Supplementary Information

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Towards an in vitro model mimicking the foreign body response: tailoring the surface properties of biomaterials to modulate extracellular matrix

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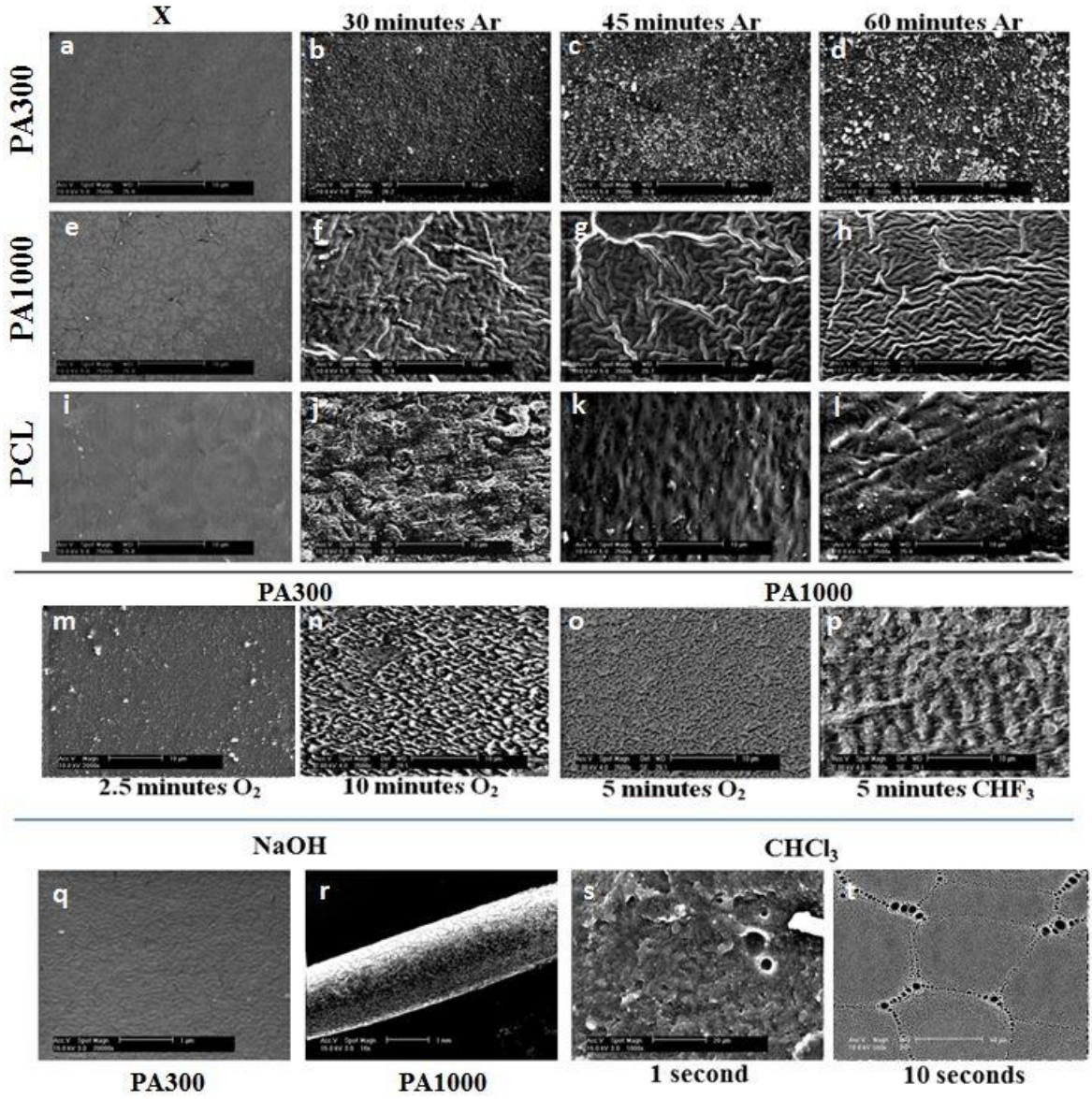
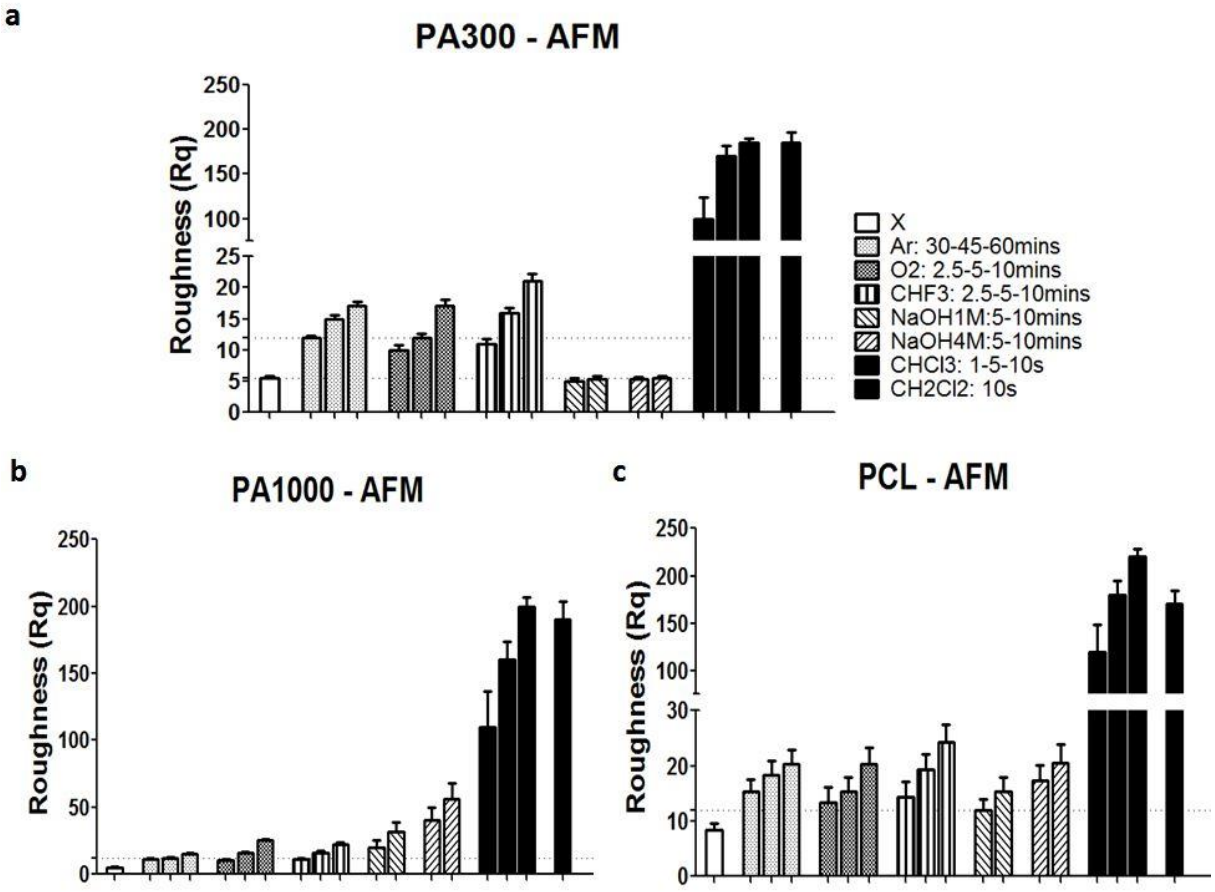
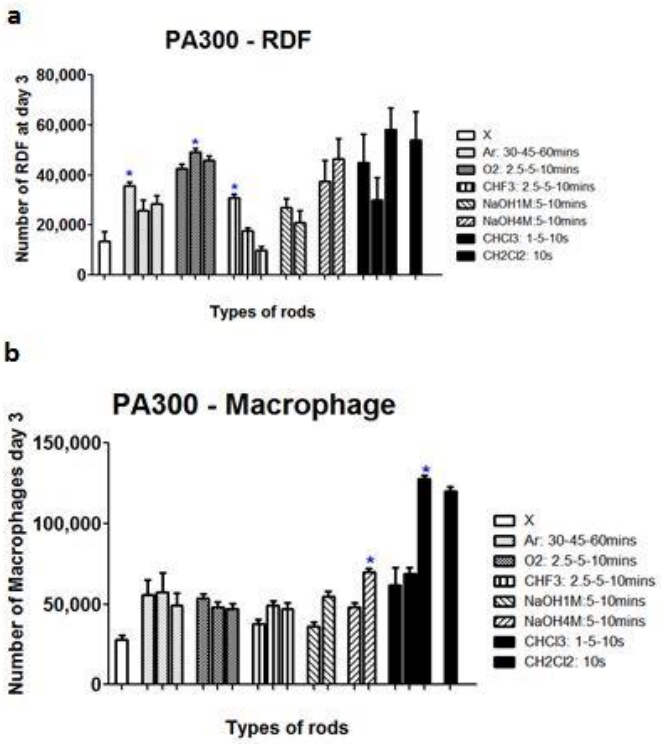


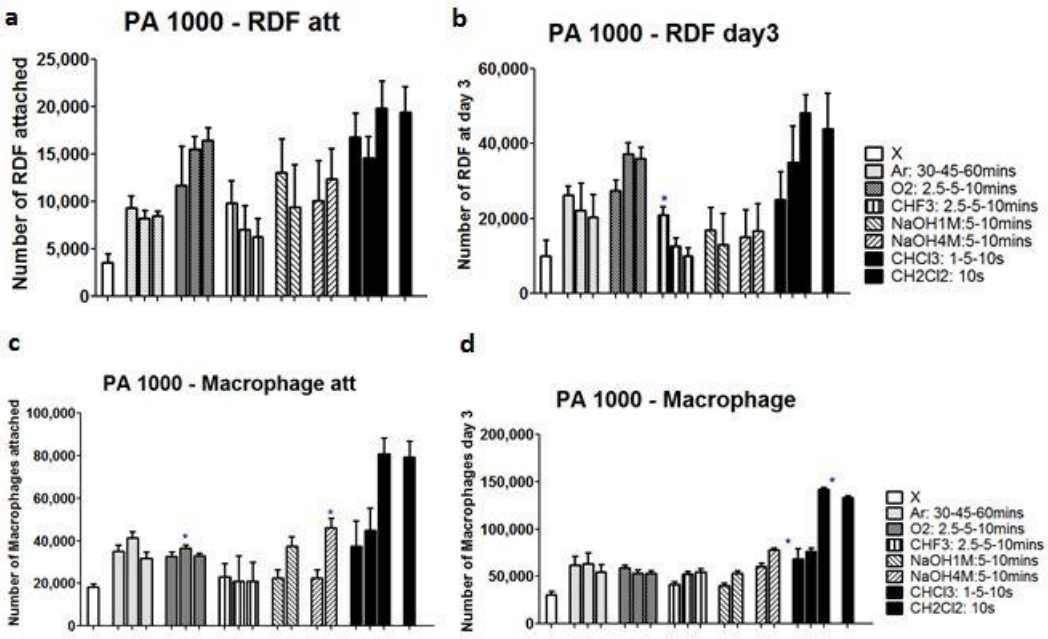
Supplementary Figure S1. SEM pre-selection of surface treatments. (a-p) SEM images of PEOT/PBT and PCL before and after gas plasma treatment (scale bar: 10 µm) at different exposure times and gases. (q-t) SEM images of PEOT/PBT after wet etching techniques with different polymer composition and exposure times. Scale bar of 1µm was chosen for PA300 NaOH rods (q) to show no surface topographical changes were seen even at high magnification. Scale bar of 1 mm was chosen for PA1000 NaOH rods (r) to provide overview of the topographical changes after treatment. Furthermore, scale bar of 50 µm for CHCl3 (t) was chosen to see homogenous small porous structure and large porous structure surrounding it.



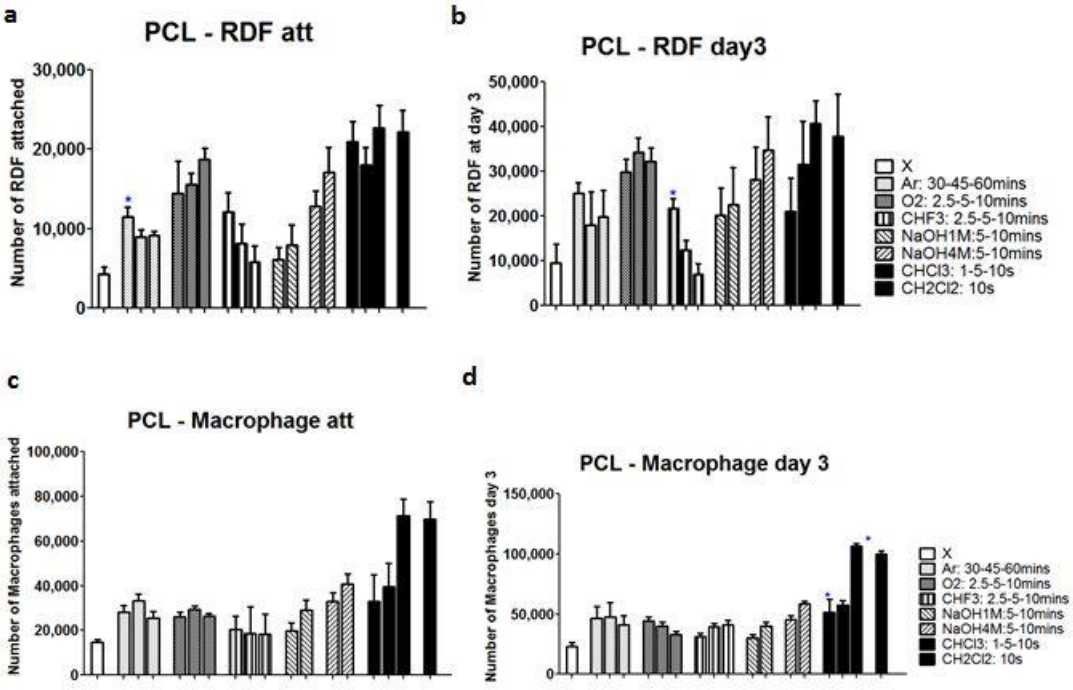
Supplementary Figure S2. AFM pre-selection of surface treatments. AFM quantification of surface roughness on PA300 (a), PA1000 (b), and PCL (c) before and after surface treatment (scan size 1 μm). For PA300 (a) horizontal ticks assembles the surface treatments into groups of 1x fold (X, NaOH), 3x fold increase (Ar30, $O_2$5, $CHF_3$2.5), and >30x fold ($CHCl_3$:5-10s, CH_2Cl_3 10s) in roughness. Similar grouping were found in PA1000 with exception to NaOH etching (b) but not in PCL (c). Data are shown as mean ±s.d. (n=3).



Supplementary Figure S3. *In vitro* pre-selection of surface treatments. DNA assay analysis of PA300 on day 3 for RDFs (a) and macrophages (b). Statistics were conducted between different parameters of each treatment type to pre-select best parameter. Data are shown as mean ± s.d. (n=3). Star (*, $P<0.05$) indicates the best parameter for each treatment type.

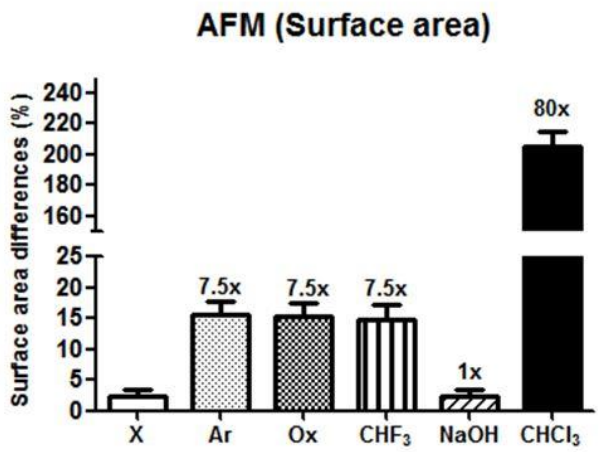


Supplementary Figure S4. *In vitro* pre-selection of surface treatments. DNA assay analysis of PA1000 at attachment after 6 hours (a) and day 3 (b) for RDFs, and attachment (c) and day 3 (d) for macrophages. Statistics were conducted between different parameters of each treatment type to pre-select best parameter. Data are shown as mean ± s.d. (n=3). Star (*, $P<0.05$) indicates the best parameter for each treatment type.



Supplementary Figure S5. ***In vitro*** **pre-selection of surface treatments.** DNA assay analysis of PCL at attachment after 6 hours (a) and day 3 (b) for RDFs, and attachment (c) and day 3 (d) for macrophages. Statistics were conducted between different parameters of each treatment type to pre-select best parameter. Data are shown as mean ± s.d. (n=3). Star (*, $P<0.05$) indicates the best parameter for each treatment type.

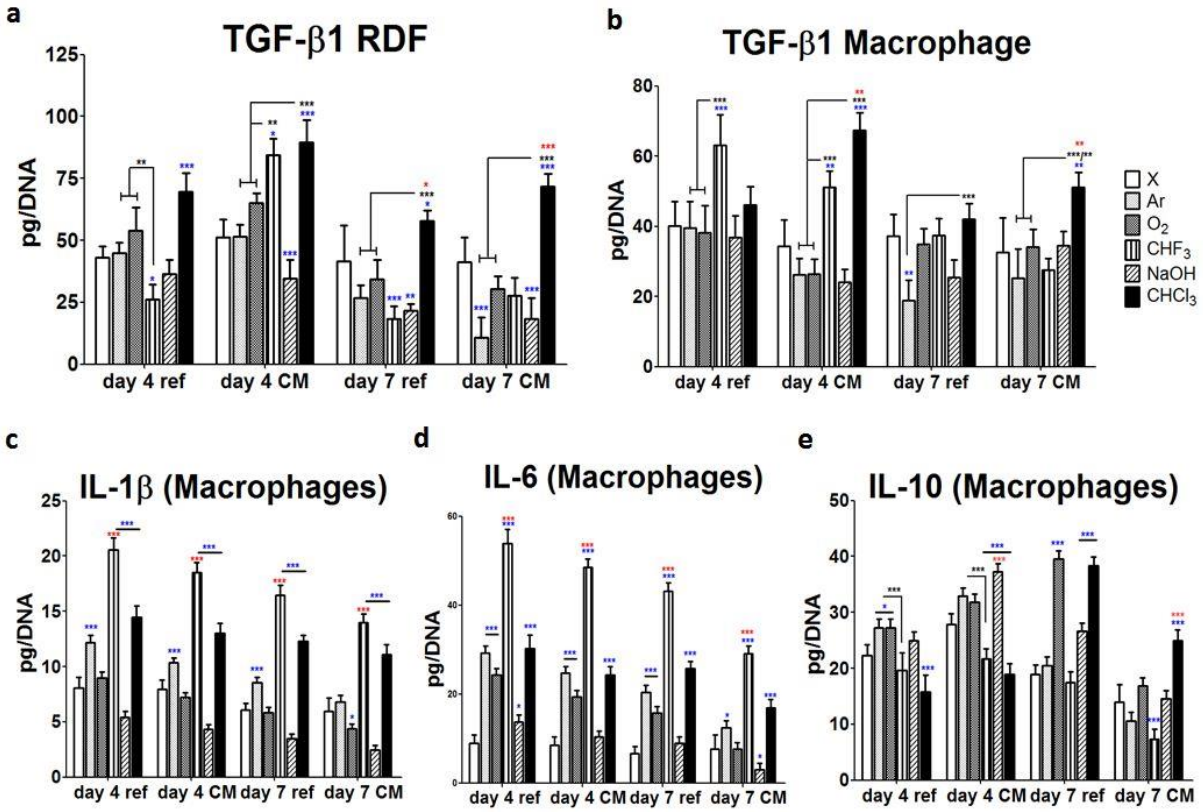
a



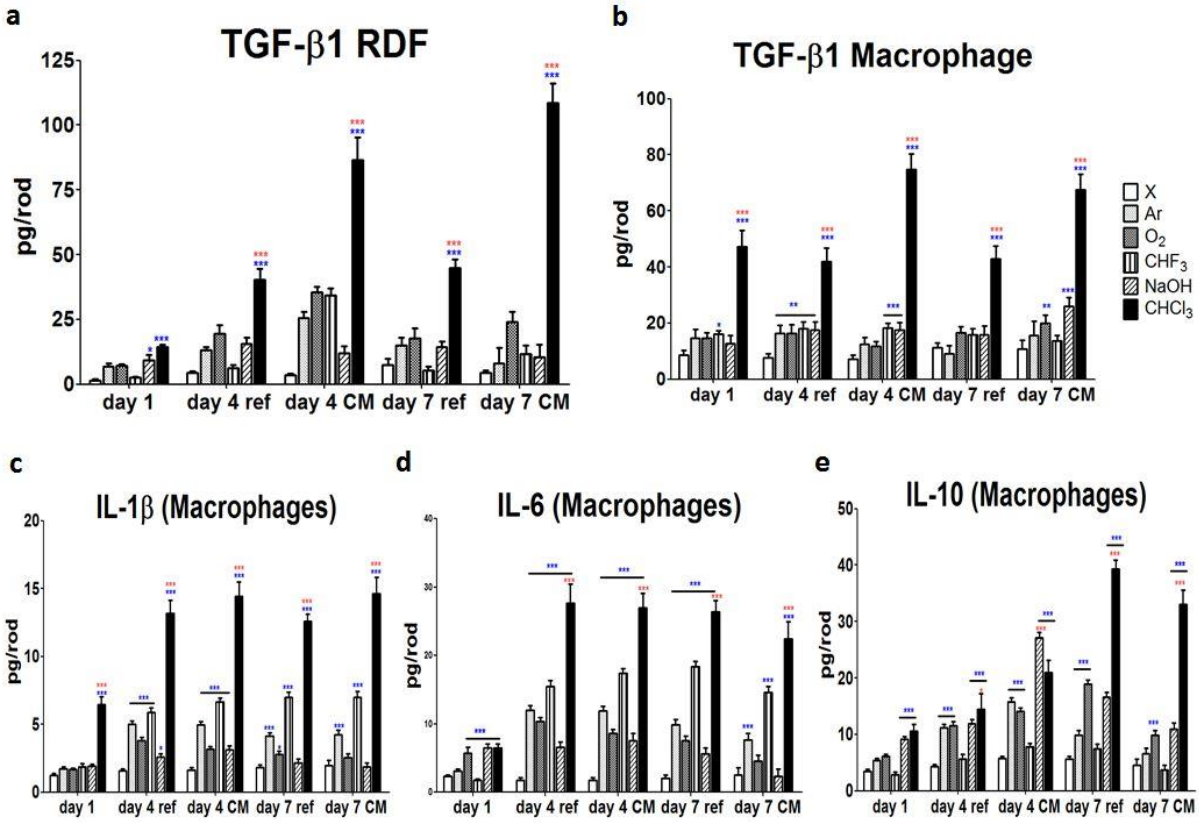
b

	F1s	Na1s	Si2p	Cl2p
C1s peak shifts				
Unmodified PA300			1.08	
Ar etched		0.33	0.35	
O2 etched			1.57	
CHF3 etched	50.96		0.05	
NaOH etched		0.23	0.10	
CHCl3 etched				0.01

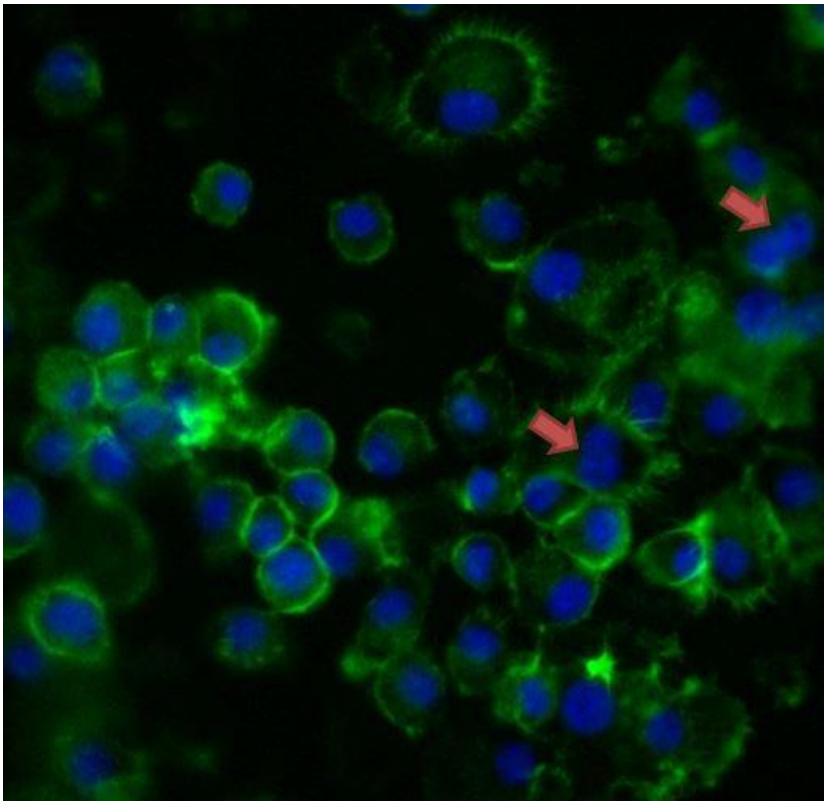
Supplementary Figure S6. **Material characterization of pre-selected treatments.** (a) AFM surface area differences in percentage. Number of folds increased is represented by the number above individual bars after surface treatment. Data are shown as mean ± s.d. (n=3). (b) XPS measurement on fluoride (F1s), sodium (Na1s), silicon (Si2p) and chloride (Cl2p).



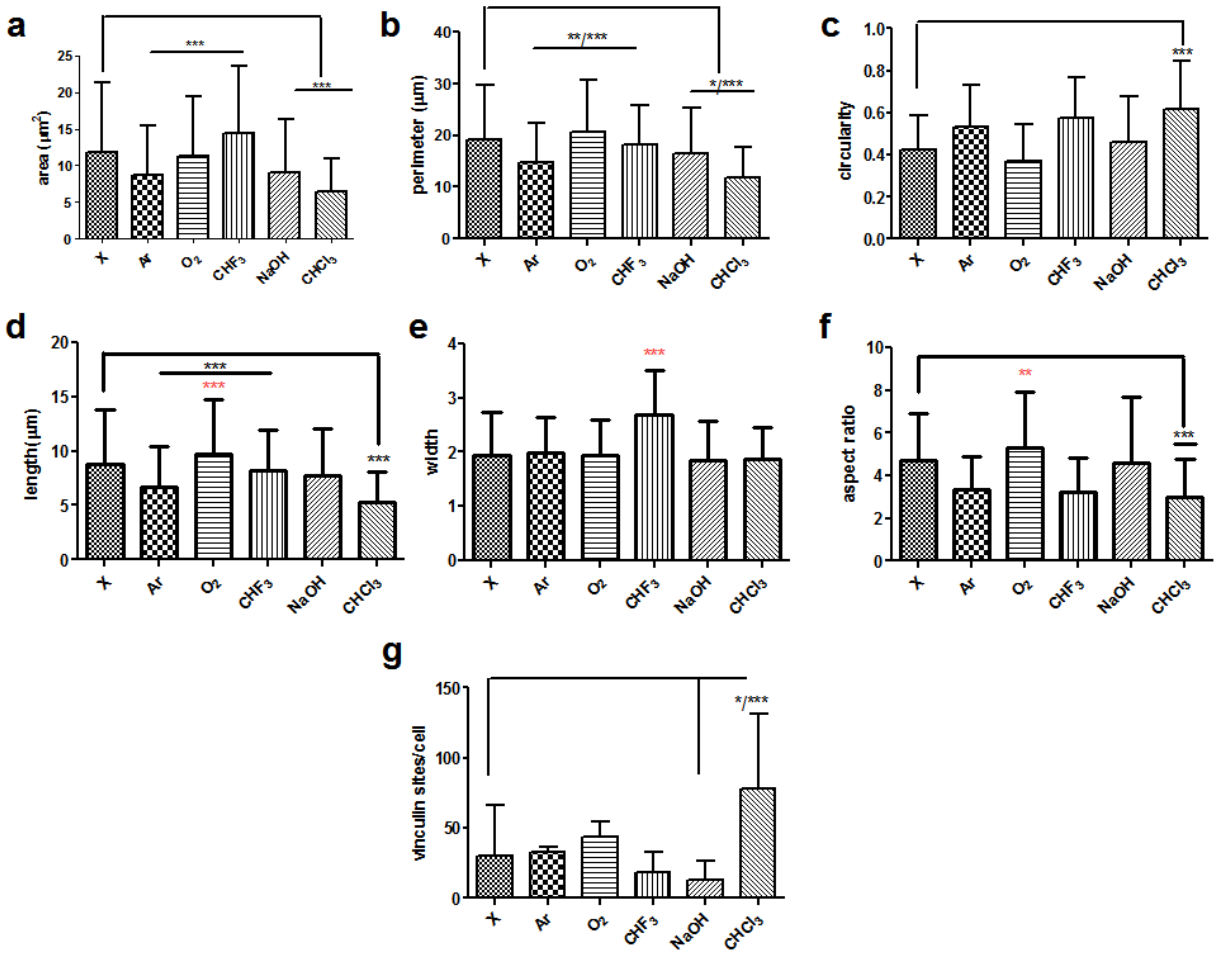
Supplementary Figure S7. Cytokine analysis on conditioned medium co-culture *in vitro* studies at day 4 and 7. (a,b) TGF-β1. Normalized per DNA represent amount secreted for 100,000 cells. In RDFs at day 4, addition of CM created an upregulation of TGF-β1. For macrophage, CM reduced TGF-β1 expression. For RDFs at day 7, TGF-β1 decreased for all treatments except for CHCl3. (c,d) IL-1β and IL-6 were down regulated by hydrophilic surfaces and up regulated by hydrophobic surfaces. Downregulation of both inflammatory cytokines IL-1β and IL-6, were seen at day 4 and day 7 with conditioned medium culture. (e) IL-10 secretion was more in hydrophillic surfaces. For all treated rods, upregulation of IL-10 was seen at day 4 CM and downregulated at day 7, with exception of CHCl3 treated rods indicating possible remodeling of the matrix. Data are shown as mean ±s.d. (n=3). Blue stars (*P<0.05, ** P<0.01, *** P<0.001) indicate statistical significances in comparision to unmodified, red stars indicate the best parameter from all the treatment types, while black stars evaluate statistical differences between the different treatments.



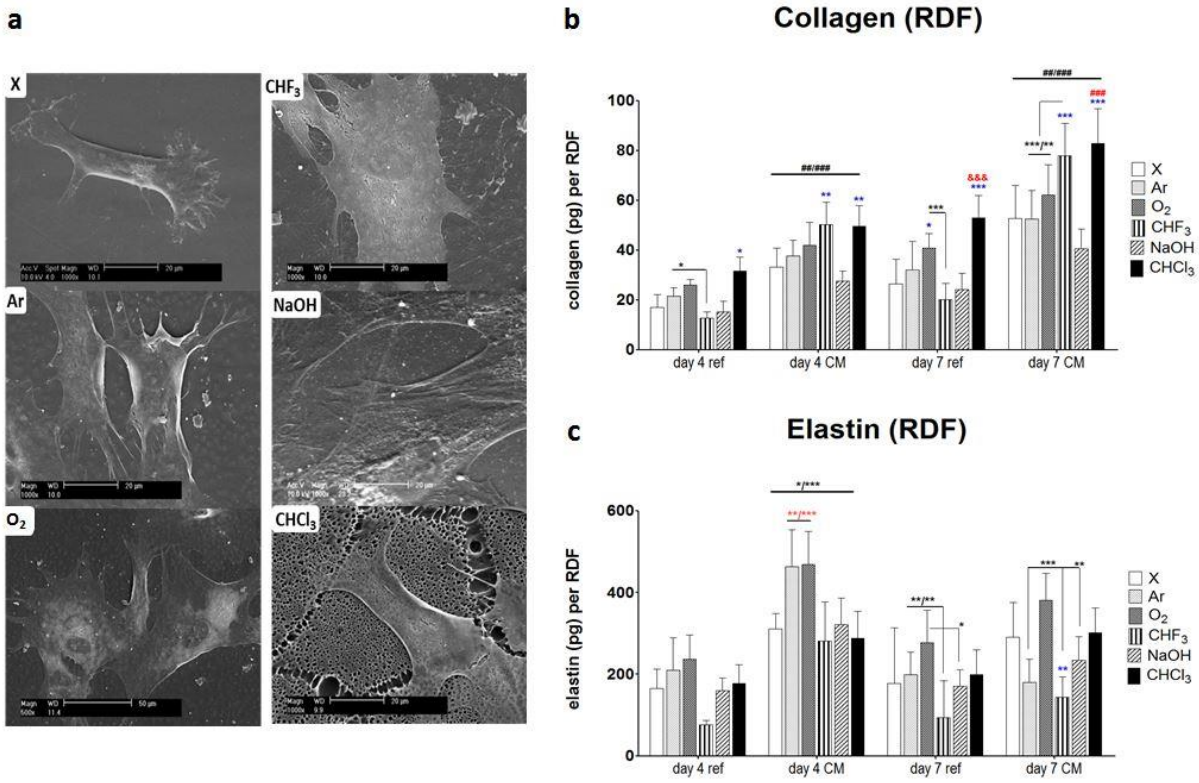
Supplementary Figure S8. Total quantification of cytokines at day 1 and conditioned medium co-culture *in vitro* studies at day 4 and day 7. (a,b) TGF-β1, (c) IL-1β, (d) IL-6 and (e) IL-10 secretion in mono (ref) or conditioned medium co-cultures (CM). CHCl3 treated rods showed highest amount of cytokines production, providing dynamic impact on soluble factors effect on culture and extracellular matrix components. Data are shown as mean ±s.d. (n=3). Blue stars (*P<0.05, ** P<0.01, *** P<0.001) indicate statistical significances in comparision to unmodified, red stars indicate the best parameter from all the treatment types, while black stars evaluate statistical differences between the different treatments.



Supplementary Figure S9. Immunostaining of macrophages. Phalloidin (green) for actin filament and dapi (blue) for nucleus was stained on macrophages seeded on polymer sheets. Filopodia of the macrophages recognize nanotopographies of substrate, and foreign body giant cells formation (arrow).



Supplementary Figure S10. Quantification of vinculin staining for cell adhesion strength. Quantification of (a) area, (b) perimeter, (c) circularity, (d) length, (e) width, (f) aspect ratio, and (g) frequency (vinculin sites/cell) analysis. Data are shown as mean ±s.d. ($n \geq 250$). Black stars evaluate statistical differences between the different treatments, and red stars indicate the highest significant difference from all the treatment types (*P<0.05, ** P<0.01, *** P<0.001).



Supplementary Figure S11. Collagen and elastin synthesis of RDFs and cell morphology on different surface treated rods. (a) SEM image analysis of RDFs on the different surface properties. Smooth hydrophilic NaOH treated rods allowed further spreading, Conversely, $CHCl_3$ topography strongly influenced cell morphology on the porous surface. Spreading was also seen for Ar, Ox, and CHF3 treated rods implying that surface roughness is a dominant factor, and CHF3 level of hydrophobicity did not significantly alter the level of cell spreading. (b) Quantification of collagen secreted (pg) per cell on different surface modifications. CM cultures triggered a significant increase in comparison to refresh medium, (# indicating exception to CHF3). $CHCl_3$ secreted the most increase in comparison to all treatments at day 7 (red *, or & indicating exception to CHF_3 and O_2) (c) Quantification of elastin secreted (pg) per cell on different surface modifications. CM cultures triggered a significant increase in comparison to refresh medium on day 4. At day 4 CM, Ar and O_2 provide the best secretion of elastin in comparison to all other surface modification per number of cells. Data are shown as mean ±s.d. (n=6). Blue symbols (*P<0.05, ** P<0.01, *** P<0.001) indicate statistical significances in comparision to unmodified, red symbols indicate the best parameter from all

the treatment types, and black symbols evaluate statistical differences between the different treatments.