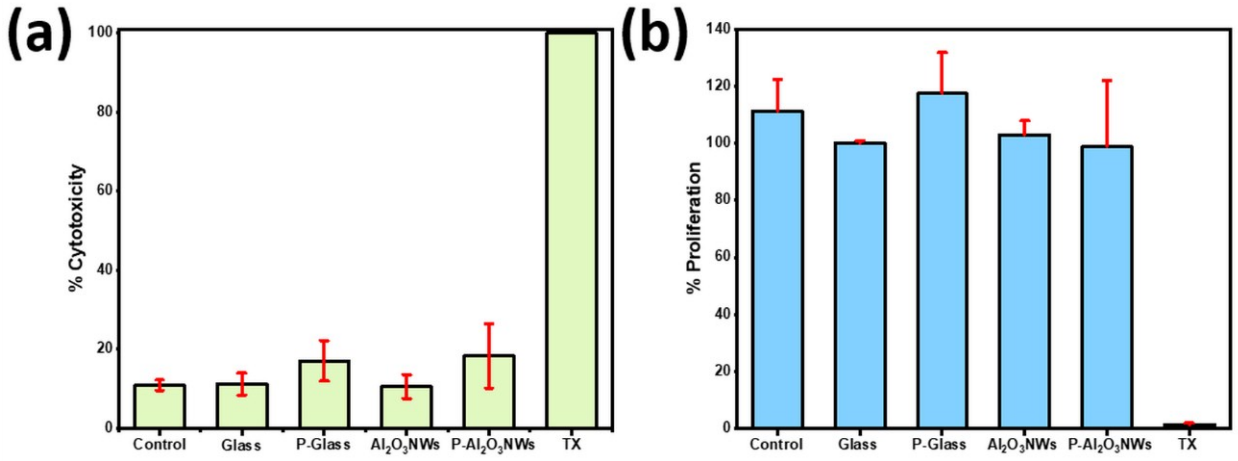

Figure S7. The metabolic activity of human cardiomyocytes (HCM) on prepared surface using **(a)** LDH and **(b)** WST-1 assays.

Materials and Methods

Synthesis of Al_2O_3 NWs and Deposition of PTFEP Layer

Al_2O_3 NWs were synthesized by chemical vapor deposition (CVD) of a single source precursor (SSP), $[tBuOAlH_2]_2$. Prior to the deposition, the SSP was synthesized as described in detail elsewhere.[2] Following the cleaning procedure (rinsing in acetone and water for 1 minutes) glass substrates were placed on a graphite holder within a vertical cold wall CVD reactor. The chamber was evacuated until pressure reached to 10^{-3} mbar and the graphite holder was heated up to 500 ℃ by a high frequency induction coil. Then the precursor (heated to 70-80 ℃) was flown into the vacuum chamber for 3 minutes. Following the deposition, substrates were left to cooling to room temperature under the vacuum.

Before coating Al_2O_3 NWs deposited samples with poly[bis(2,2,2-trifluoroethoxy)phosphazene] (PTFEP), (a solution of PTFEP in tetrahydrofuran, methyl ethyl ketone and acetone) was prepared. Then PTFEP solution was placed in a syringe pump which was connected to the atomizing nozzle. The pump flow rate was set to 0.05 ml/min and the ultrasonic atomizing nozzle was actuated at a frequency of 120 kHz. Al_2O_3 NWs coated samples were scanned under the nozzle (focused beam) with the help of an automated XY stage. The distance between the stage and the nozzle was kept around 2 mm and scanning speed was set to 0.5 mm/sec. Each sample was coated with 8 continuous passes.

Platelet Adhesion and Aggregation

Complete human blood from the same donor was used for the observation of platelets adhesion and aggregation. Each time, the four different samples: Glass, P-Glass, Al_2O_3 NWs and P-Al_2O_3 NWs were inserted in a special designed holder. The holder was then coupled to an established closed flow system activated by a miniaturized perfusion pump (Medos Gmbh) with a volume capacity of 16 ml. The samples were perfused within the system at 25 °C for 10 min under a flow rate of 1500 rpm. After that,

the holder containing samples was removed, samples were detached and directly washed with DPBS (Dulbecco's Phosphate-Buffered Saline - GIBCO®) 3 times, followed by a fixation with a freshly prepared 2,5% Gluteraldehyde in 0.1 M Cacodylate buffer solution for 15 min at RT. After fixation, samples were washed with 0.1 M Cacodylate buffer solution (3 times, for 10 min each), then incubated for 1 h at RT in 1% Osmiumtetroxide (OsO_4) in 0.2 M Cacodylate buffer solution for contrasting. The incubations were performed in a dark room. Following the contrasting, samples were again washed with distilled water dH_2O for 4 times at 10 min in order to remove every OsO_4 contamination. Samples were then dehydrated with a series of 70%, 80%, 90%, 96% and 100% Ethanol solutions (incubation for 10 min). Then samples were dried by incubation once for 10 min in 50% Ethanol and 50% Hexamethyldisalazane (HMDS) solution, then twice for 10 min in pure HMDS. At the end, samples were covered with HMDS and stayed overnight under the hood. For analysis, samples were sputtered with Platinum-Gold and observed under scanning electron microscope (SEM). The experiment was repeated six times (n=6) using the same donor.

Protein Adsorption

The four different samples: Glass, P-Glass, Al_2O_3 NWs and P-Al_2O_3 NWs were perfused in 1 ml Platelet poor plasma (PPP) for 30 min at 37 °C. Platelet poor plasma (PPP) was separated from a donor blood. In order to visually analyses the protein adsorption similar as mentioned above, washing, fixation, contrasting and drying steps were applied, and observations of the protein layers were performed under SEM. The experiment was repeated 3 times (n=3) using the same donor.

Cell mediated cytotoxicity

LDH assay (Cytotoxicity Detection Kit; Roche Diagnostics, Mannheim, Germany) was used to determine cytotoxicity. As previously described, Cells were incubated at 37 °C and 5% CO_2 for 24 h. Medium supernatants of each samples were collected in 500 µl Eppendorf tubes and centrifuged under 300xg for 10 min. After centrifugation, a 3 x 100 µl volume of each sample supernatant was transferred to 3 wells of a 96 multi-well-plate. Subsequently, LDH mix was prepared as described in instruction manual and 100 µl of the reagent mix is added to each well followed by incubation in a dark room and slight shuttling for 30 min. To block the reaction 50 µl stop solution was given to each well and shacked for 1 min. The mean absorbance of cells cultured with normal medium was defined as control indicating minimal cytotoxicity. Accordingly, the absorbance of cells grown under prepared mediums was related to this value. 2% Triton X-100 treated cells were used as positive control indicating a 100% cytotoxicity. Statistical analysis was performed using the Student's t-test. The differences in viability between cells grown on glass and those grown on structured surfaces were: significant when $P < 0.05$ (*), very significant when $P < 0.01$ (**) and extremely significant when $P < 0.001$ (***).

Cell proliferation

WST-1 assay (Cell Proliferation Reagent; Roche Diagnostics, Mannheim, Germany) was used to determine the cellular viability. As previously described[3], Cells were incubated at 37 °C and 5% CO_2 for

24h. Accordingly, the different culture medium were discarded and a volume of new 300 µl fresh medium was given to each plate. Subsequently, 1:100 WST-1 reagent was added to each sample and incubated for 4h in 37°C. The formazan formation was measured as a sign of cell viability according to the instruction manual provided by the manufacturer. The mean absorbance of cells cultivated with normal medium was defined as 100% proliferation. Accordingly, the absorbance of cells cultivated with prepared mediums was related to this value. 2% Triton X-100 treated cells were used as negative control indicating the minimal proliferation. Statistical analysis was performed using the Student's t-test. The differences in viability between cells grown on glass and those grown on structured surfaces were: significant when $P < 0.05$ (*), very significant when $P < 0.01$ (**) and extremely significant when $P < 0.001$ (***).

Bacterial Adhesion

For the preparation of the bacterial suspension two to three colonies of 16 hours inoculated Staphylococcus epidermidis isolate RP62a sheep blood agar plate was taken from by cotton swab and stirred in 2 ml of PBS until a McFarland turbidity of 0.5 has been reached. This solution was then serially diluted in 10-fold increments in PBS to a 10-4 dilution level. Surfaces to be tested (9 mm in diameter) were fixed by agar in sterile 24-well plates (Greiner) and subsequently overlaid with 1 ml of PBS (phosphate-buffered saline, pH 7.4). The agar-fixed surfaces were overlaid with 1 ml of the diluted to 10-4 bacterial solution after incubation of the PBS and incubated for 30 min at 37 °C. Subsequently, the bacterial solution was completely sucked off and the surfaces were washed twice with PBS. Subsequently, the surfaces were removed from the 24-well plate using sterile tweezers and placed upside down on a Luria-Bertani agar plate. This was incubated for 24 hours at 37 °C and then determined the number of colonies forming units (CFU) on the surfaces.

References

1 I. H. Jaffer, J. C. Fredenburgh, J. Hirsh and J. I. Weitz, *J. Thromb. Haemost.*, 2015, 13, S72–S81.

2 M. Veith, J. Lee, M. Martinez Miró, C. K. Akkan, C. Dufloux and O. C. Aktas, *Chem. Soc. Rev.*, 2012, **41**, 5117–30.

3 K. Kiefer, G. Akpınar, A. Haidar, T. Ikier, C. K. Akkan, E. Akman, J. Lee, M. M. Miró, E. Kaçar, A. Demir, M. Veith, D. Ural, M. Kasap, M. Kesmez, H. Abdul-Khaliq and C. Aktas, *RSC Adv.*, 2016, **6**, 17460–17469.