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EVENT ABSTRACT

Chemical aspects of cell adhesion and -growth for vascular grafts

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Introduction: Various routes enable one to functionalize polymer surfaces for enhanced cell adhesion and -growth. For example, commercial products [1] like tissue-culture poly(styrene) (TCP) have oxygen (O)-containing groups (e.g. OH, C=O, COOR) that originate from plasma-based surface modification, while Primaria™ in addition contains N-bearing functionalities like primary amines, NH_2; Parylene diX-AM [2] is a commercial coating with only NH_2. Such polar surfaces also enable covalent immobilization of bioactive molecules, to foster specific cell response to implants such as vascular grafts. This study compares various functionalization processes in their ability to create high densities of functional groups, and compares the efficacies of O- and N-rich polymer surfaces, along with "hybrid" (O+N) ones, for culture of hMSCs (human mesenchymal stem cells) and HUVECs (human umbilical vein endothelial cells).

Materials and Methods: Low-pressure plasma-polymerized ("L-PP") coatings were prepared on polyethylene terephthalate (PET) films from mixtures of C2H4 with NH_3 ("L-PPE:N"), N_2O ("L-PPE:O,N"), or O_2 (diluted in Ar, "L-PPE:O"); and simple surface modification of PET with NH_3 plasma ("PETf"). Commercial Primaria™ and Parylene diX-AM were used for comparison. PET films were also treated with polyallylamine (PAAm) by aminolysis in an alkaline (pH=12.5) PAAm solution. Compositions and bond types of these surfaces were obtained by X-ray photoelectron spectroscopy (XPS); prior derivatization by TFBA (4-trifluoromethybenzaldehyde) allowed us to determine amine concentrations, $[NH_2]$. A bioactive molecule, chondroitin sulfate (CS), was grafted onto $[NH_2]$-rich surfaces by EDC/NHS chemistry. Finally, hMSCs and HUVECs were seeded onto the surfaces, the Alamar Blue test being used to evaluate cell adhesion and -growth at three time-points (24h, 4d, 6d).

Results:

Table 1: Elemental compositions and $[NH_2]$ values obtained by derivatization and XPS

	XPS composition (at. %)			
	[C]	[N]	[O]	$[NH_2]$
L-PPE:N	81.4 ± 2.3	14.6 ± 1.6	4.1 ± 1.7	**6.4 ± 1.0**
PETf	63.3 ± 0.9	11.4 ± 1.6	25.2 ± 0.9	**2.8 ± 0.8**
PAAm	72.5 ± 2.4	4.0 ± 0.9	23.5 ± 1.6	**3.9 ± 0.8**
Parylene diX-AM	93.5 ± 0.2	5.3 ± 0.2	1.1 ± 0.3	**5.6 ± 0.4**
Primaria™	83.5 ± 1.5	4.9 ± 0.3	11.6 ± 1.2	**0.5 ± 0.2**
L-PPE:O,N	83.2 ± 0.4	5.7 ± 0.6	11.2 ± 0.6	**1.7 ± 0.1**
TCP	83.6 ± 0.9	-	16.4 ± 0.9	-
L-PPE:O	65.2 ± 0.5	-	34.8 ± 0.5	-

Table 1 shows compositions and $[NH_2]$ values; L-PPE:N, PETf, PAAm and Parylene diX AM manifest a wide $[NH_2]$ range, up to 6.4 at.%. For PAAm, $[NH_2]$ could be varied by controlling pH. "Hybrid" surfaces, Primaria™ and L-PPE:O,N, show lower $[NH_2]$, in spite of high [N], ca. 5 at.%, suggesting more varied, complex functionalities (confirmed by high-resolution C1s spectra, not shown).

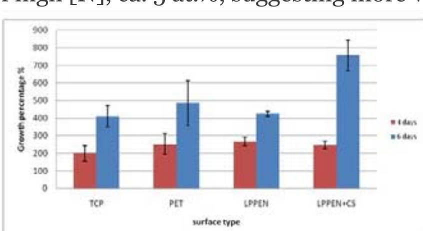

Figure 1: Growth ratio of hMSCs on various surfaces at two different time-points, as compared to t=24h

L-PPE:N greatly enhanced hMSC adhesion, but grafting of CS reduced it; however, this was compensated by an increased growth ratio (Figure 1). HUVEC culture confirmed that O- and NH_2-rich surfaces enhance cell adhesion and growth, whereby L-PPE:N and L-PPE:O appear to yield similar results. Although Primaria™ showed excellent behavior, L-PPE:O,N did not, despite its high [O] and [NH_2].

Conclusion: Both N- and O-rich surfaces have good cell-colonization properties, particularly plasma polymers, while “hybrid” surfaces are more ambiguous and call for further investigation. Samples with [NH_2] enabled grafting of CS; while its low-fouling properties limited adhesion, it still allowed substantial cell growth.

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