SUPPORTING INFORMATION

SPECIFIC ADSORPTION VIA PEPTIDE TAGS: ORIENTED GRAFTING AND RELEASE OF GROWTH FACTORS FOR TISSUE ENGINEERING

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XPS analysis of PS-based microplates

XPS measurements were performed on a VG ESCALAB 3 MkII instrument using non-monochromatic Mg Kα radiation (12 kV, 18 mA). The samples were extensively washed and immersed in an ultrasonic bath prior to cutting (4 mm x 4 mm) from a 48-well microplate, in order to remove any possible trace of chemical residues that could alter XPS measurements. A compositional survey and high-resolution scans were acquired at 0° emission angle, normal to the surface, using an analyser pass energy of 100 eV and 20 eV, respectively. XPS data analysis was performed by applying a Shirley-type background subtraction, using sensitivity factors from the Wagner table and charging correction by referencing to the C 1s peak at 285.0 eV[1].

XPS data showed that the plasma-treated CB surfaces featured high oxygen content (16 at. %) and, more specifically, carboxyl (O*-C=O), carbonyl (C=O) and hydroxyl (C-O) groups (**Error! Reference source not found.**), in good agreement with the manufacturer's guide[2]. The high-resolution survey also indicated that the density of polystyrene phenyl groups was strongly diminished by the CB treatment although a significant proportion remained detectable (O 1s peak, 7.2 and 2.0 at. % on pristine PS and CB, respectively). The low amino content (N 1s peak, 1.6 at. %) was attributed to unidentified contaminants. We further modified the CB surface by incubating a poly(allylamine) (PAAm) solution supplemented with NHS and EDC (see Methods). Formation of a covalent bond between activated carboxyl groups on the surface and primary amino groups of the polymer was confirmed by the increase in amide content on CB-PAAm (O-C=N peak, 1.7 at. %). Accordingly, the density of primary amino groups increased on the latter (NH_2 and NH_3^+ peaks, total 3.8 at. %).

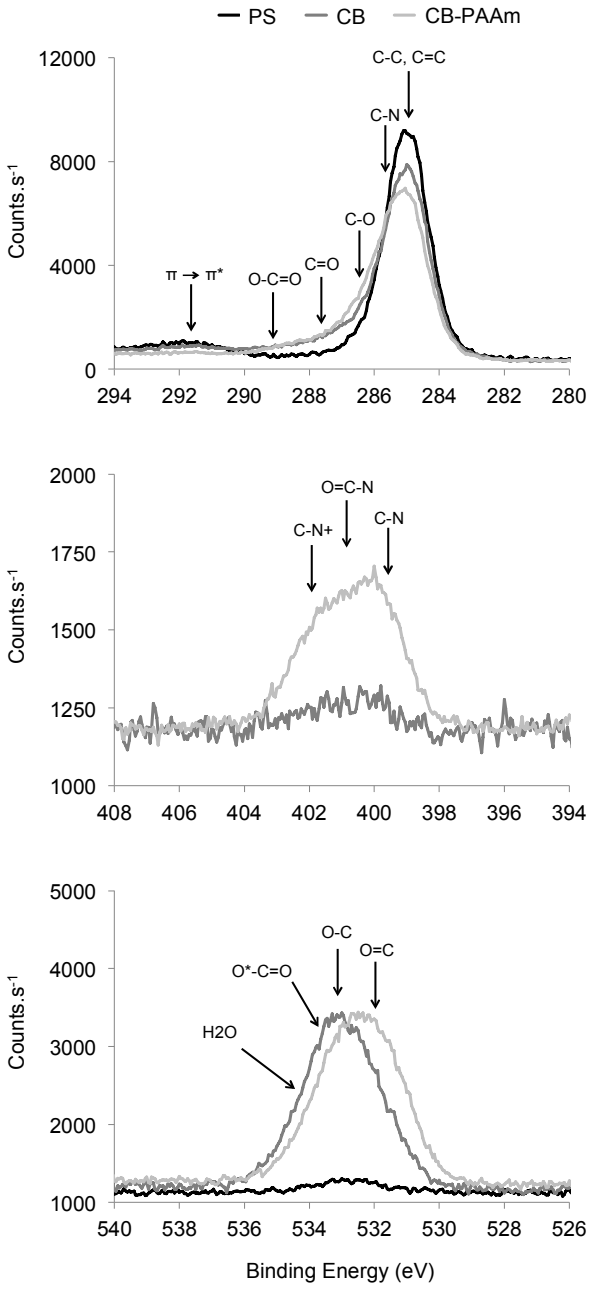

Figure 1. XPS spectra obtained from high-resolution scans of polystyrene, CellBIND® (CB) and CellBIND-PAAm (CB-PAAm) well-plates

Table 1. Atomic percentages derived from XPS analysis of samples from PS, CB and CB-PAAm well-plates

	Binding Energy (eV)	Identification	Plate		
			PS	CB	CB-PAAm
C1s Peak (at. %)	285.0	C 1s	98.6	82.5	78.8
	285.0	C-C, C=C	89.7	69.3	56.5
	285.7	C-N	•	•	5.7
	286.5	C-O	1.7	7.5	10.4
	287.8	C=O	•	2.8	4.3
	289.1	O-C=O	•	0.9	1.3
	291.7	"Shake-up"[a]	7.2	2.0	0.6
N1s Peak (at. %)	400.2	N 1s	•	1.6	5.5
	399.5	NH_2	•	•	2.3
	400.7	O-C=N	•	1.0	1.7
	402.0	NH_3^+	•	0.6	1.5
O1s Peak (at. %)	532.8	O 1s	1.4	16.0	15.7
	532.0	C=O	•	5.0	5.6
	533.0	C-O	1.4	8.3	8.8
	533.7	O*-C=O	•	1.0	1.3
	534.2	H_2O	•	1.7	•

Adsorption of E5-VEGF onto aminated poly(ethylene terephthalate) films

PET-PAAm films were incubated with various concentrations of VEGF or E5-VEGF and protein density was assessed by ELISA (**Figure 2**). The tagged chimera showed considerably higher levels of adsorption on PET-PAAm when compared to pristine PET, or to untagged VEGF incubated on PET-PAAm, in good agreement with the results obtained on CB-PAAm (Main Text, Figure 1).

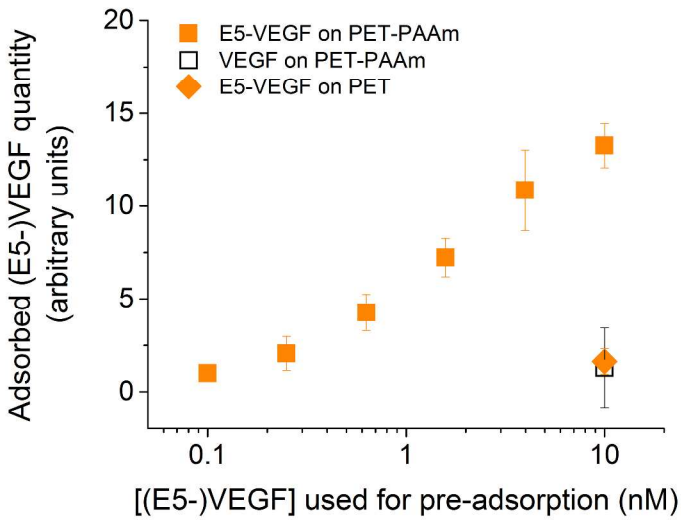

Figure 2. Presence of a coil-tag enables E5-VEGF adsorption on PET-PAAm films. ELISA response obtained with native and E5-tagged VEGF incubated over pristine and aminated PET films

Endothelial cell survival triggered by adsorbed E5-VEGF

Primary human umbilical vein endothelial cells (HUVECs) were seeded on CB-PAAm previously decorated with E5-VEGF at concentrations ranging from 0.5 to 100 nM. The cells were allowed to adhere for 3 h, then further cultivated for 48 h in serum-free basal medium, that is, in pro-apoptotic conditions[3]. The adhesion levels were unaffected by the protein density (data not shown). After 48 h, the normalized response of HUVECs increased with the amount of E5-VEGF used for surface preparation, following a sigmoid-like trend very similar to the results obtained in proliferation assays (Main Text, Figure 1) and in good agreement with the results we previously obtained on aminated glass with E5-VEGF grafted via coiled-coil interactions[3].

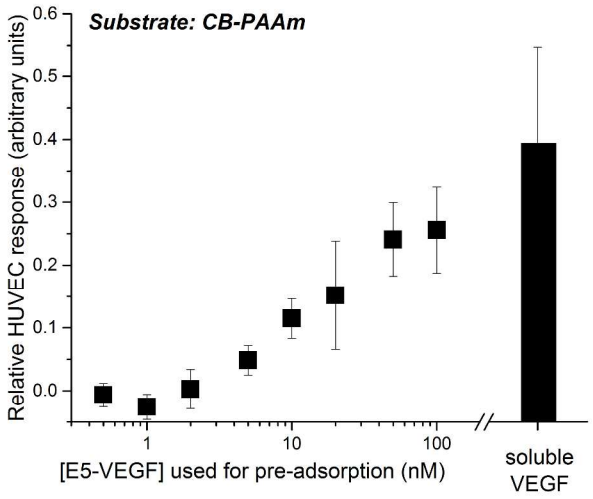

Figure 3. E5-VEGF adsorbed on CB-PAAm retains bioactivity. HUVEC survival in 96-well plates decorated with various densities of E5-VEGF in basal medium, evaluated by resazurin assays after 48 h. Data are represented as assay response relative to the response obtained with cells in complete medium and in basal medium.

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