Chemically Treated 3D Printed Polymer Scaffolds for Biomineral Formation-Supporting information

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Supporting Information

This work details the manufacture of the printed objects, their subsequent imaging by electron microscopy, x-ray computed tomography and analysis via gas sorbtion and x-ray photoelectron spectroscopy in order to characterise the print treatments. Further details on the cell culture and differentiation methods are given, as well as the process of heating ferrofluid impregnated prints with an alternating magnetic field. The method to evaluating the hydrophobicity of each treated print surface via water contact angle measurement is also given. Extra images of the treated prints and adhesion test work are also shown.

Instrumentation

SLS 3D printer

CAD files were loaded into an EOS formiga P100 selective laser sintering machine using its Magics software and printed at a speed of 1500 mm s^{-1} with a laser power of 16 W, at zero beam offset and at a layer height of 100 µm using the powder feedstock PA2200, consisting of Nylon 12 polyamide polymer at an average grain size of 60 µm.

Electron microscopy

Scanning electron microscopy (SEM) images were recorded on a Jeol JSM-6301F SEM at an acceleration voltage of 15 kV. EDS spectra were acquired using and Oxford Instruments x-act EDS detector running INCA software.

X-ray computed tomography (CT:

CT was acquired using a Nanoscan PET/CT (Mediso), with tube voltage of 50 keV, 30 ms exposure, and 720 projections, operated using Nucline 2 software (Mediso). Total scan time was 3 minutes 46 seconds per sample. Reconstructions were done with Nucline 2 software, analysis and volume rendering was done in Interview Fusion (Bartec) software.

Gas sorption analysis

Surface areas were measured by nitrogen adsorption and desorption at 77.3 K. Powder samples were degassed offline at 100 °C for 15 h under dynamic vacuum (10–5 bar) before analysis, followed by degassing on the analysis port under vacuum, also at 100 °C. Isotherms were measured using Micromeritics 2020, or 2050 volumetric adsorption analyzer.

X-ray photoelectron spectroscopy

XPS spectra were recorded on a K-alpha instrument (Thermo Fisher Scientific, East Grinstead, UK) using a monochromated Al Kα source. All spectra were recorded using a charge neutralizer to limit

differential charging and subsequently calibrated to the main adventitious C_xH_y carbon peak at a binding energy of 284.8 eV. Survey scans were recorded at a pass energy of 200 eV and step size of 1 eV. High resolution scans of C (1s), Ca (2p), N (1s), O (1s), P (2p), Si (2p) and Ti (2p) were recorded at a pass energy of 50 eV with 0.1 eV step size. Data was fitted using CASA XPS with Shirley backgrounds.

Well plate reader/assays

To measure cell growth, D-luciferin (Promega) was added to each well (10uL, 15mg/mL solution), and after 4 minutes the photon count measured over 1000ms per well using a plate reader (Varioskan Lux, Thermofisher).

Growth and differentiation of bone-stem cells

For osteocytic differentiation the cell culture medium was supplemented with adenosine at a final concentration of 30 μg/mL, which was replenished every 2 days.

MACH system

Magnetic heating experiments were undertaken using a MACH (Magnetic Alternating Current Hyperthermia) system designed and built by Resonant Circuits Limited (15 kA/m and the frequency is 930 kHz). The temperature was monitored using a fluoroptic (fibreoptic) temperature probe (Luxtron FOT Lab Kit, Lumasense California USA). Thermal images were recorded with a Jenoptik VarioCam HD thermal camera, temperature measurements and images were extracted with the Infratec IRBIS 3 software package.

Water contact angle measurements

Static water contact angle measurements were undertaken with a FTA 1000 B drop-shape analyser. A 5 μl water droplet was placed onto a flat substrate *via* a syringe, a photograph taken and the water

contact angle analysed using OEG Surftens software. For the nylon-12 treated with the NeverWet spray, the needle had to be kept in the droplet, as the extreme superhydrophobicity of the sample caused an isolated droplet to immediately roll off.

Additional figures

Additional SEM characterisation

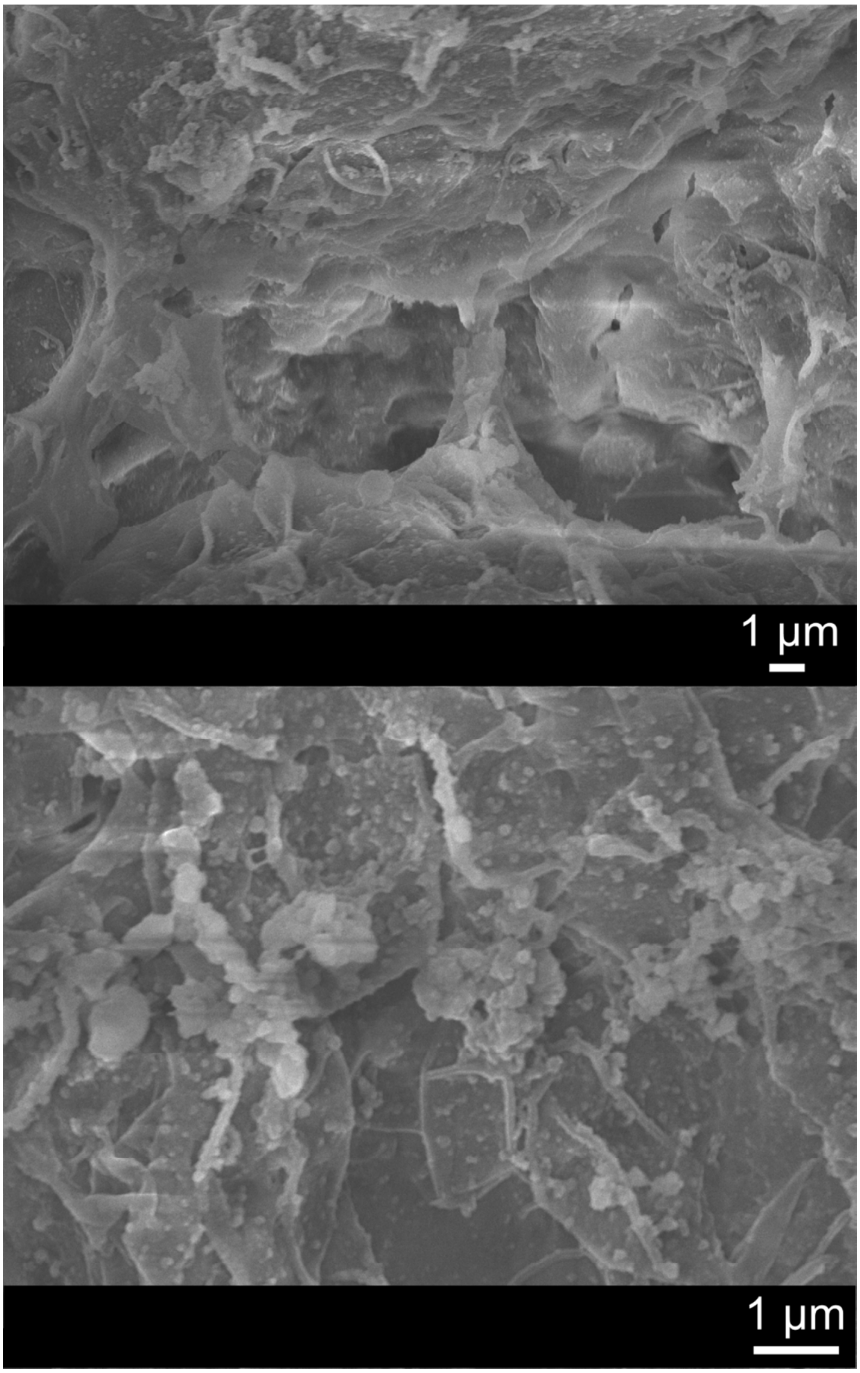

Figure S1: Scanning electron micrographs of (3-aminopropyl)triethoxysilane functionalised nylon-12 substrates showing a rough morphology.

EDS spectra

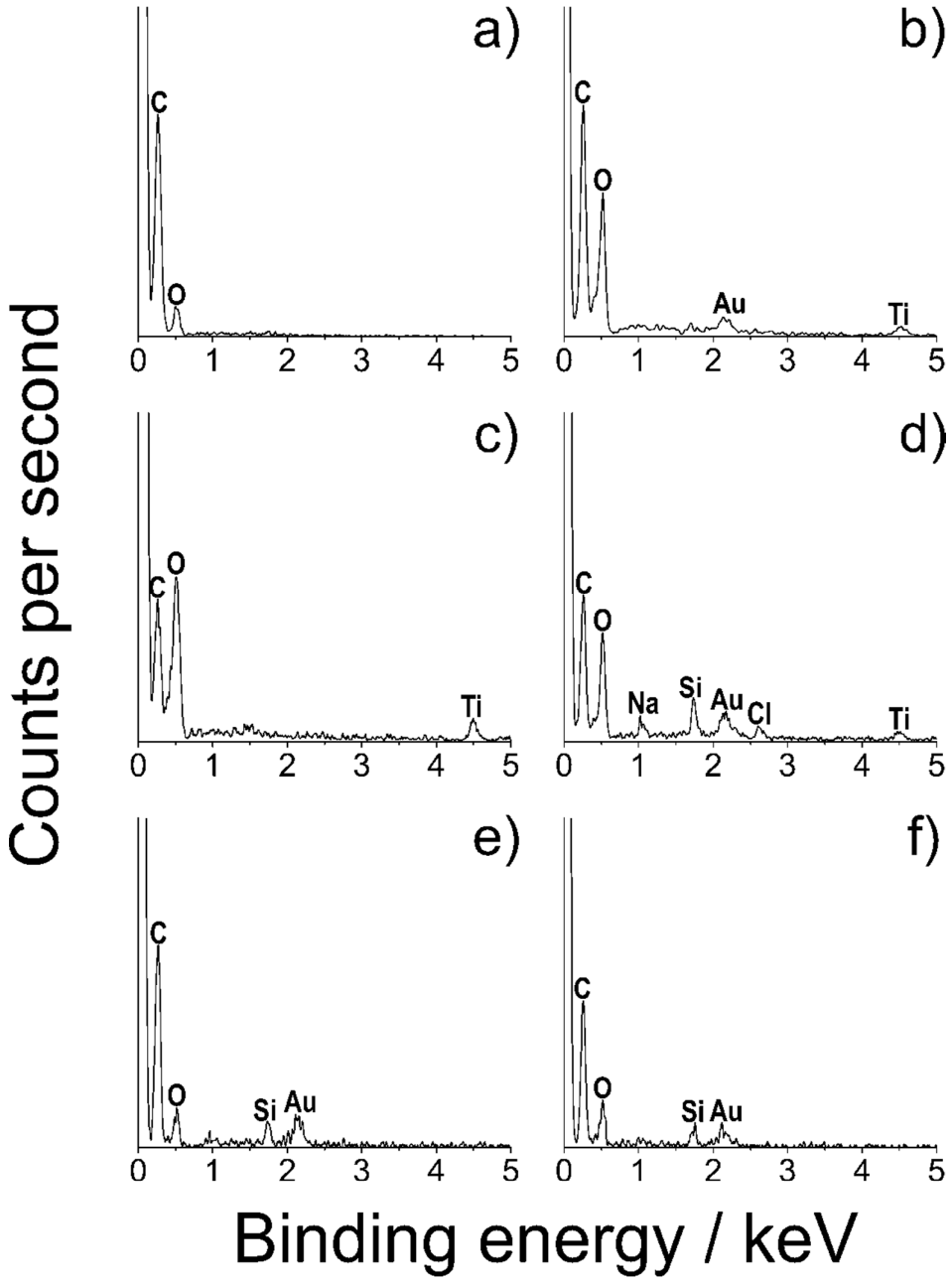

Figure S2: Energy dispersive X-ray spectra of chemically functionalised nylon-12 substrates: a) untreated nylon-12, b) TiO_2 sol, c) titanium(IV) butoxide, d) titanium(IV) butoxide–tetraethylorthosilicate mix, e) tetraethyl orthosilicate and f) (3-aminopropyl)triethoxysilane. Gold emanated from gold coating used in SEM imaging.

Superhydrophobic treatment characterisation

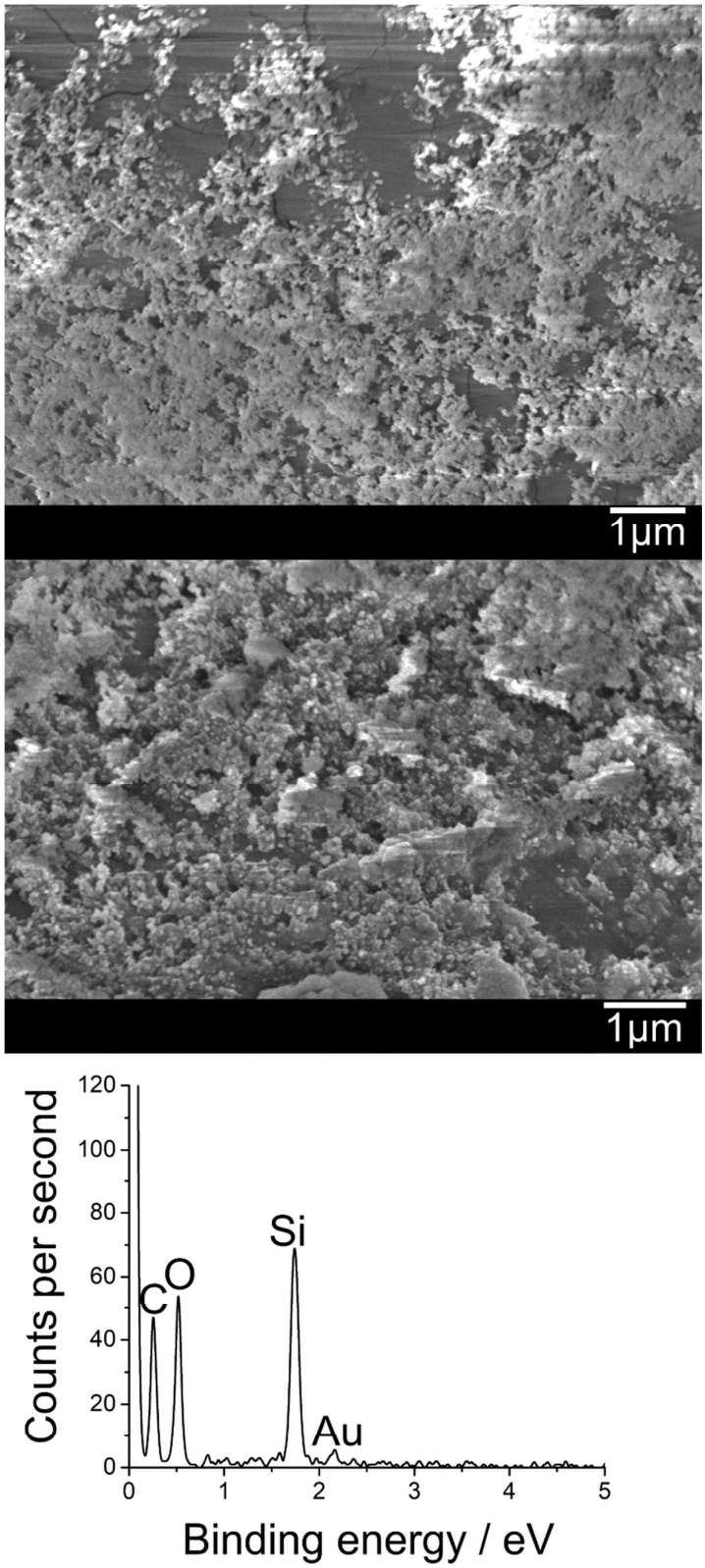

Figure S3: (Top and middle): Scanning electron microscope images of a nylon-12 substrate treated with hydrophobic NeverWet spray and (bottom): the corresponding Energy dispersive X-ray spectrum.

Static water contact angles

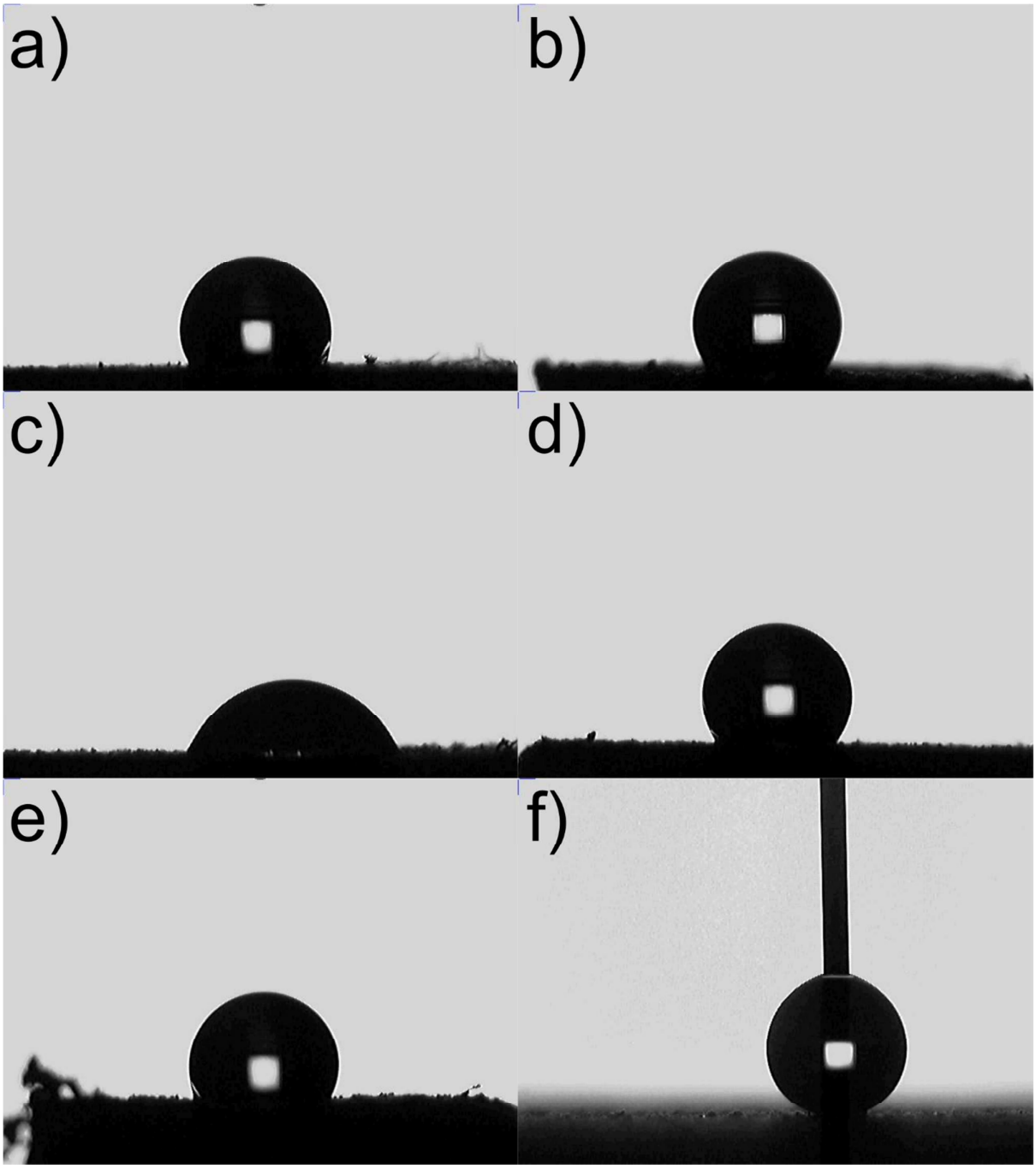

Figure S4: Water droplets resting on chemically treated substrates showing the range of hydrophobicities with different chemical treatments: a) (3-aminopropyl)triethoxysilane, b) titanium(IV) butoxide, c) tetraethylorthosilicate, d) TiO_2 sol, e) titanium(IV) butoxide–tetraethylorthosilicate mix and f) a nylon-12 surface treated with the NeverWet spray. Note that the untreated nylon-12 sample was hydrophilic and absorbed the water droplet.

Additional photographs

Figure S5: The Formiga P 100 3D-printer.

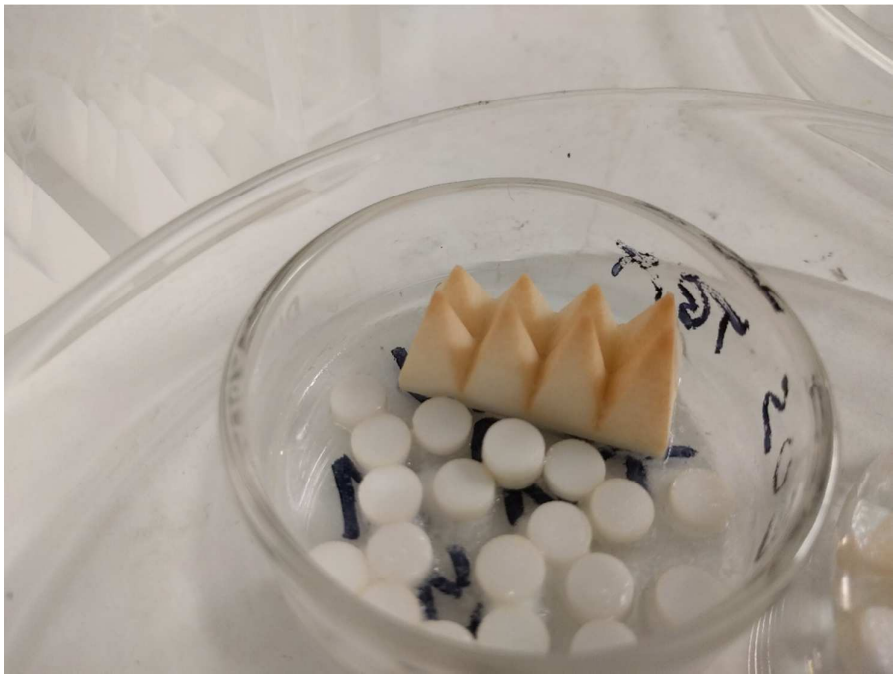

Figure S6: Nylon-12 “spikes” treated with a titanium(IV) butoxide– tetraethylorthosilicate mix after annealing in air at 90 °C for a 2-week period.

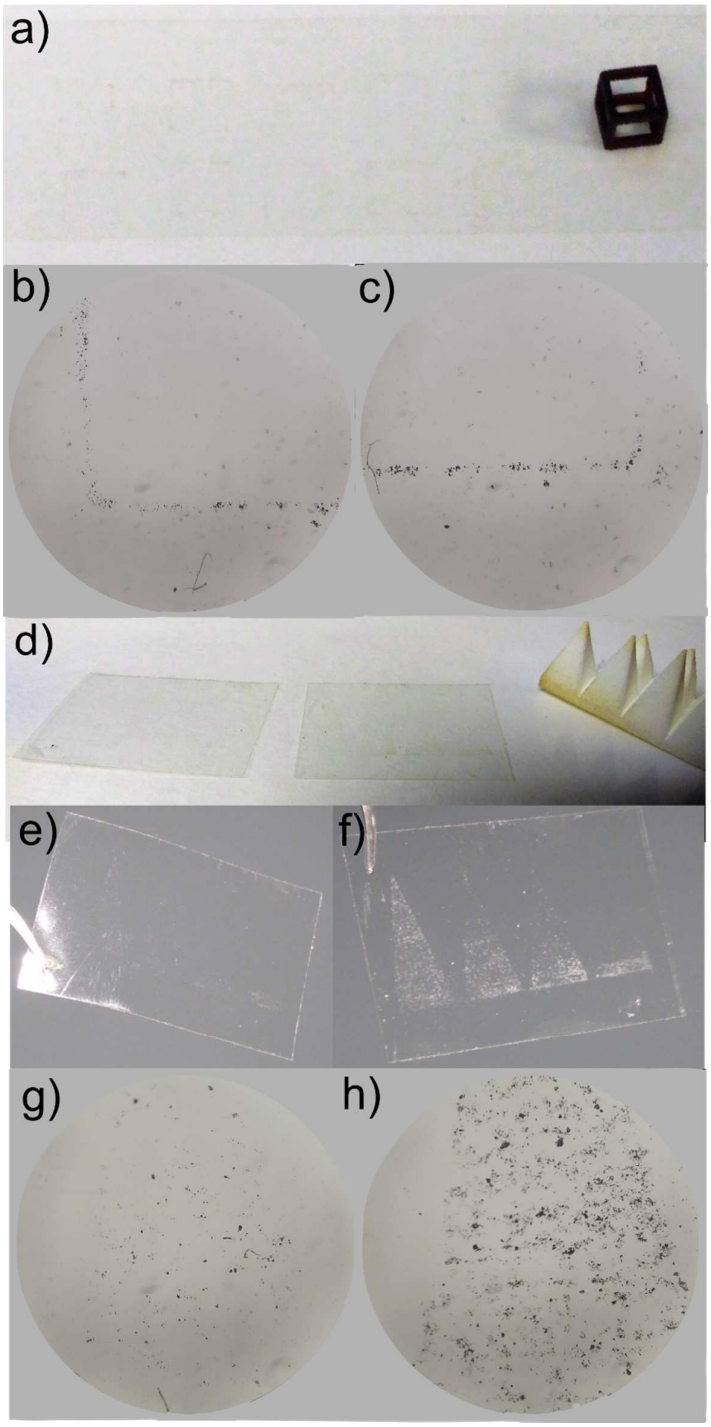

Figure S7. Adhesion tests of a) ferrofluid treated cube with ten impressions on scotch tape b) tenth impression under 10x magnification, c) first impression under 10x magnification, d) TEOS treated spike array with fifth (l) and first (r) impression, e) fifth impression under direct overhead lighting, f) first impression under direct overhead lighting, g) fifth impression under 10x magnification, and h) first impression under 10x magnification.

Tables

XPS hydroxyapatite table

	Peak positions, binding energy [eV]				
Sample	$Ca2p_{3/2}$	$Ca2p_{1/2}$	$P2p_{3/2}$	$P2p_{1/2}$	Ca:P ratio / % conc.
Nylon-12	347.13	350.63	133.10	133.95	58.2 : 41.8
TiO_2 "sol"	347.04	350.54	132.90	133.76	60.0 : 40.0
Titanium(IV) butoxide	347.12	350.62	132.97	133.84	65.7 : 34.3
Titanium(IV) butoxide – tetraethylorthosilicate mix	347.09	350.59	132.98	133.83	64.0 : 36.0
Tetraethyl orthosilicate	347.04	350.54	133.13	134.00	64.7 : 35.3
(3-aminopropyl)triethoxysilane	347.14	350.64	133.04	133.89	60.0 : 40.0

Table S1: This table shows the peak positions for Ca2p and P2p chemical environments in X-ray photoelectron spectroscopy positions for samples after cell differentiation. Doublet separation was 3.50 eV for Ca2p and 0.85 eV for P2p. The optimal ratio of Ca : P for hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) is 62.5 : 37.5. Peak positions are indicative of hydroxyapatite.

Cell growth Student's "t" test data

	Sample				
Days	TiO_2	TBX	TEOS	TEOS TBX	APTES
1	ns	ns	ns	ns	ns
2	ns	ns	ns	ns	ns
3	ns	ns	ns	ns	ns
4	ns	ns	ns	ns	ns
7	ns	ns	ns	ns	ns
8	ns	ns	*	ns	ns
9	*	*	****	ns	ns
10	**	**	***	*	*
11	****	****	***	***	**
14	****	****	**	****	ns
15	****	****	****	****	ns
16	****	****	ns	****	ns

Table S2. Student's "t" Test with Dunnett correction for multiple comparisons shows significant differences in viable cell number compared to the control untreated substrate. * $p<0.05$; ** $p<0.01$; *** $p<0.001$ **** $p< 0.0001$.