Electrospun scaffold preparation. Polycaprolactone (PCL) was dissolved at 10 % (w/v) in 1,1,1,3,3,3-hexafluoro-2-propanol (HFP) (Sigma, USA) and electrospun into microfibers as previously described (15). PCL solutions were loaded into a syringe and fed through a 0.6 mm diameter needle at a flow rate of 4 mL/hr using a syringe pump. The needle was connected to a 20 kV positive power supply and directed at a rotating circular drum (12 cm diameter, 500 rpm) or a stainless steel mandrel (0.5 mm diameter, 500 rpm) at a distance of 20 cm for the fabrication of flat sheets or mouse vascular grafts, respectively. Scaffold sheets were peeled from the drum and then cut into circular discs using a 5 mm diameter biopsy punch. Before implantation, scaffolds were sterilized by UV light for 30 min and washed three times with and stored in sterile PBS. Vascular grafts were spun using the same method at dimensions of 0.5mm internal diameter x 6mm length.

Surface Characterisation. Fourier transform infrared spectroscopy in total attenuated reflectance mode (FTIR-ATR) was carried out to examine the surface chemistry of scaffolds, using a Digilab FTS7000 spectrometer (Holliston, MA, USA) equipped with a trapezium germanium crystal at an incidence angle of 45˚. Scaffolds were cut into 2 cm × 2 cm strips, and spectra were collected by averaging a total of 500 scans at a spectral resolution of 4 cm^{-1} in the wavenumber range 650-4000 cm^{-1}. Electron paramagnetic resonance (EPR) was carried out to detect the density of unpaired electrons on 6 cm × 1 cm samples as previously described (18). Spectra were acquired with a centre field of 3510 G, sampling time of 90 ms, modulation amplitude and frequency of 3 G and 10^5 Hz, and microwave frequency and power of 9.8 GHz and 25 mW, respectively. The absolute spin density was calculated through quantitative analysis using a weak pitch (~10^{13} spin/cm) Bruker standard sample.

In vitro characterization of bioactive IL-4 surfaces. Both murine-derived macrophage cell line RAW 264.7 cells and primary macrophages were used in this study. RAW 264.7 cells were seeded onto scaffolds for 8 h. Actin cytoskeleton was stained with Alexa Fluor 594 labelled-phalloidin (Life Technologies Pty Ltd., Australia) and examined under confocal laser scanning microscopy (Nikon Air Confocal). Scanning electron microscopy (SEM) images were taken under Zeiss SEM (FEI, USA). Murine bone marrow primary macrophages were isolated using a pre-established protocol (19). Primary macrophages were seeded onto scaffolds for 8 h. The quantitative real-time reverse-transcription polymerase chain reaction (qRT-PCR) was performed using QuantStudio™ Real-Time PCR System instrument (Applied Biosystems, USA).

Histology and immunohistochemistry. Tissue from both models were fixed in PFA (4 %) and dehydrated through an ethanol gradient and embedded in paraffin and sectioned at 5 µm (22). Paraffin sections were deparaffinised and rehydrated for haematoxylin and eosin staining and Milligans trichrome staining. For immunohistochemistry staining, sections were deparaffinised and stained with antibodies against anti-CD68 (Abcam, USA), anti-CD31 (Abcam, USA), anti-CD206 (Abcam, USA), anti-IL-1β (Abcam, USA), anti-TNF-α (Abcam, USA), anti-IL-10 (Santa Cruz, USA), and anti TGF-β (Abcam, USA). Immuno-stained sections were then mounted and coverslipped with DAPI-containing mounting media (VECTASHIELD).

Quantitative image analysis. Histological and immunohistochemical sections were imaged using a Zeiss Upright Olympus fluorescence multi-channel microscope, captured with a Nikon DP Controller 2.2 (Olympus, Japan). Immunohistochemical and histopathological analysis was done using ImageJ. Briefly, 20 × magnification images were taken of the entire

implant. For H&E and $CD206^{+}/CD68^{+}$ analysis, positive staining was quantified as individual particles counted based on a common threshold intensity. For Milligan's Trichrome and IL-1β/TNF-α/IL-10/TGF-β cytokine analysis, positive staining was quantified as total area stained based on a common threshold intensity. All quantifications were standardized to scaffold cross-sectional area (n = 5 sections per scaffold group per time point). For vascular grafts, each graft was sectioned entirely from proximal to distal and slides were selected from three points evenly distributed along the graft (n = 5 grafts per group). Immunohistochemical analysis for macrophage response and cytokines was identical to subcutaneous scaffold methodology. Neointimal area was represented as a percentage of total lumen area defined by the inner graft wall. Collagen, PCNA and elastin was quantified using a threshold intensity and expressed as a percentage of the neointimal area. Endothelial coverage was quantified by measuring the length of CD31 positive staining at the surface of the lumen and expressed as a percentage of the total lumen circumference.

Statistical analysis. Data are expressed as mean ± SEM (Standard Error of the Mean), and statistical analysis was performed using GraphPad Prism 7 (Version 7.02) for Windows (GraphPad Software Inc., San Diego, California). The normal distribution of data sets was confirmed using Komogorov-Smirnov tests and parametric analysis was performed. Where appropriate, data were analyzed by unpaired Student t test when comparing 2 samples at different time points. For analysis of Figures 1E and 2C, one-way analysis of variance (ANOVA) was performed. For analysis of Figure 8B, all points through the graph were averaged for each animal (n = 5 animals). Post hoc comparisons were analysed with Tukey multiple comparisons test. We considered p values <0.05 to be significant.

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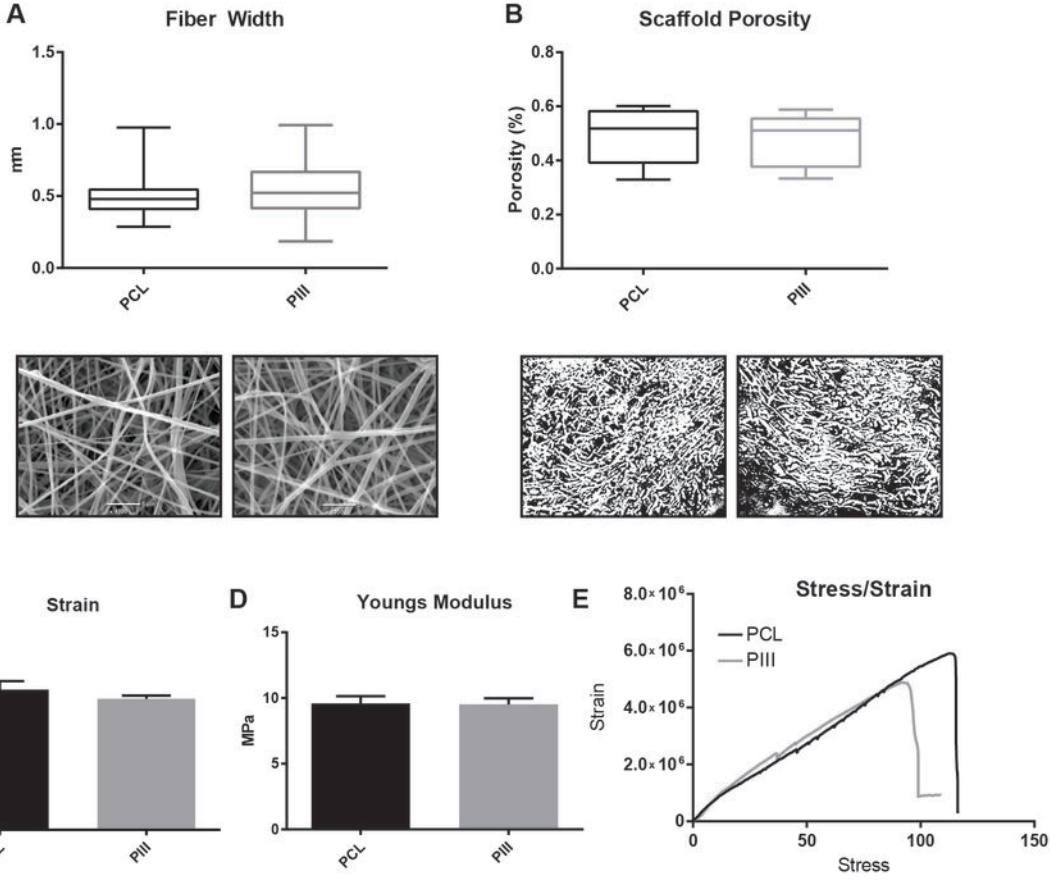
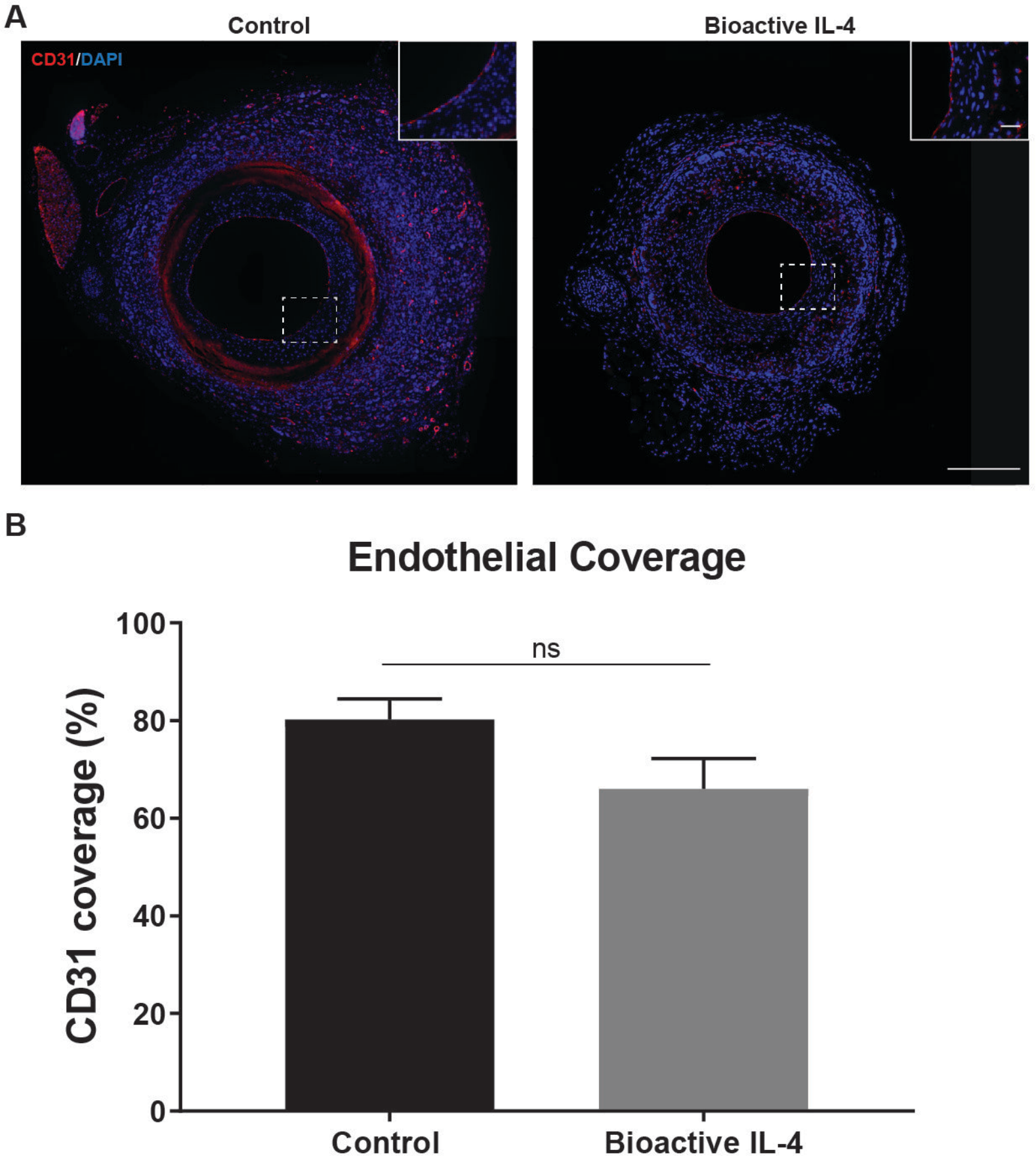
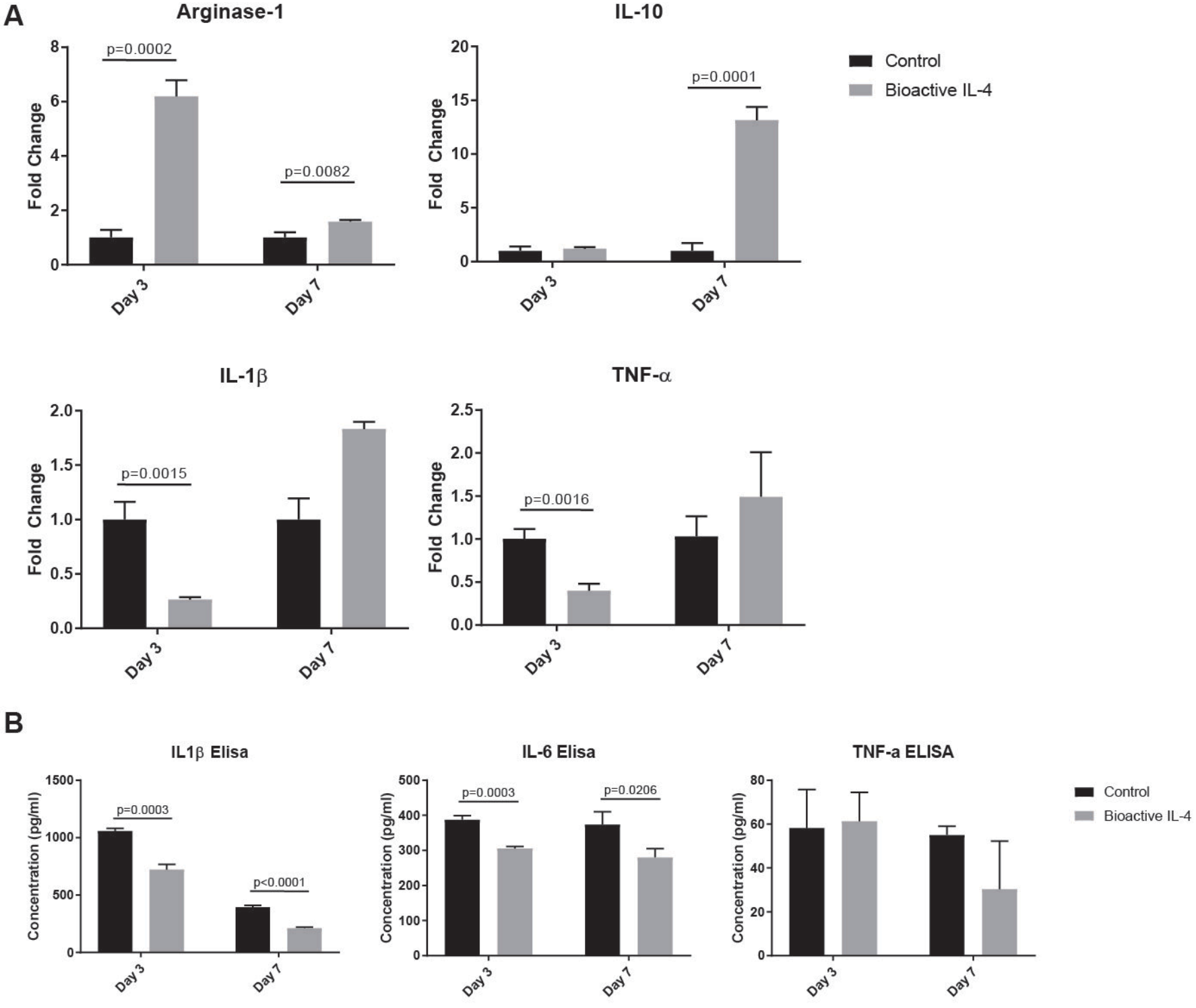
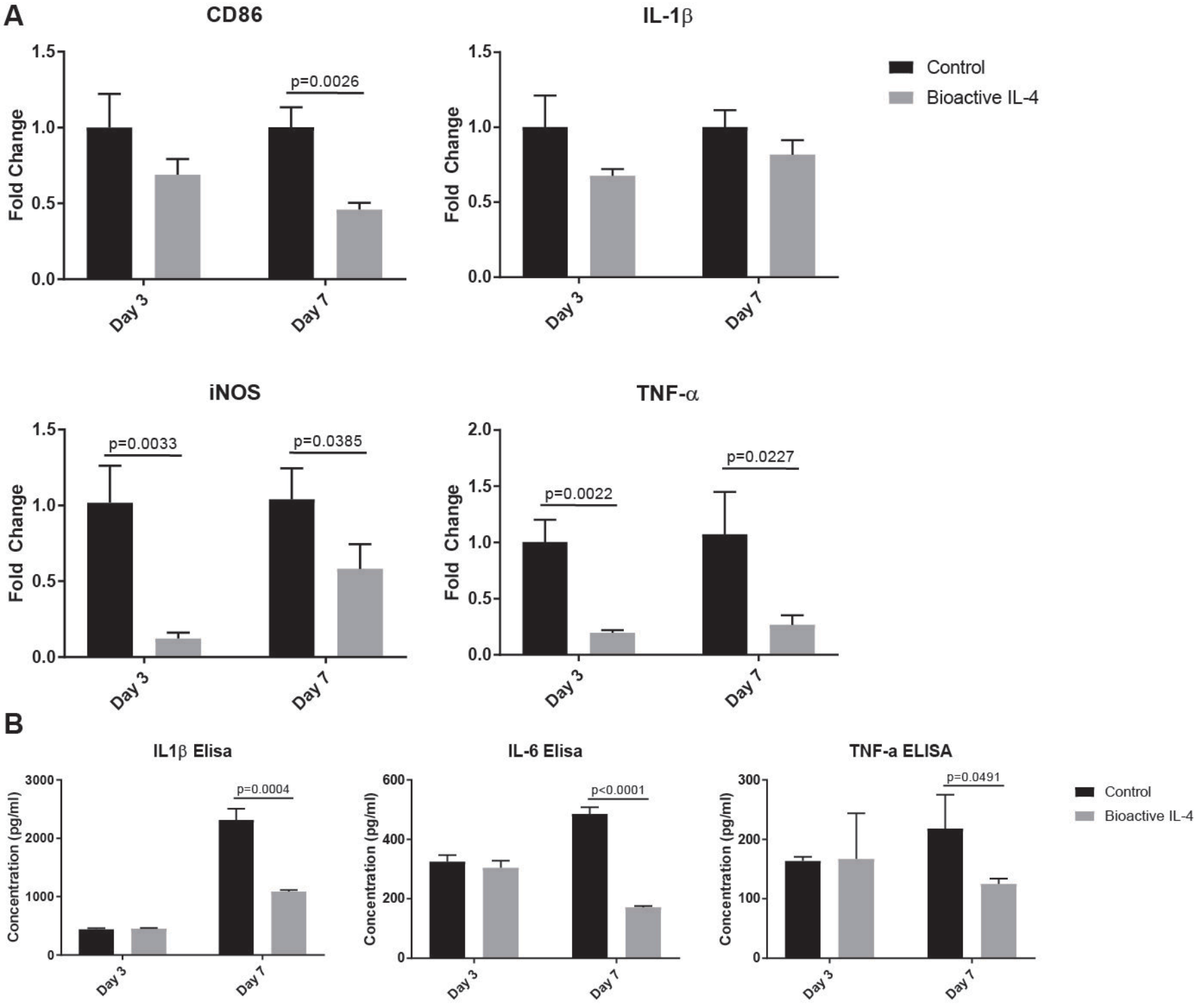
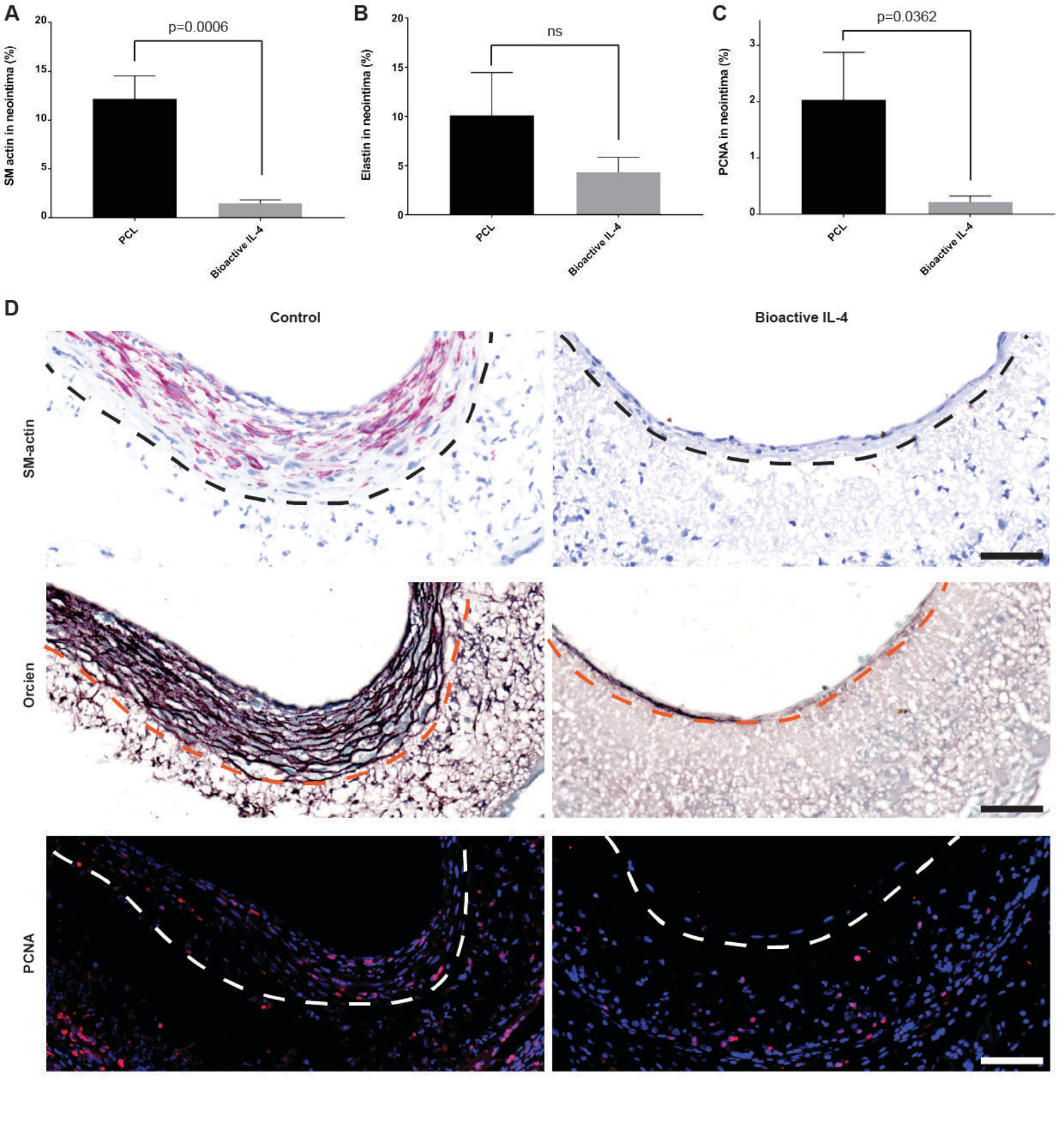
Characterisation of PIII treated surfaces. Changes to the surface chemistry of PIII treated scaffolds were confirmed by an increase in the absorbance of O-H and NH (3660 cm^{-1} – 3050 cm^{-1}), C=O (1741 cm^{-1} and 1714 cm^{-1}), C=C, C=N, N=O (1710 cm^{-1} - 1512 cm^{-1}) and C≡N (2300 cm^{-1} - 2000 cm^{-1}) peaks in the FT-IR spectrum, indicative of new bonds in the treated surface layer (Figure 1B). These new bonds suggest the formation of functional groups, which arise upon the bombardment of nitrogen ions during the PIII treatment and subsequent radical-mediated surface oxidation due to exposure of the modified PCL scaffold to air. Quantitative EPR spectroscopy further confirmed the formation of radicals (Figure 1C), as shown by a significant increase in the density of unpaired electrons on PIII treated scaffolds ($3.65 \pm 0.69 \times 10^{13}$ spins/cm^2) compared with untreated samples ($4.08 \pm 1.14 \times 10^{12}$ spins/cm^2). The bombardment of energetic nitrogen ions displaces carbon atoms throughout the penetration depth of the nitrogen, ultimately forming a modified layer rich in long-lived radicals stabilized in π conjugated carbon clusters.


A
Fiber Width
mm
1.5
1.0
0.5
0.0
PCL
PIII
B
Scaffold Porosity
Porosity (%)
0.8
0.6
0.4
0.2
0.0
PCL
PIII
C
Strain
1.5
1.0
0.5
0.0
PCL
PIII
D
Youngs Modulus
MPa
15
10
5
0
PCL
PIII
E
Stress/Strain
8.0x10^6
6.0x10^6
4.0x10^6
2.0x10^6
0
PCL
PIII
Strain
0
50
100
150
Stress


A
Control
Bioactive IL-4
CD31/DAPI
B
Endothelial Coverage
CD31 coverage (%)
100
80
60
40
20
0
ns
Control
Bioactive IL-4


A
Arginase-1
p=0.0002
p=0.0082
Fold Change
Day 3
Day 7
IL-10
p=0.0001
Control
Bioactive IL-4
IL-1β
p=0.0015
TNF-α
p=0.0016
B
IL1β Elisa
p=0.0003
p<0.0001
Concentration (pg/ml)
IL-6 Elisa
p=0.0003
p=0.0206
TNF-a ELISA


A
CD86
IL-1β
iNOS
TNF-α
Fold Change
p=0.0026
p=0.0033
p=0.0385
p=0.0022
p=0.0227
Day 3
Day 7
Control
Bioactive IL-4
B
IL1β Elisa
IL-6 Elisa
TNF-a ELISA
Concentration (pg/ml)
p=0.0004
p<0.0001
p=0.0491


A
SM actin in neointima (%)
p=0.0006
PCL
Bioactive IL-4
B
Elastin in neointima (%)
ns
PCL
Bioactive IL-4
C
PCNA in neointima (%)
p=0.0362
PCL
Bioactive IL-4
D
Control
Bioactive IL-4
SM-actin
Orcien
PCNA

Supplementary Figure 1 – Physical characterization of scaffolds following PIII treatment (A) Quantification and representative scanning electron microscopy images of scaffold surfaces.(B) Quantification and representative cross-sectional images of scaffold porosity. Mechanical testing of scaffold (C) strain, (D) Young's modulus, and (E) stress/strain curves, n = 5 per group.

Supplementary Figure 2 – Endothelialization of vascular grafts. (A) Representative $CD31^{+}$ immunostains of endothelial cell coverage of graft luminal surfaces, scale bar represents 1mm (inset - magnified images, scale bar represents 20μm) and (B) quantification. N = 5 per group

Supplementary Figure 3 – Subcutaneous implant qPCR and ELISA analysis at 3 and 7 days post-implantation. (A) Gene expression changes for M2 markers, Arginase-1 and IL-10 (top row), and for M1 markers IL-1β and TNF-α (bottom row) (B) Protein expression of IL-1β, IL-6, and TNF-α. N = 5 per group.

Supplementary Figure 4 Vascular Graft qPCR and ELISA Analysis at 3 and 7 Days' Postimplantation

(A) Gene expression changes for M1 markers CD86 and IL-1β **(top row)**, and for inducible nitric oxide synthase (iNOS) and TNF-α **(bottom row)**. **(B)** Protein expression of IL-1β, IL-6, and TNF-α. N = 5 per group. Abbreviations as in Supplemental Figure 3.

Supplementary Figure 5 Characterization of Vascular Graft Neointima

(A) Quantification of smooth muscle (SM) cells, SM-α-actin+ stained area expressed as percentage of total neointimal area. **(B)** Quantification of elastin as percentage of total neointimal area. **(C)** Quantification of PCNA+ (proliferating) cells in the neointima, expressed as percentage of neointimal area. **(D)** Representative images of the respective histological stains; **dotted lines** indicate the interface between the luminal graft wall and neointima. SM-α-actin in **red (top row)**, elastin in **black (middle row)**, and PCNA in **red (bottom row)**. Scale bar = 50 μm; n = 5 per group. Other abbreviations as in Supplemental Figure 3.