Electronic Supplementary Material (ESI) for Biomaterials Science.

Surface grafting of Fc-binding peptides as a simple platform to immobilize and identify antibodies that selectively capture circulating endothelial progenitor cells

Supplementary Materials

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X-Ray Photoelectron Spectroscopy [XPS]

To measure the elemental compositions on the PureCoat™ aminated polystyrene, X-ray photoelectron spectroscopy (XPS, Thermo Scientific K-Alpha™) was used. First, chemical derivatization was initiated with 4-(trifluoromethyl) benzaldehyde (TFBA, #224944, Sigma-Aldrich®), which reacts with primary amines as previously described.[1] The reaction between the primary amines on the surface and the vapor phase of TFBA was left to proceed for 3 h at 45°C. After incubation, XPS was used directly at 200eV pass energy by analyzing a 400 µm spot size. To calculate the concentration of primary amines on the samples, the following formula was used:

$$[NH_2]=[N]_u \times \frac{[F]_d}{3[N]_d} \times 100 \quad (1)$$

Where u and d represent underivatized and derivatized compositions, respectively.

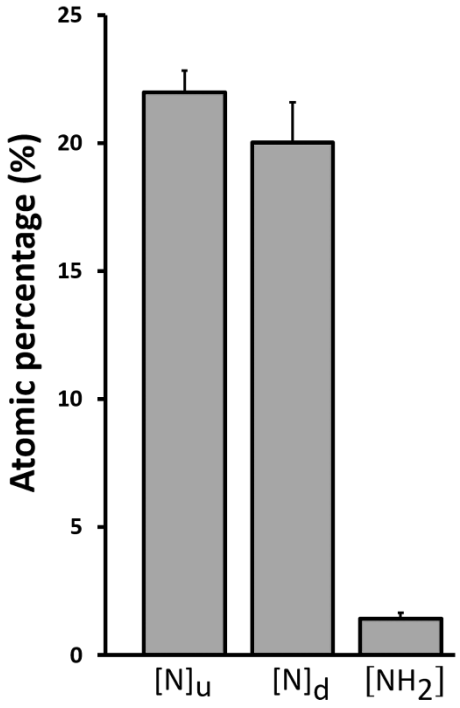

Figure S1. Surface characterization of the PureCoat™ aminated surface. XPS analysis accompanied by TFBA for chemical derivatization was used to measure the percentages of nitrogen (%N) and amine (%NH_2) on the aminated surfaces. $[N]_u$, $[N]_d$, $[NH_2]$ represent underivatized nitrogen, derivatized nitrogen and amine conditions, respectively. *N*=3 experimental replicates.

Water Contact Angle Measurements

Optical contact angle (OCA 150 instrument, DataPhysics, Germany) was used to measure the wettability of the aminated polystyrene surface after each modification step. The water contact angle measurements were performed on the non-modified aminated substrate, after adding sulfo-SMPB and after adding RRGW peptides to the surface. To do so, 5 µL reverse osmosis water (RO water) drops were deposited onto the surfaces at a rate of 0.5 µL/s. The average between the left and the right contact angles was measured using SCA-20 software (DataPhysics, Germany).

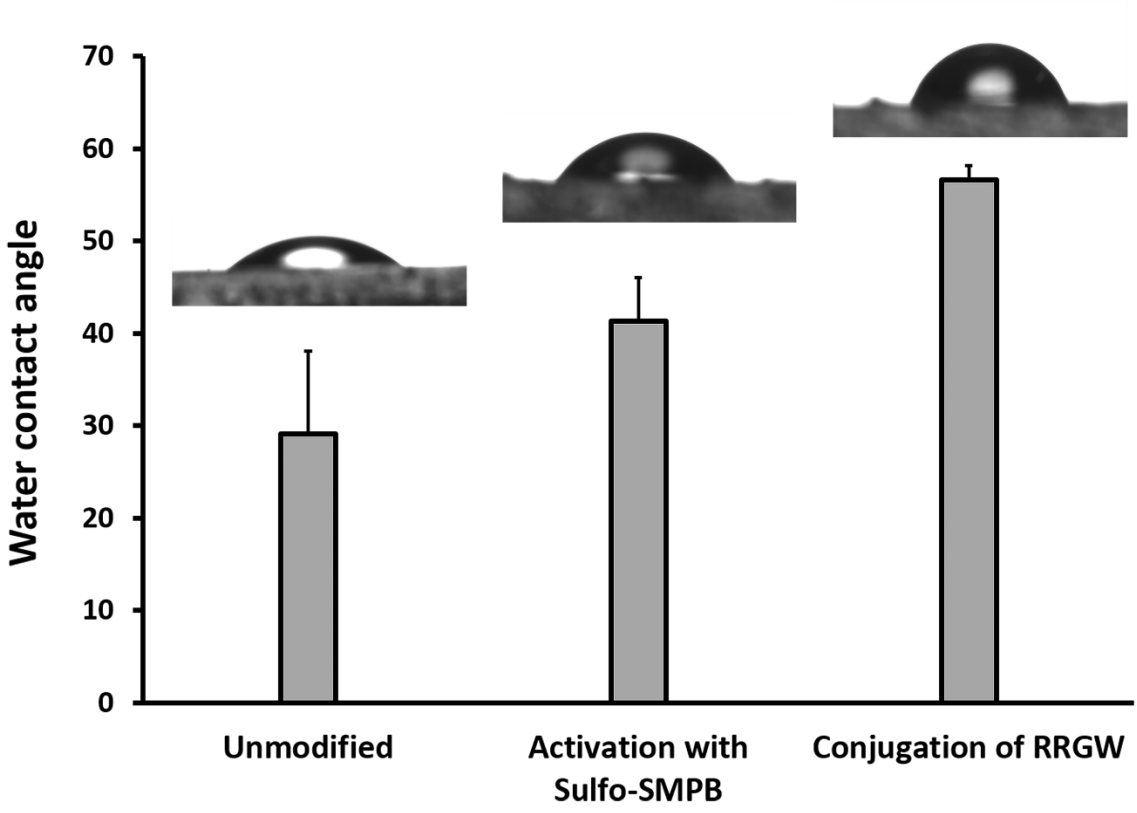

Figure S2. Surface wettability of PureCoat™ aminated surfaces before and after each reaction step. The figure shows the average water contact angle measurements between 5 µl drops of RO water and the surface before and after modifications with sulfo-SMPB and RRGW. *N*=3 experimental replicates (8 different images were taken per experimental replicate).

Standard Curve to Quantify Immobilized Antibodies

The following concentrations of secondary antibodies (F(ab′)2-goat anti-mouse IgG conjugated with Alexa Fluor 488) were prepared by resuspension in phosphate-buffered saline solution (PBS, pH 7.4): 20 µg/mL, 10 µg/mL, 5 µg/mL, 1 µg/mL and 0.1 µg/mL. Spots of 0.2 µL of each solution were deposited on the aminated polystyrene substrate and left to dry for approximately 2 h. Dried spots were then imaged using a 10X objective of a confocal microscope (Zeiss LSM 710 Exciter, Germany). For the intersection with the Y-axis – fluorescence intensity – or at a concentration of 0 µg/mL of the secondary antibodies, images at the same magnification (same surface area) were taken outside the spot regions. For each concentration, 3 spots were analyzed, and the mean intensities for 0.013 ± 0.001 cm^2 of surface area per each spot were quantified and extracted using CellProfiler™ software with "measure object intensity" plug-in**.** The standard curve was built using the Fit Model option in JMP®. The standard curve was used to estimate the number of immobilized primary antibody molecules on the surface, assuming that primary and secondary antibodies interact in 1:1 ratio. Images of test samples were acquired in the same conditions on the same day (i.e., in dry conditions without the addition of mounting medium, with the same lamp power, magnification, objectives and exposure times).

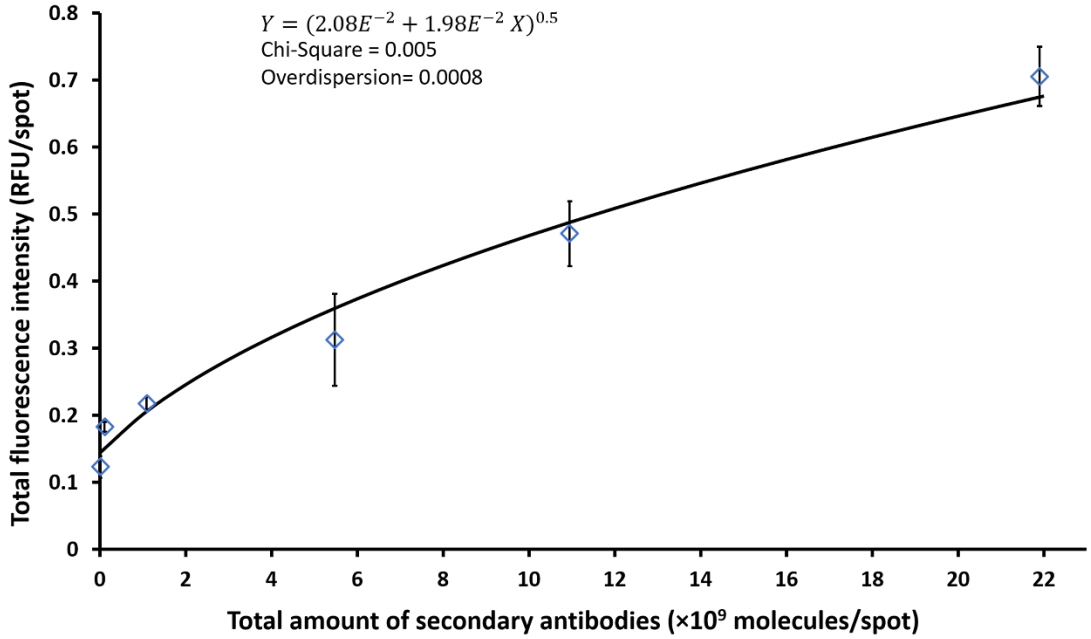

Figure S3. Fluorescence intensity of secondary antibody (Alexa 488 F(ab′)2-goat anti-mouse IgG) spots as a function of the total number of molecules. For each concentration, a total of 3 spots were analyzed for each replicate to extract the mean intensity (RFU) per each spot. The values of Chi-Square (0.005) and overdispersion (0.0008) are great indications of the goodness of the model fit. *N*=3 experimental.

References

1. E. Yegen, U. Zimmermann, W. E. S. Unger and T. Braun, *Plasma Processes and Polymers*, 2009, **6**, 11-16.