Supporting Information

Surface engineering of porous silicon to optimise therapeutic antibody loading and release

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Infliximab loading optimisation

Table S1: Loading of Infliximab at concentrations > 1 mg/mL.

Infliximab Concentration (mg/mL)	Loading (mg/mg)	Loading Efficiency (%)
1.0	0.063 ± 0.010	94.5
2.4	0.159 ± 0.010	85.0
5.2	0.225 ± 0.004	65.0
8.3	0.321 ± 0.004	58.3

XPS Results

XPS was performed using an AXIS Ultra DLD spectrometer (Kratos Analytical, UK) equipped with a monochromatic Al Kα radiation source (hν - 1486.6 eV) at a power of 225 W. The pass energy for survey spectra, recorded over the energy range 0 - 1000 eV, was 160 eV with 0.5 eV step size and the pass energy for high-resolution C 1s spectra was 20 eV with 0.1 eV step size. The analysis area was approximately 700 μm x 300 μm. The spectra were acquired at a takeoff angle of 90°. Elements present on the surface were identified from survey spectra and quantified in atomic percentage (at. %) with CasaXPS Software (version 2.3.14, www.casaxps.com) using a Shirley-type background and applying the relative sensitivity factors supplied by the manufacturer of the instrument. In order to minimise X-ray-induced sample degradation, the exposure time was kept to the minimum required to obtain an adequate signal-to noise ratio. Charging effect of the samples during analysis was corrected using a reference value of 285.0 eV for the C 1s component of neutral hydrocarbon.[1]

Infliximab loaded and blank pSi-Ox surfaces were investigated via XPS to confirm the presence of protein in the pores after loading. Table 2 shows the at. % of elements found on both surfaces.

Table S2: At. % of loaded and non-loaded oxidised pSi surfaces.

Sample/Element	Non-loaded Oxidised pSi	Loaded Oxidised pSi
Si 2p	42.26	31.56
O 1s	46.54	40.07
C 1s	11.19	24.75
N 1s	0.00	3.62

XPS analysis of unloaded and loaded pSi-Ox corroborated the findings from IR spectroscopy. The unloaded pSi-Ox showed high surface atomic percentage (at. %) signal for Si (42.26 at. %) and O (46.54 at. %), as expected from the presence of a silicon oxide layer. There was also the presence of slight contamination on the surface mainly from hydrocarbons. Importantly, all the fluorine from the etching process has been successfully removed. After loading oxidised pSi with Infliximab, the carbon increased significantly from 11.19 at. % to 24.75 at. %, whereas silicon decreased by 10.7 at. % to 31.56 at. %; attributed to the drug covering the surface causing a partial attenuation of the Si signal. The atomic percentage of oxygen did not change as much due to the protein also containing oxygen species (46.54 at. % to 40.07 at. %). As expected, nitrogen from the Infliximab could be detected on the loaded pSi-Ox surface at (3.62 at.%), whilst none appeared on the unloaded sample.

L929 BioAssay

To screen the optimal release conditions for the Infliximab loaded into pSi MPs we used a bioassay based on L929 cells and their sensitivity to TNF-α. TNF-α is cytotoxic to L929 fibroblasts and hence can be used as a cell-based bioassay for TNF-α.[2] In this bioassay the addition of TNF-α causes cell death and a reduced ability of the L929 fibroblasts to convert the tetrazolium salt (MTT) to formazan, hence producing a decrease in the optical density at 570 nm. Supp Figure 5 shows the dose dependent response of the L929 cells with varying concentrations of TNF-α (0 - 100 μg/mL). These results are comparable to the earlier work of Cowin et al 2006.[3] When increasing concentrations of Infliximab (1 - 1000 μg/mL), a TNF-α neutralising antibody, were added to the cultures in the presence of a single dose of TNF-α (1 μg/mL), the antibody dose-dependently prevented TNF-α-induced cell death (Supp Figure 6). These results confirmed the L929 fibroblast as a sensitive bioassay for TNF-α. Sufficient recovery of L929 cells was observed at concentrations as low as 5 mg/mL of Infliximab.

ToF-SIMS

Table S3: Positive ion fragments of Infliximab detected on loaded pSi-Ox surfaces and their corresponding amino acids over mass range 0 – 150 *m*/*z*.

Mass (*m*/*z*)	Positive fragment	Amino acid
30.036	CH_4N	Glycine, Lysine
44.049	C_2H_6N	Alanine
56.05	C_3H_6N	Lysine
59.05	CH_5N_3	Arginine
60.054	C_2H_6NO	Serine
61.018	C_2H_5S	Methionine
68.05	C_4H_6N	Proline
69.043	C_4H_5O	Threonine
70.029	C_3H_4NO	Asparagine
70.066	C_4H_8N	Proline, arginine
71.014	$C_3H_3O_2$	Serine
72.081	$C_4H_{10}N$	Valine
73.064	$C_2H_7N_3$	Arginine
74.067	C_3H_8NO	Threonine
81.054	$C_4H_5N_2$	Histidine
82.052	$C_4H_6N_2$	Histidine
83.052	C_5H_7O	Valine
84.054	C_4H_6NO	Glutamine, Glutamic acid
84.088	$C_5H_{10}N$	Lysine
86.097	$C_5H_{12}N$	Leucine, isoleucine
87.06	$C_3H_7N_2O$	Asparagine
88.046	$C_3H_6NO_2$	Asparagine, aspartic acid
91.055	C_7H_7	Phenylalanine
98.019	$C_4H_4NO_2$	Asparagine
100.089	$C_4H_{10}N_3$	Arginine
101.091	$C_4H_{11}N_3$	Arginine
102.058	$C_4H_8NO_2$	Glutamic acid
104.062	$C_4H_{10}NS$	Methionine
107.059	C_7H_7O	Tyrosine
110.077	$C_5H_8N_3$	Histidine, arginine
120.089	$C_8H_{10}N$	Phenylalanine
127.1	$C_5H_{11}N_4$	Arginine
130.073	C_9H_8N	Tryptophane
131.055	C_9H_7O	Phenylalanine
132.064	C_9H_8O	Phenylalanine
136.083	$C_8H_{10}NO$	Tyrosine

Characterization of microporous surface layers

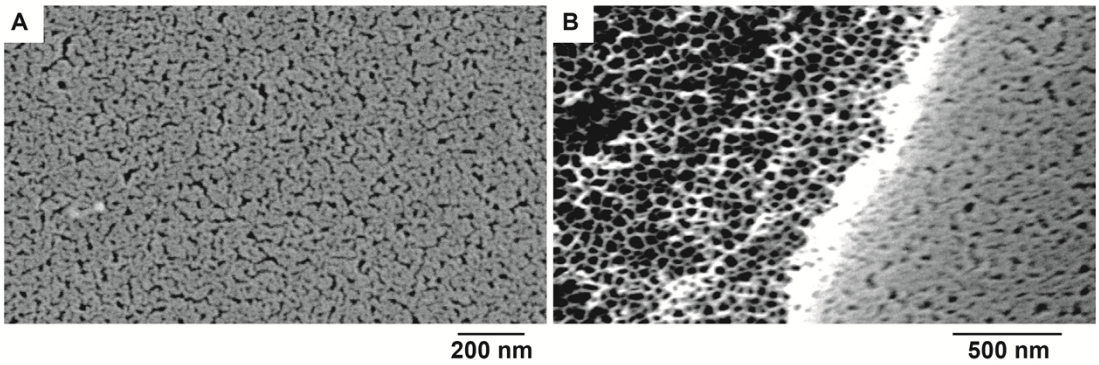

Figure S1: (A) SEM of the microporous layer remaining above pSi etches if not removed via techniques such as NaOH dissolution or sacrificial etching and (B) A defect site in the films shown in (A) showing both the microporous layer and the desired porous layer beneath it.

Error analysis in EOT readings

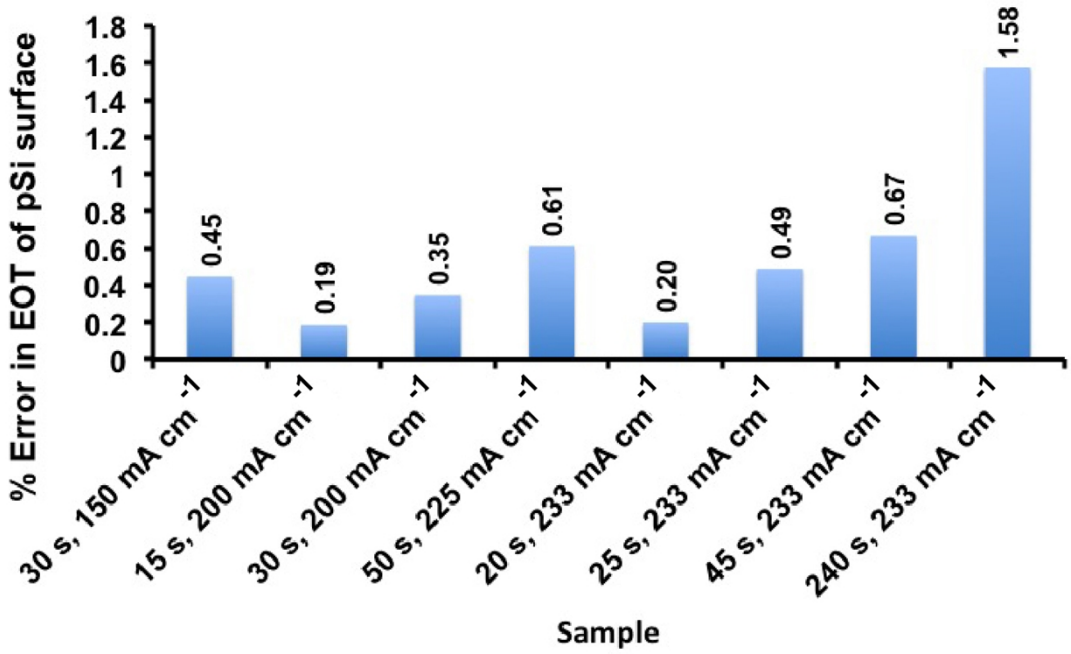

Figure S2: EOT readings of the different etching conditions (time and current) (n = 20).

Zeta potential measurements at pH 6.5 and 5.5

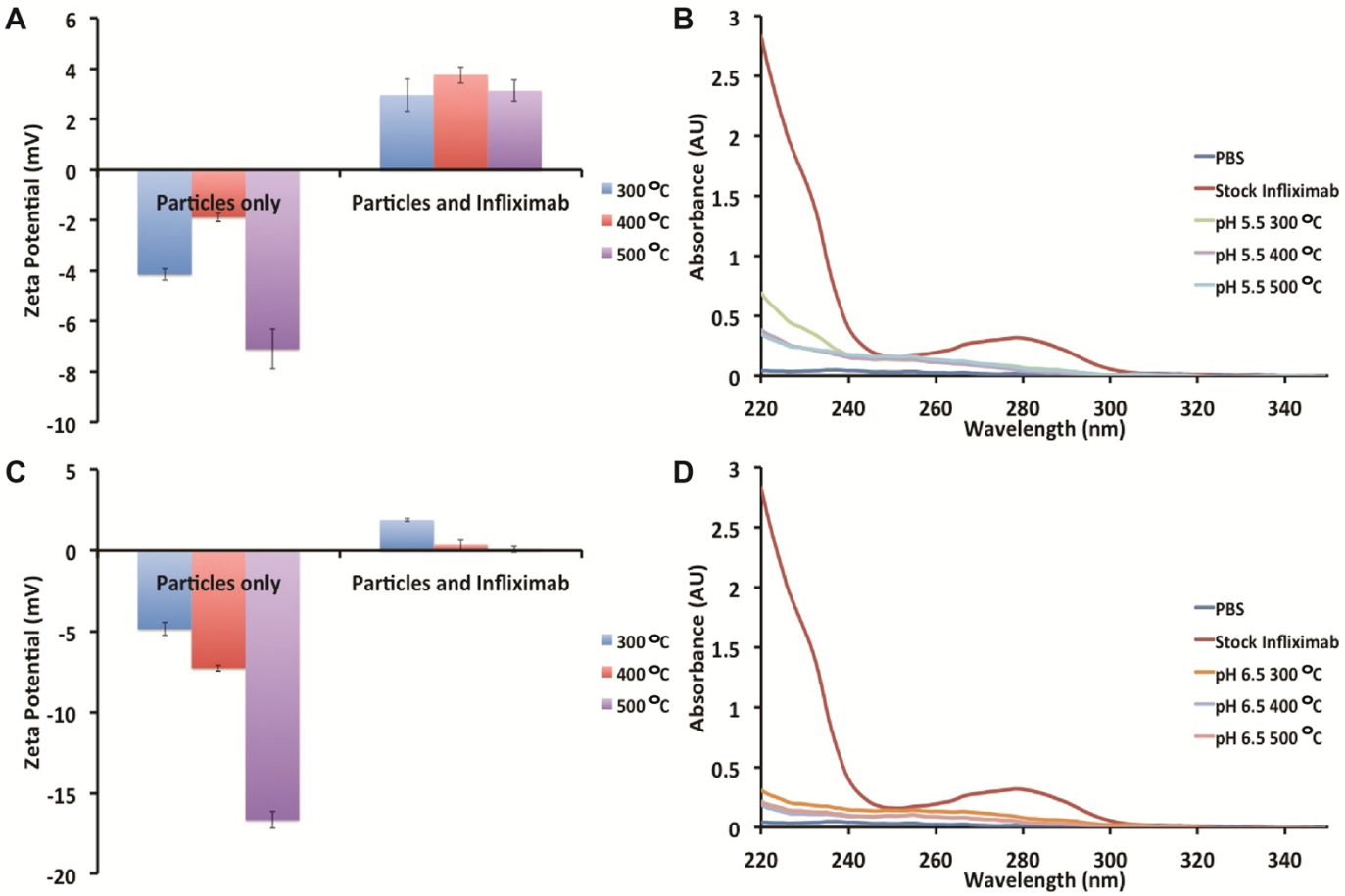

Figure S3: Zeta potential measurements of the Infliximab binding at to 300 °C, 400 °C and 500 °C oxidised pSi at (A) pH 5.5 and (B) corresponding UV-Vis monitoring of the Infliximab in solution during the zeta-potential binding experiment in panel (A). (C) Zeta potential measurements of the Infliximab binding at to 300 °C, 400 °C and 500 °C oxidised pSi at pH 6.5 for different oxidation conditions (n=3) and (D) UV-Vis monitoring of the Infliximab in solution during the zeta-potential binding experiment in panel C at pH 6.5 (n = 3).

ToF-SIMS mass spectra

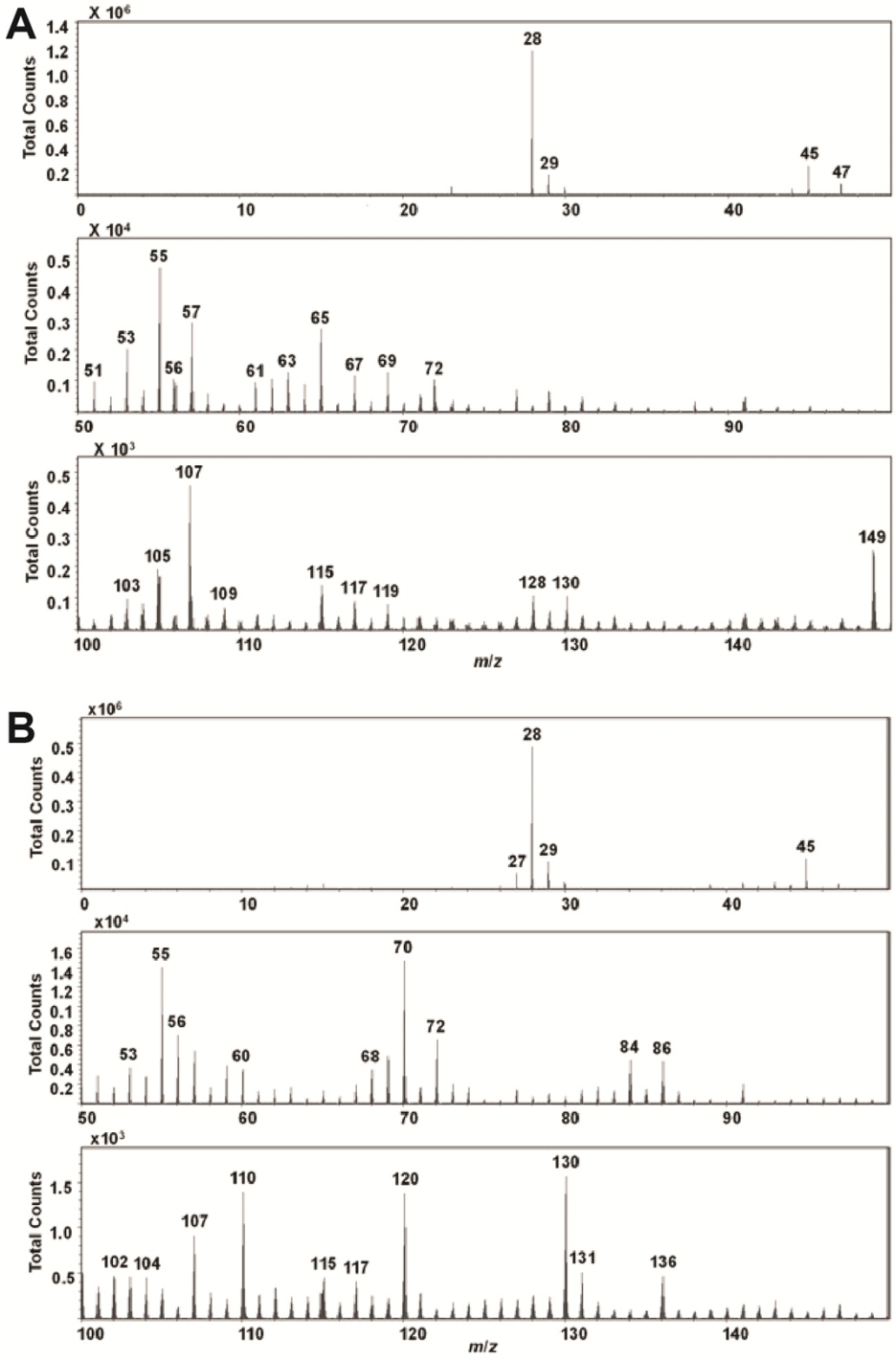

Figure S4: Positive ion ToF-SIMS mass spectra (0 – 150 *m*/*z*) for (A) unloaded and (B) Infliximab-loaded pSi MPs.

L929 bioassay optimization

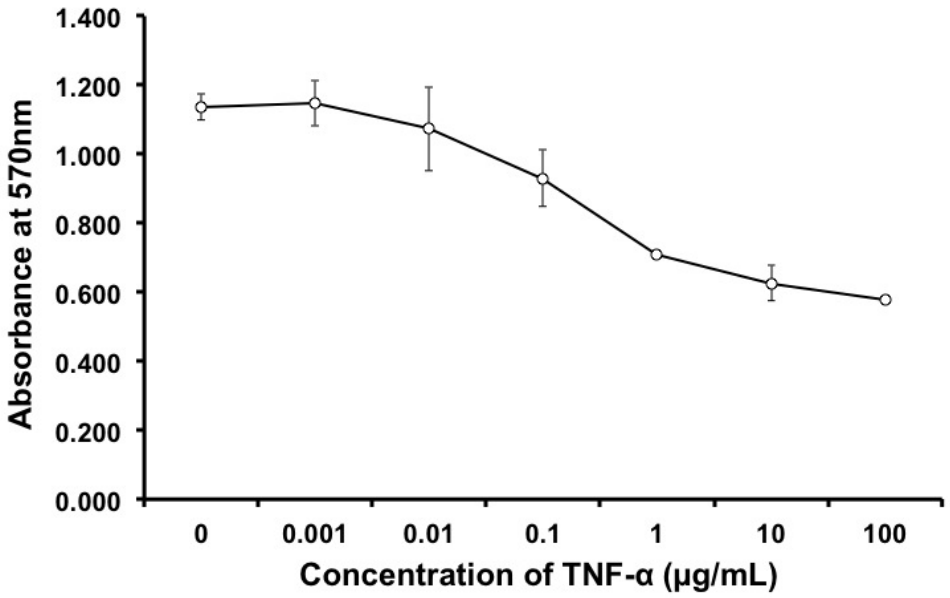

Figure S5: MTT assay to determine the viability of TNF-α-treated L929 cells. Increasing doses of human TNF-α was added to L929 cells, with viability measured using the absorbance at 570 nm. Data is presented as mean +/- one standard deviation (n = 4).

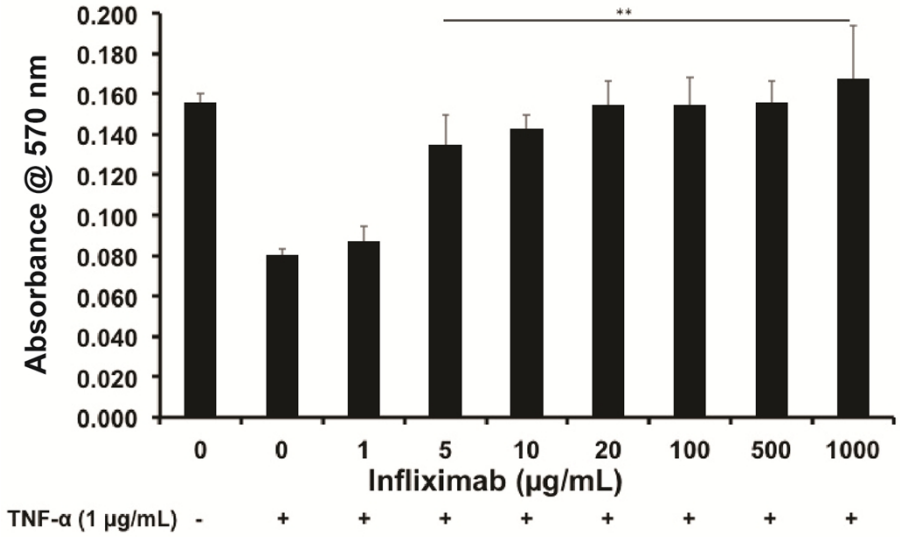

Figure S6: MTT assay to measure Infliximab-induced recovery of TNF-α-treated L929 cells. Increasing concentrations of Infliximab was incubated with 1 µg/mL human TNF-α for 10 minutes at 37˚C, and then added to L929 cells. MTT assay was used to measure L929 cell viability using absorbance at 570 nm. Data is presented as mean +/- one standard deviation (n = 4).

Stability of Infliximab at various pH, temperatures and incubation times

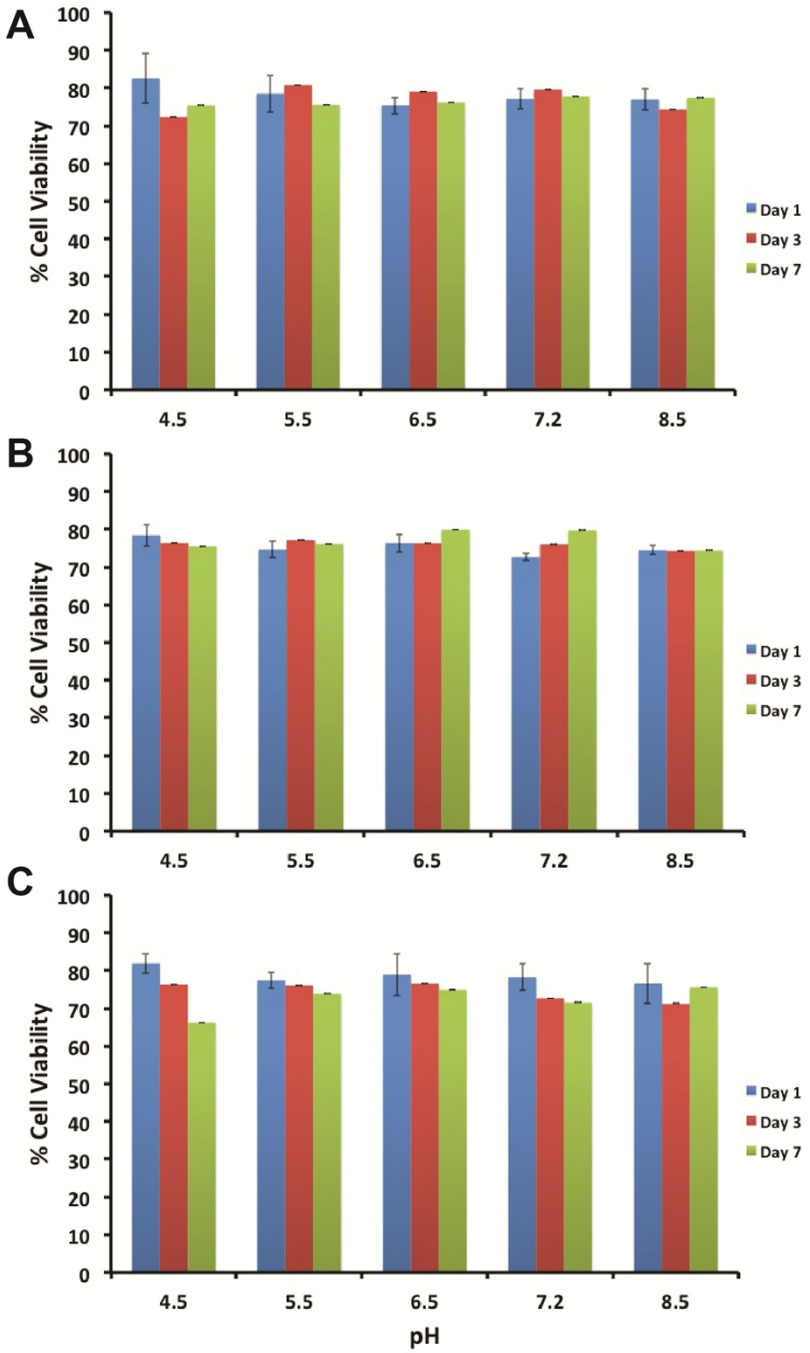

Figure S7: Effect of pH and temperature on the functionality of Infliximab. Infliximab (1 mg/mL) was incubated in pH adjusted PBS at 4 ºC (A), 25 ºC (B) and 37 ºC (C). Samples were then incubated with 1 µg/mL human TNF-α for 10 minutes at 37˚C, and then added to L929 cells. MTT assay was used to measure L929 cell viability using absorbance at 570 nm. Data is presented as mean +/- one standard deviation (n = 3). Data is presented as a % of cell viability.

References

1. G. Beamson and D. Briggs, *High resolution XPS of organic polymers*, John Wiley and Sons, Chichester, UK, 1992.
2. F. Denizot and R. Lang, *J. Immunol. Methods*, 1986, **89**, 271–277.
3. A. J. Cowin, N. Hatzirodos, J. Rigden, R. Fitridge, and D. A. Belford, *Wound Repair Regen.*, 2006, **14**, 421–426.