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Electronic Supplementary information

Nanoparticle functionalized laser patterned substrate: an innovative route towards low cost biomimetic platforms

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Substrate processing

No.	Type of Pattern	Fluence (J/cm^2)	Scan Rate (mm/s)	Line Spacing (µm)	No. of Passes
1	Crosshatch	High: 0.582	1.2	150	1 High + 3 Low
2	Concentric Circles	Low: 0.388		150	
3	"APT" Test Pattern			-	
4	Light Texture	0.689	22	50*	6

* Scans overlap due to line spacing being less than beam width (w_0 = 140 µm)

Table1S: Laser conditions for different patterns obtained in Figure 2 (main manuscript)

XPS Spectroscopy

To investigate the effect of laser ablation on surface chemistry of the COP, X-ray photoelectron spectroscopy (XPS) analysis was performed. Wide-scan XPS spectra confirmed the absence of any impurities induced in the samples during preparation or measurement (Fig 1S)

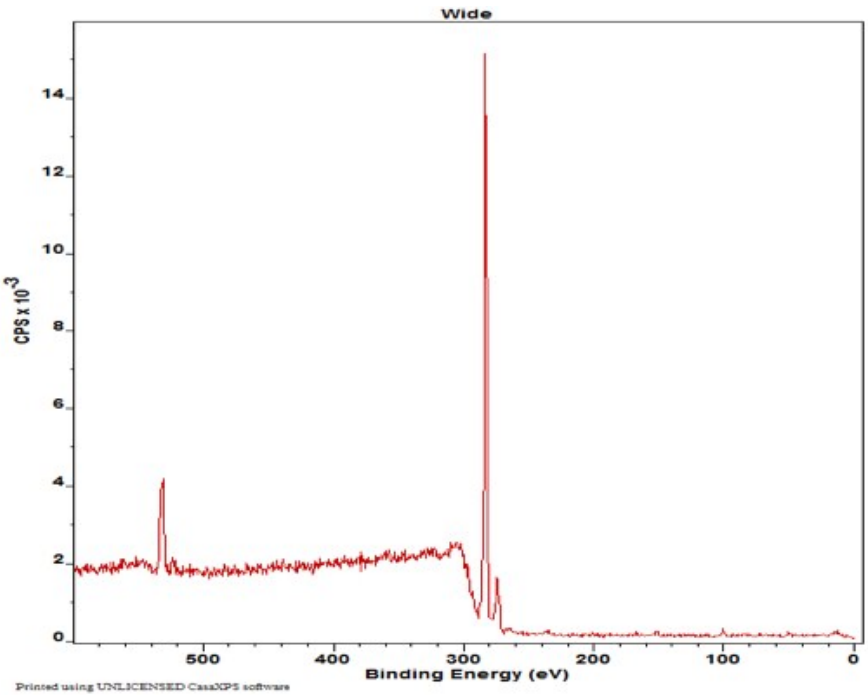

Fig 1S: Wide-scan XPS spectra of laser treated sample

Laser optimization experiment

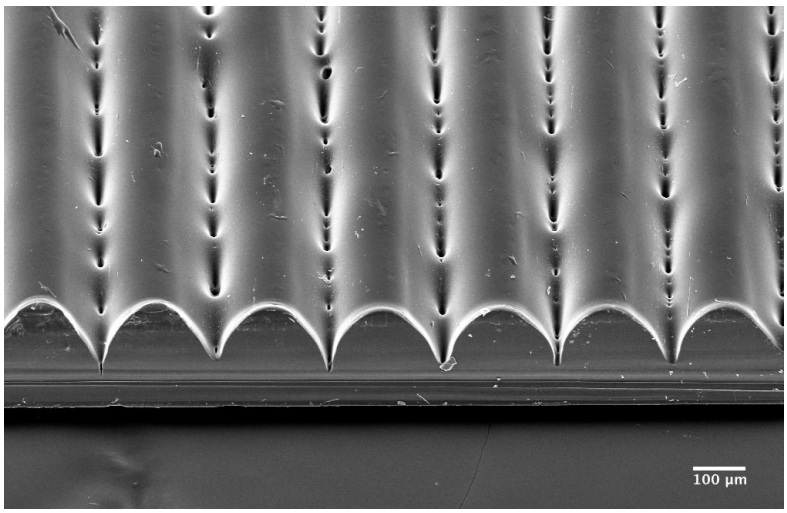

Fig 2S: Scanning electron microscopy images of the COP substrate at test parameters.

Several laser optimization experiments were performed to achieve clean continuous channels. Correlation of multiple laser passes and fluence was examined for fine control of dimensions and dimensional uniformity. Fig 2S represents one of the test experiments at higher fluence 0.79 J/cm^2 with 1 pass which resulted in non-continuous localized deeper ablation sites.

NMR Spectroscopy

Complementary to X-ray photoelectron spectroscopy (XPS), NMR analysis was performed to eliminate possibility of other functional groups, and confirm the presence of C-O bond. The obtained 1H-NMR, Correlation Spectroscopy (COSY) NMR and Heteronuclear Multiple Quantum Coherence (HMQC) NMR spectra confirmed the presence of the C-O-H hydroxyl moiety in the sample (Fig 3S, 4S, 5S respectively).Peak at 3.56ppm on ^{1}H NMR (fig 2S) suggests presence of protons vicinal to a hydroxyl(OH) or carbonyl (C=O) functionality. To clarify the origin of 3.56ppm peak, Heteronuclear Multiple Quantum Coherence (HMQC) NMR (fig 3S) spectra was obtained. HMQC suggests peak at 3.56 ppm correlates to ^{13}C shift of 70.5 ppm which correlates to protons vicinal to a hydroxyl (OH) and not carbonyl. All the

other alternative possibilities are eliminated by Correlation Spectroscopy (COSY) spectrum (fig 4S).

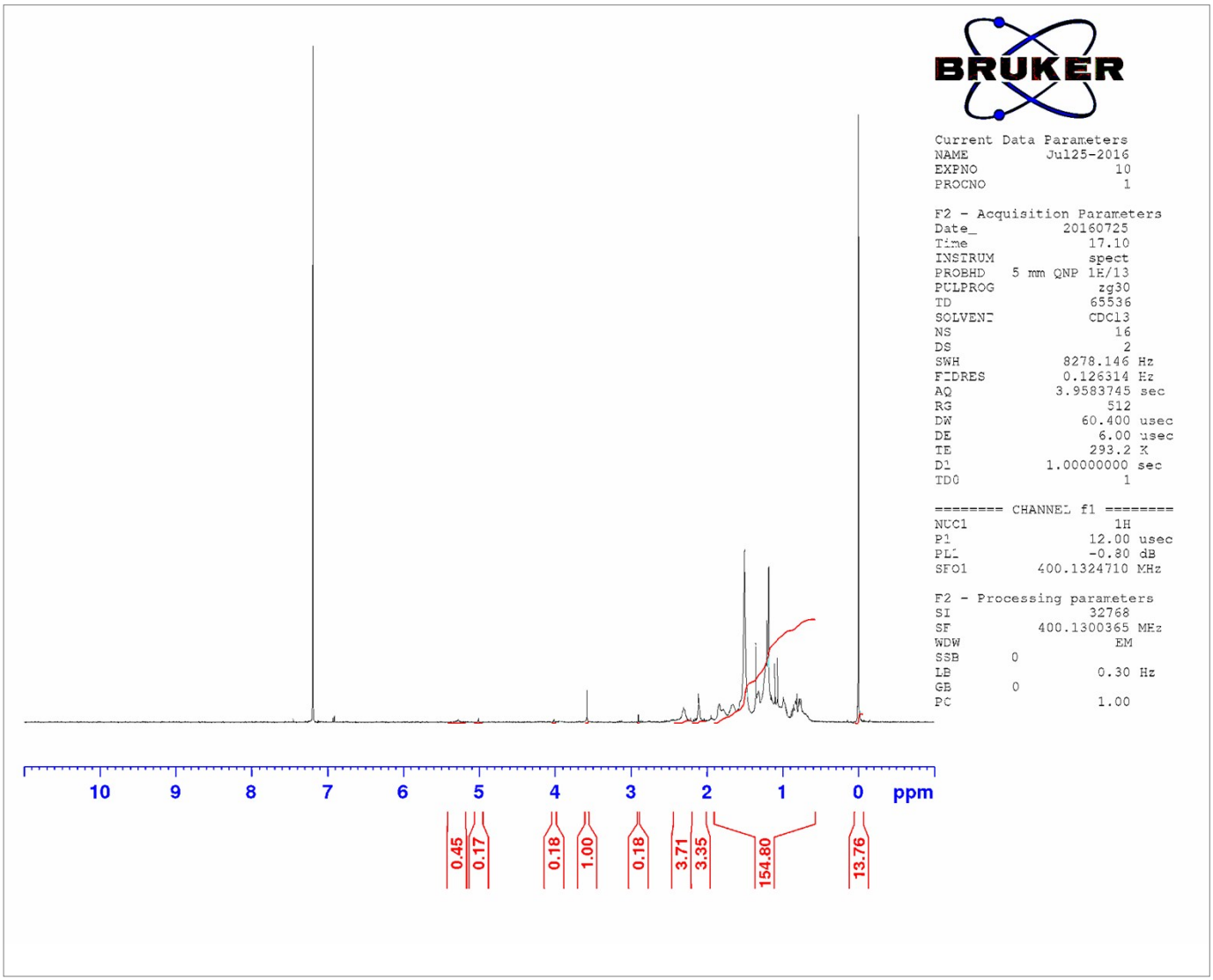

Figure 3S: ^{1}H-NMR spectrum of laser textured COP

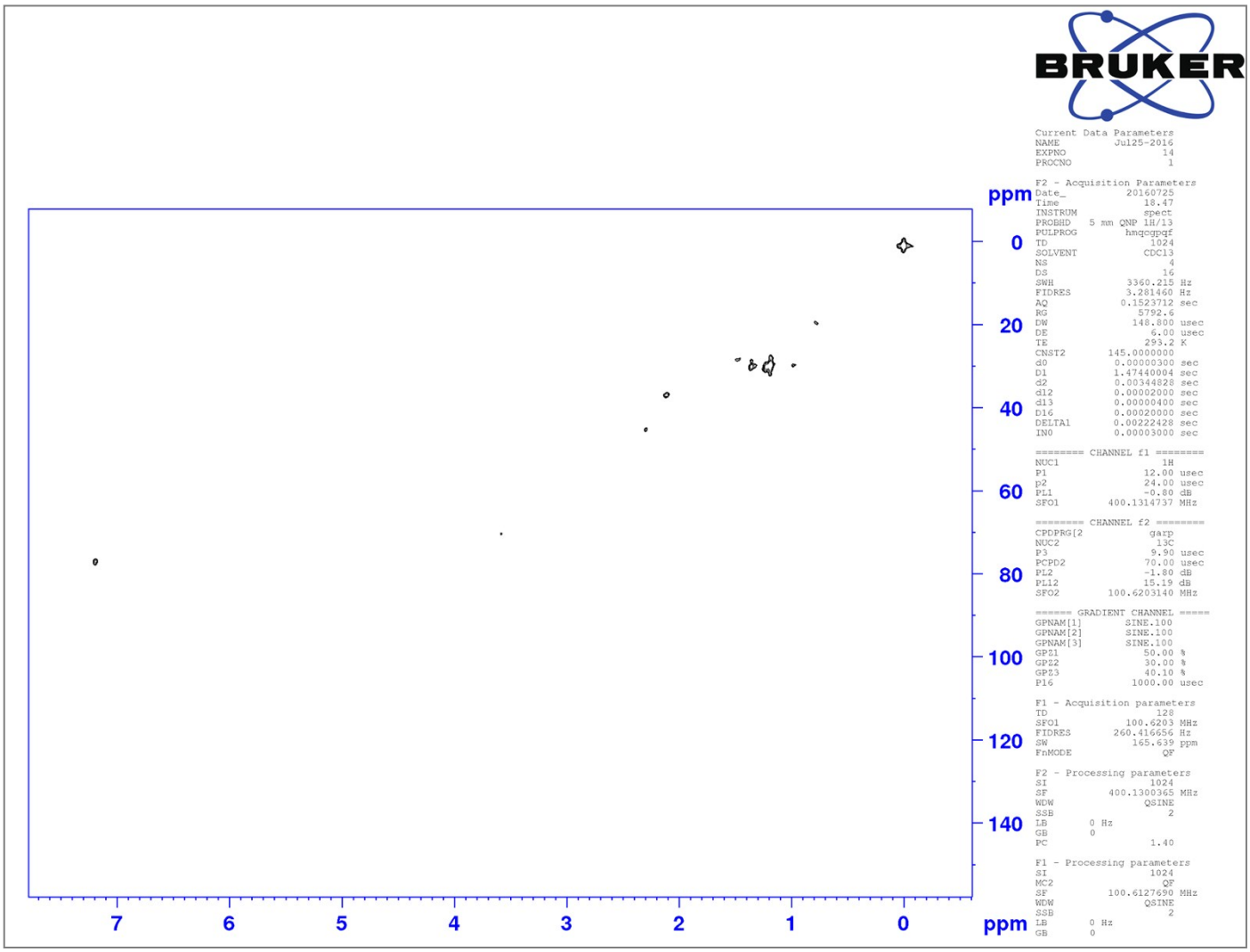

Figure 4S: Heteronuclear Multiple Quantum Coherence (HMQC) NMR of laser textured COP

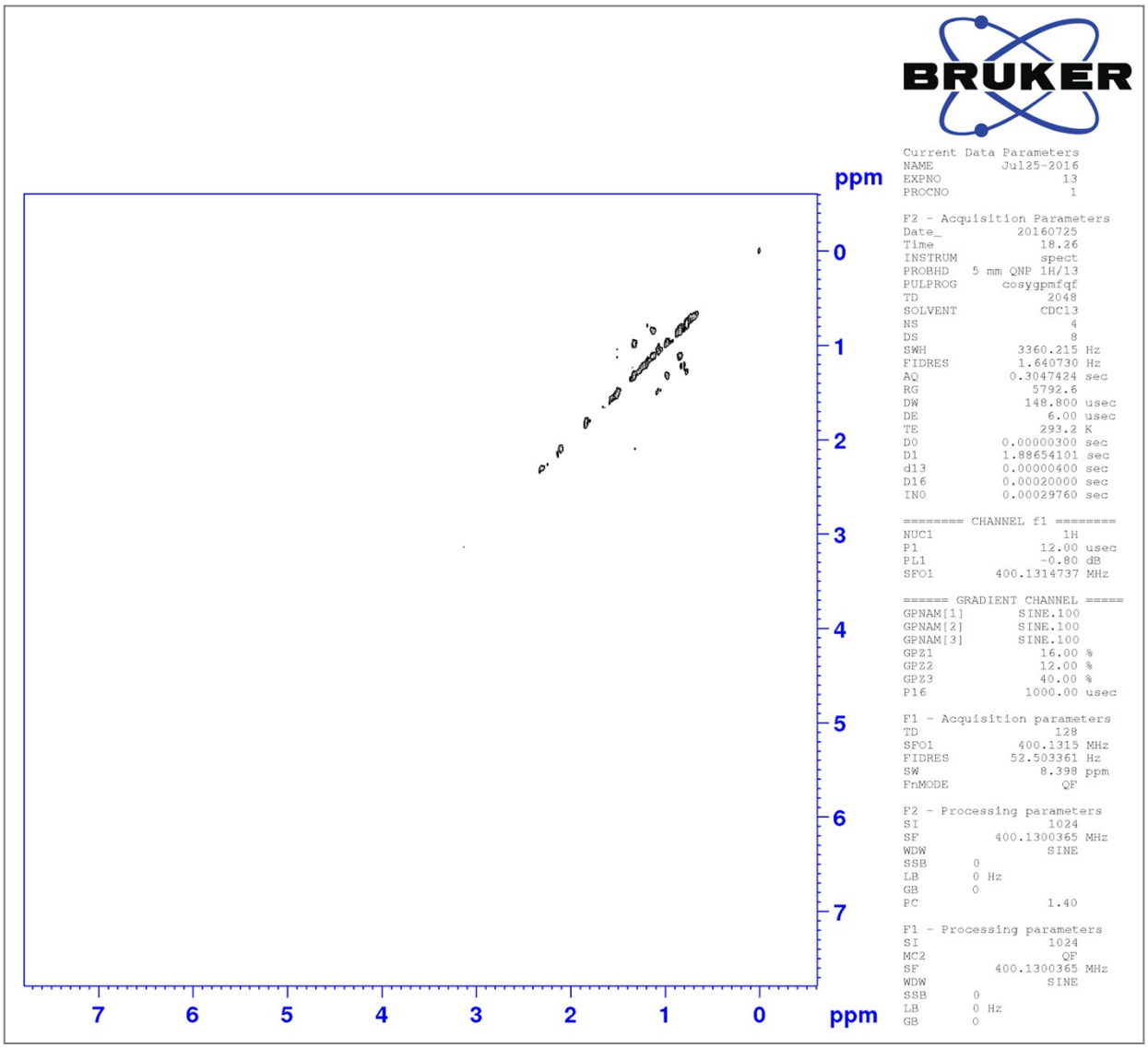

Figure 5S: Correlation Spectroscopy (COSY) NMR spectrum of laser textured COP

Wettability Studies

Systematic investigation was carried out to study the effect of the different plasma treatment on surface wettability by conducting Water Contact Angle measurements. for 5-day measurement period after plasma treatments (Figure 6S) Characterizing the duration of the plasma-induced hydrophilicity is critical for mapping out a stable working window to perform further surface functionalization.

Time	**Contact Angle**				
Days	*Ar/O_2 RIE*	*O_2 (5 min)*	*O_2 (10 min)*	*O_2 + Piranha*	*Native COP*
0	25.06	30.72	20.18	24.21	88
1	31.96	39.95	20.67	35.03	88
2	64.63	55.65	41.81	47.55	88
3	75.69	59.89	44.51	57.99	88
4	73	56.09	70.76	60.29	88

Figure 6S: Water contact angle measurements for 5-day measurement period after different plasma treatments

Scanning Electron Microscopy

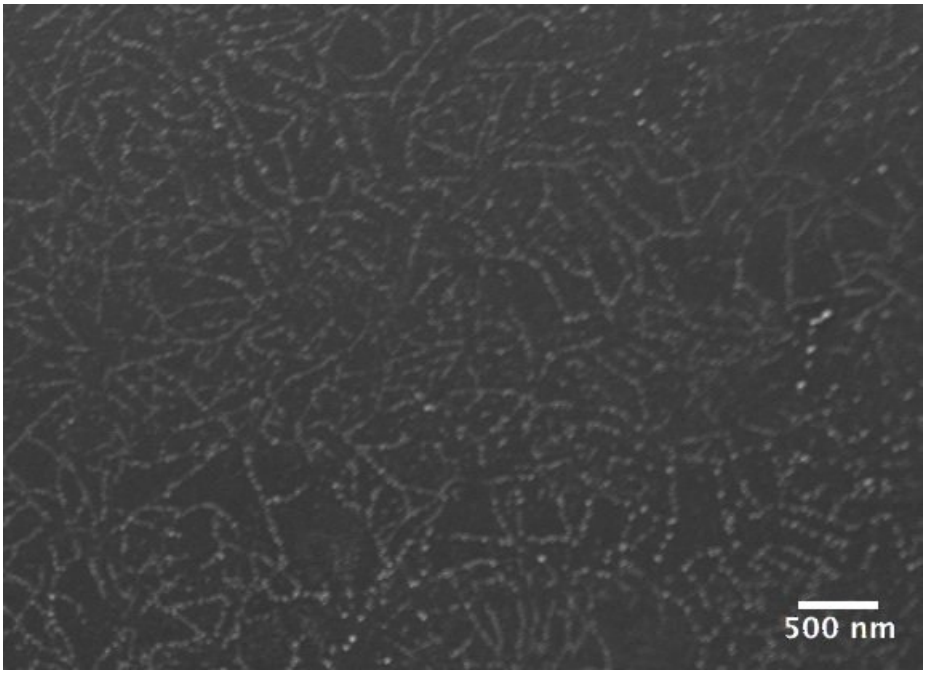

Fig 7S: SEM image of carbon nanoparticles deposited on non-textured COP.

The formation of nanoparticles clusters can be seen as a result of drop casting of nanoparticle solution. Due to peculiarity of the drop casting technique conducted at room temperature nanoparticles self-assemble and form small clusters as the water evaporates. To note: the self-assembly pattern depends on various factors such as rate of evaporation, concentration of nanoparticle solution and medium of colloidal suspension.

Biocompatibility studies

Concentration	2.4ug/ml	6ug/ml	36ug/ml	48ug/ml
Cell count 1	443	428	387	391
Cell count 2	413	441	311	227
Cell count 3	360	378	289	366
Average	405.33333	415.6666667	329	328

Table 2S: Cell count on each substrate post 7-day exposure to different concentration of CNps.

Table 2S represents the cell count for each substrate on 340682 um^2 area post 7-day exposure to different concentration of CNps. These values are calculated taking in account the area close to the channel (refer Fig 10 in the manuscript). It should be noted that the quantification of the cell count inside the channels as compared to the non-textured surface was not possible due to the high density of the cells and optical scatter.

The control experiments were performed on non-textured non-functionalized COP substrates (Fig 8S) to record the proliferation of HaCat cells. Compared to the nanoparticle functionalized and textured surfaces, cells did not appear to attach and proliferate on control surfaces.

To understand the effect of different nanoparticle material onto the laser textured substrate, similar set of biocompatibility studies were performed on titanium oxide (TiOx) and silicon oxide (SiOx) nanoparticles functionalized substrates (Fig 9S). The experimental conditions and cell line were same as for CNPs (discussed in the main article). The fluorescent microscopy

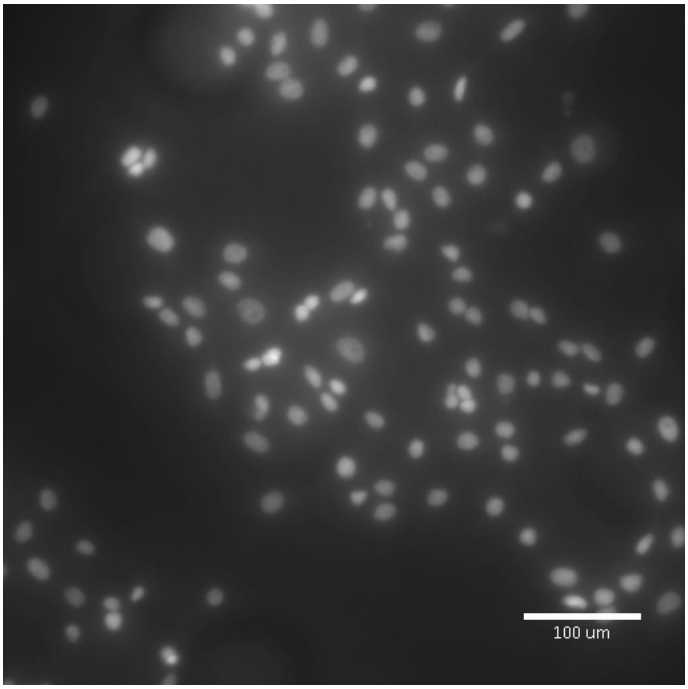

Figure 8S: Fluorescent microscopy images of nuclei stained HaCaT cells on non textured non NP functionalized substrate. The length of the scale bars in the micrograph images is 100 μm.

images of nuclei stained HaCat cells after 7 days of exposure to SiOx NPs and TiOx NPs reveal no change in shape of cells. The cell nuclei were round and appeared healthy with no evident cytotoxicity. These results present an opportunity to create a versatile biocompatible platform with desired properties through nanoparticle functionalization.

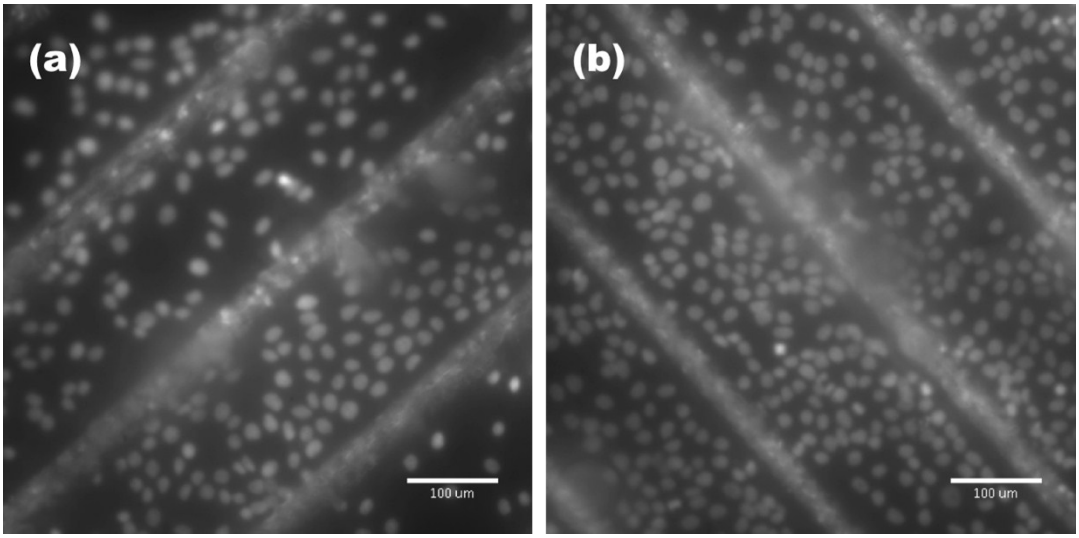

Figure 9S: Fluorescent microscopy images of nuclei stained HaCat cells after exposure of (a) SiOx NPs and (b) TiOx NPs The length of the scale bars in the micrograph images is 100 μm.