Implanted biomaterials often fail because they elicit a foreign body response (FBR) and concomitant fibrotic encapsulation. To design clinically relevant interference approaches, it is crucial to first examine the FBR mechanisms. Here, we report the development and validation of infrared-excited nonlinear microscopy to resolve the three-dimensional (3D) organization and fate of 3D-electrospun scaffolds implanted deep into the skin of mice and the following step-wise FBR process. We observed that immigrating myeloid cells (predominantly macrophages of the M1 type) engaged and became immobilized along the scaffold/tissue interface, before forming multinucleated giant cells. Both macrophages and giant cells locally produced vascular endothelial growth factor (VEGF), which initiated and maintained an immature neovessel network, followed by the formation of a dense collagen capsule two- to four-weeks post-implantation. Elimination of the macrophage/giant-cell compartment, by clodronate and/or neutralization of VEGF by VEGF Trap, significantly diminished giant-cell accumulation, neovascularization and fibrosis. Our findings identify macrophages and giant cells as incendiaries of the fibrotic encapsulation of engrafted biomaterials via VEGF release and neovascularization, and therefore as targets for therapy.

Nonlinear imaging of biomaterials
To explore the cause–consequence relationships of the FBR, we developed an intravital window model and applied nonlinear multiphoton microscopy to monitor the fate of engrafted material in mice and in response to molecular intervention. As a currently clinically investigated scaffold design and material, different concepts have been explored to attenuate the FBR and fibrosis experimentally, including improving the biomimetic properties of the biomaterial, for example, by geometrical and/or biochemical surface modifications. Likewise, molecular interference has been explored, including: local immunosuppression by corticosteroid deposition; dampening leukocyte and fibroblast activation by anti-transforming growth factor-antibody or halofunginone; or stimulating vessel development to improve perfusion and performance of the bioactive implants by pro-angiogenic vascular endothelial growth factor (VEGF). These approaches have resulted in incomplete and often transient efficacy, with ongoing chronic inflammation, fibrosis and even graft failure as outcomes. As a potentially stringent approach, the depletion of phagocytes by liposomal clodronate bisphosphonate or genetic phagocytes by liposomal clodronate bisphosphonate or genetic

**Examination of the foreign body response to biomaterials by nonlinear intravital microscopy**

Eleonora Dondossola1*, Boris M. Holzapfel2,3, Stephanie Alexander1,†, Stefano Filippini1, Dietmar W. Hutmacher2,4 and Peter Friedl1,5,6*

Implanted biomaterials often fail because they elicit a foreign body response (FBR) and concomitant fibrotic encapsulation. To design clinically relevant interference approaches, it is crucial to first examine the FBR mechanisms. Here, we report the development and validation of infrared-excited nonlinear microscopy to resolve the three-dimensional (3D) organization and fate of 3D-electrospun scaffolds implanted deep into the skin of mice and the following step-wise FBR process. We observed that immigrating myeloid cells (predominantly macrophages of the M1 type) engaged and became immobilized along the scaffold/tissue interface, before forming multinucleated giant cells. Both macrophages and giant cells locally produced vascular endothelial growth factor (VEGF), which initiated and maintained an immature neovessel network, followed by the formation of a dense collagen capsule two- to four-weeks post-implantation. Elimination of the macrophage/giant-cell compartment, by clodronate and/or neutralization of VEGF by VEGF Trap, significantly diminished giant-cell accumulation, neovascularization and fibrosis. Our findings identify macrophages and giant cells as incendiaries of the fibrotic encapsulation of engrafted biomaterials via VEGF release and neovascularization, and therefore as targets for therapy.

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platform technology, we applied calcium phosphate-coated medical grade poly(ɛ-caprolactone) (mPCL-CaP) implants, which were fabricated by melt-electrospinning with a fully interconnected honeycomb pore network (average pore size 100 μm, 90% porosity, Fig. 1a)25–28. In previous studies, we observed that mPCL-CaP scaffolds combined with bone morphogenetic protein-7 (BMP-7) form bone after ectopic implantation in mice, whereas BMP-7 free scaffolds induce a physiological FBR29,30. Hence, we hypothesized that these scaffolds would be a suitable porous implant to explore the mechanisms of the FBR in four dimensions. To detect both mPCL-CaP fibres and deriving biological responses by multiphoton microscopy, infrared excitation and higher harmonic generation were used for deep tissue penetration, for improved detection of red fluorophores and minimized phototoxicity (Supplementary Fig. 1)31,32.

Scaffold fibres elicited strong second (SHG) and third harmonic generation (THG) signals, using excitation/emission wavelengths of 1,090/545 and 1,180/393 nm, respectively (Fig. 1b). SHG/THG z-scanning resolved both individual fibre geometry in three dimensions (Fig. 1b) and an additional weak THG signal from the calcium phosphate coating (Fig. 1b, arrowheads). Remarkably, SHG-/THG-positive substructures were present throughout the fibre core, with strong individual signal profiles and insignificant co-localization ($R^2=0.06$, $P<0.001$, Fig. 1c,d). Thus, SHG/THG signals resolve different structural PCL subdomains after three-dimensional (3D) printing, such as the crystalline phase of the melt electrospun fibres33.

**Figure 1 | Higher harmonic multiphoton microscopy of 3D-printed mPCL-CaP fibres in vitro.** a, Macroscopic overview of the melt-electrospun PCL scaffold by bright-field microscopy. Scale bar, 300 μm. b, 3D reconstruction of the mPCL-CaP scaffold by multiphoton-excited label-free SHG and THG detection (forward direction). Left, overview image; the inset on the right panel shows calcium deposits (arrowheads). Scale bars, 0.5 mm (left) and 200 μm (right). c, High-resolution SHG/THG projection of a single fibre in the horizontal (xy) and orthogonal (xz) directions, and signal intensity analysis in the x-direction. Lines denote calcium coating. Detailed representations in the bottom three panels show a PCL fibre as single and merged channels (SHG and THG). d, Co-localization analysis of SHG and THG signal intensity ($R^2=0.06$, $P>0.001$). e, Forward and backward direction detection of a PCL fibre shown as single channels (SHG and THG) and merged. Scale bar, 10 μm. f, Comparison of forward versus backward detection of orthogonal projections of (e). SHG and THG signal intensities decay in a single fibre in the z-direction.
Figure 2 | Generation and characterization of an in vivo model to study the FBR by longitudinal intravital imaging. a, Schematic representation of the model, showing a dorsal skin-fold chamber (DSFC) on the back of a C57BL/6 GFP mouse. A mPCL-CaP scaffold was implanted inside the DSFC within the subcutaneous tissue (x–y and x–z projections of the implant site). b, Longitudinal macroscopic overview of the FBR after scaffold implantation within the DSFC. Dotted lines denote the position of the scaffold. c, Histology of the FBR in response to the scaffold implantation within the DSFC at day 14. Green arrowhead, giant FBR cells; blue arrowhead, blood vessels; asterisk, scaffold fibre. Right panels show magnified images of boxes in the left panel. Scale bar, 100 μm. d, CD68 expression detected by immunohistochemistry on day 14 post-implantation and graphical representation of all mononucleated (m) and giant cells (g) as percentage of total CD68-positive cells. The number of mononucleated cells that stably associated with the fibres was negligible (four fields per mouse; three mice). Image area, 500 × 700 μm (x5 μm). e, IRF5 and CD163 expression detected by immunohistochemistry at day 14 post-implantation. Scale bar, 50 μm. f, g, Longitudinal intravital imaging of FBR at days 4, 7 and 14, performed for different areas of the same lesions, chosen randomly. f, Merged multiparameter images representing: THG (red); GFP-positive cells (cyan); 70 kDa dextran (magenta); and SHG (green). g, Single-channel representations (top panels) and image-based quantification of scaffold fibres (THG), GFP-positive infiltrate cells (white arrowheads), dextran-positive blood vessels (magenta arrowhead) and SHG, detecting PCL fibres (white asterisks) and fibrillar collagen (days 7 and 14; green asterisks). Scale bar, 50 μm. Bottom panel of (g) quantifies the signal-positive area (% of total image), obtained from four to eight independent fields per implant from four mice, for each channel (*P < 0.05; **P < 0.01; ***P < 0.001; one-way analysis of variance followed by Tukey’s HSD post hoc test).

To extend the applicability of nonlinear SHG/THG microscopy for other implant types used in clinical applications, we analysed a series of biomaterials next to mPCL-CaP, including polyester, polysulfone (PSU), and biodegradable ones, such as polyglycolic lactic acid (PGLA) and gelatin. All tested materials elicited a strong THG signal, which predominantly originated from the scaffold interface independently from their microarchitecture (Supplementary Fig. 2). Likewise, a SHG signal was emitted by most materials, except PSU (Supplementary Fig. 2). The results establish the THG signal, in particular, as a universal interface-detection strategy for robust analysis across implant types.

As a prerequisite for intravital imaging in animals, we tested whether the SHG/THG signals induced by scaffold fibres were sufficient for reliable detection in the backward direction, which is mandatory for thick tissue microscopy22 (Fig. 1e,f). Signal intensity was fourfold decreased when detected in the backward, as compared with the forward, direction, and an additional 50% of signal decayed per 25 μm penetration into the fibre for each detection mode (Fig. 1e,f). Despite this scatter-induced signal loss, 40-μm-thick fibres were completely detected in either configuration (Fig. 1e). This establishes the combined SHG/THG signal detection as a tool for the non-destructive analysis of scaffold fibre geometry and of substrutures of semicrystalline mPCL polymers.

**Intravital longitudinal imaging of the FBR**

To monitor both extracellular matrix and cellular kinetics during the FBR in vivo, a mPCL-CaP scaffold of 5 × 5 × 0.2 mm was implanted in parallel to the deep dermis/subcutis interface in GFP-C57BL/6 mice expressing GFP in all cells and in nude (nu/nu) mice, and analysed through an optical imaging window over time (Fig. 2a and Supplementary Fig. 3a). The scaffolds integrated without macroscopic tissue trauma, inflammation or bleeding (Fig. 2b), similarly to the integration of scaffolds in window-free mice23. Fourteen days after implantation, a histologically significant FBR response emerged, characterized by a cell-rich infiltrate and mononucleated giant cells decorating the surface of fibres, high density of neovessels and emerging fibrosis between and surrounding the scaffold fibres (Fig. 2c). The composition of the inflammatory infiltrate, which indeed may vary with material type and geometry19,25,34,35, was further delineated by flow cytometry; macrophages/giant cells represented the predominant cell type of the immune infiltrate (47.2%), followed by T-cells (20%), granulocytes (9.1%) and B-cells (4%)...
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Kinetic and mapping of infiltrating cells

Unlike their composition, the dynamics of FBR infiltrate cells remain unknown. We mapped infiltrate kinetics and the accumulation of foreign body giant cells along the scaffold surface and performed time-lapse recordings and single-cell tracking of infiltrating GFP-positive cells. Relative to the implant position, we identified two kinetic subpopulations that were recruited over time, including interstitially moving and fibre-attached resident cells (Fig. 3a–c and Supplementary Videos 1–3). A population of mononuclear interstitial cells retained mostly kinetic behaviours and moved along and between collagen fibrils, without directly contacting scaffold fibres (Fig. 3a,d; speed up to 3 μm min⁻¹). These were interstitial leukocytes, also detected by histology (Fig. 2c). Conversely, scaffold-attached cells were typically multinucleated, as detected by intravital injection of Hoechst dye (Fig. 3b, arrowhead), with high positional stability despite vigorous cytoplasmic dynamics (Fig. 3d and Supplementary Video 4). These were stably anchored resident giant cells, as predicted; they nonetheless retained slow dynamic behaviours, locally exploring the material surface by ruffling lamellipodia (Fig. 3b,d; average speed at day 14: 0.07 μm min⁻¹).

Neangiogenesis and fibrotic capsule development during FBR

Whether neovessels contribute to enhance or resolve the FBR remains unclear. Both immunodeficient and immunocompetent mice carrying mPCL-CaP material developed a dense neovascular network (Fig. 2f,g and Supplementary Fig. 3b,c), with

Figure 3 | Dynamics of infiltrate cells of the FBR monitored by intravital microscopy. a, b, Dynamics of GFP-positive infiltrate cells at day 7, monitored by time-lapse intravital microscopy and analysed by single-cell tracking. Sequential frames obtained at different time points from Supplementary Video 2, which was used to track cells and classify them as interstitial (a) or scaffold-associated (b). White arrowheads denote single-cell positioning over time. GFP-positive cells, green; SHG and Hoechst, red and grayscale (single channel). Scale bar, 20 μm. Lower panels denote the speed as heatmaps from ten representative cells. c, Mean ± s.d. of interstitial or mPCL-CaP fibre-associated infiltrate cells per imaging field over time. Area, 360 x 360 μm (x40 μm). *P < 0.05; **P < 0.01; n.s., non significant; analysed by unpaired two-tailed Student’s t-test. d, Frequency distributions of the average speed for interstitial and scaffold-associated cells over time. Three mice per time point and two fields per mouse were analysed. Data show one representative image sequence per time point from three independent experiments.

(Supplementary Fig. 4a). Giant cells and macrophages accumulated with a ratio of about 1:6 and retained monocyte differentiation marker CD68 (Fig. 2d) and M1 polarization (IRF-5, interferon regulatory factor 5), but lacked the M2 profile marker CD163 over time (up to seven weeks; Fig. 2e and Supplementary Fig. 4b). Thus, a macrophage-dominated pro-inflammatory M1-type response was induced by mPCL-CaP implants during early and late stages of the FBR, with no evidence for M2 polarization over time, in contrast with what occurs with other material types. When monitored longitudinally by intravital microscopy, the tissue surrounding the THG-positive mPCL-CaP scaffold became gradually infiltrated by GFP-positive cells (Fig. 2f,g), followed by sprouting of perfused neovessels and deposition of, initially loose and later bundled, fibrillar collagen (SHG) throughout the initially SHG-free wound bed (Fig. 2f,g and Supplementary Fig. 3b,c). The SHG signal intensity in the scaffold fibres was diminished in parallel to progressing collagen deposition, whereas the THG signal intensity remained largely unperturbed (Fig. 2g). This established the THG signal as the most robust detection mode in vivo, to monitor the positioning and integrity of the scaffold over time. Although the FBR kinetics in the GFP-C57BL/6 and nude mice were similar, neovascularization was enhanced in the nu/nu mice, consistent with angiogenic strength being dependent on the genetic background of mouse strains. Despite severe inflammatory tissue remodelling, mPCL-CaP fibres were neither resorbed nor infiltrated by cells (Supplementary Fig. 3d,e), confirming the documented stability of this material in vivo.

To demonstrate in vivo applicability of THG imaging for an independent material, PSU implants were efficiently monitored, including early recruitment of GFP-positive cells, robust...
Neovessel development and collagen deposition in the scaffold-elicited FBR monitored by intravital microscopy. a, Regular skin and scaffold-elicited vasculature. Scale bar, 100 μm. b, Quantification of neovessel organization, including: number of branches, interbranching distance and total vessel length. Mean ± s.d., three fields per mouse from three mice. **P < 0.01; ***P < 0.001 (unpaired two-tailed Student t-test). c, Frequency distribution of vessel-to-scaffold angle. Dashed line, boundary of PCL-CaP fibre. Data represent three fields per mouse from three mice. d, Collagen staining by Masson’s Trichrome on day 14. The red boxes indicate the location of magnified image(s) in panels to the right. Dotted line (left panel), region of implant. Asterisks, scaffold fibre. Scale bar, 1 mm (left panel). e, Collagen-wrapped mPLC-CaP fibres at indicated imaging depths (SHG, green; THG, red). Scale bar, 100 μm. f, Frequency distribution of the angle of collagen fibrils relative to the scaffold fibre orientation (three fields per mouse, three mice).

in parallel to neoangiogenesis, collagen deposition occurred in an incremental fashion (Fig. 2f,g), histologically forming a fibrillar capsule surrounding and penetrating the scaffold architecture by day 14 and thereafter (Fig. 4d). In a 3D reconstruction, two subsets of SHG-positive collagen fibres and bundles were detected, including fibres wrapping around (60–90° orientation) or aligning in parallel (0–15°) to scaffold fibres (Fig. 4e,f). This indicated fibrous scarring as a mechanically precise process, leading to functionally distinct subsets of collagen bundles. In aggregate, giant-cell development is accompanied by a desmoplasia-like process consisting of leukocyte influx, primordial vascularization, and progressive collagen deposition and encapsulation of the porous implant.

Therapeutic targeting of macrophages and neoangiogenesis

To test whether infiltrating macrophages and multinucleated giant cells are the cause or consequence of neovascularization and late-stage fibrosis, we depleted the macrophage lineage of scaffold-bearing mice with clodronate liposomes before graft implantation. After internalization, clodronate induces apoptosis of phagocytes but no other cells. Clodronate treatment stopped the recruitment of infiltrate cells and giant-cell development, as previously reported19,24, and further blocked scaffold-associated neovascularization and fibrosis (Fig. 5a,b and Supplementary Fig. 6a). To directly test whether neovessel development and late-stage scarring were linked and dependent on the presence of macrophages and giant cells, lesions containing the scaffold were stained for VEGF-A, the predominant proangiogenic cytokine in regenerating and cancerous tissue44 and an important cytokine produced by M1 macrophages8,9. VEGF-A was prominently expressed in mononucleated stromal cells and giant cells (Fig. 5c, left panel); after clodronate treatment both VEGF-positive cell subsets were absent, without compensatory up-regulation of VEGF-A in other tissue regions (Fig. 5c, right panel), and there was an absence of neoangiogenesis (Fig. 5a). This identified myeloid and giant cells as the main source for VEGF-A and as drivers of neovessel formation.

We next aimed to clarify whether neovascularization is an independent mechanism that enhances or counteracts the severity of the FBR and scarring. To minimize neovessel establishment...
directly, without targeting giant cells, we administered VEGF Trap to prevent VEGF availability\(^4^5\), using a dosing scheme effective for anti-angiogenic therapy in patients\(^4^6,4^7\). VEGF Trap stopped vessel formation and significantly diminished the numbers of giant cells and collagen deposition (Fig. 5a, b and Supplementary Fig. 6a). VEGF Trap monotherapy was slightly less efficient than clodronate treatment, but combining both agents resulted in near-complete blocking of giant cells and fibrosis development; although, this was not an improvement on the already profound effects observed by clodronate treatment alone (Fig. 5a, b).

When addressed longitudinally by intravital microscopy, clodronate-mediated cell depletion, effective by day 7, preceded the blocking of neovascularization and fibrous encapsulation of scaffold fibres by day 14 (Fig. 5d and Supplementary Fig. 6b).
This failure to mount both neovessels and fibrosis after macrophage/giant-cell targeting indicated that myeloid cells are non-redundant drivers of FBR-associated tissue remodelling. VEGF Trap inhibited tissue infiltration by GFP-positive cells after, but not before, day 7 (Fig. 5d), suggesting a two-step process with early-onset inflammation mediated by VEGF-independent pre-existing vessels, followed by secondary exacerbation via VEGF-dependent neovessels.

Cell accumulation near the implant was strongly diminished by clodronate and/or VEGF Trap (Supplementary Fig. 7a,b), but the positional stability of residual scaffold-associated cells and the mobility distribution of interstitial cells remained unperturbed, compared with untreated lesions (Supplementary Fig. 7c–e). These results indicated that both clodronate and VEGF targeting act by limiting cell entry into the tissue rather than mobility within the lesion.

To investigate the long-term efficacy of macrophage and neovessel depletion on the FBR and fibrotic encapsulation beyond the two-week observation period achievable using the window system, window-free mice bearing scaffolds were treated with clodronate liposomes and/or VEGF Trap and assessed by ex vivo 3D SHG/THG microscopy and histology on day 28 (Fig. 6a–c and Supplementary Fig. 6a, lower panels). Suppression of the FBR, including diminished inflammation, vascularization and fibrosis, was sustained by anti-angiogenesis therapy and, with stronger effects, by clodronate and combined clodronate and VEGF Trap treatment (Fig. 6b,c).

Lastly, we tested whether the therapeutic effects persisted after discontinuation of clodronate and/or VEGF Trap administration, by monitoring FBR giant cells, vessels and collagen deposition 14 and 28 days after the treatment was suspended (Fig. 6d). For all therapy settings, the FBR severity remained significantly diminished until at least four weeks post therapy withdrawal, including stable collagen levels and vessel numbers, and a decreased but partly relapsing density of giant cells (Fig. 6e–h). This indicated that dampening the initial inflammatory phase is crucial to reach a sustained anti-angiogenic and anti-fibrotic response.
Besides confirming macrophages and giant cells as central drivers of the FBR and late-stage fibrosis, including implanted prosthesis, valves, pacemakers, catheters and defibrillators.

Depleting myeloid cells and neovascularization using targeted (combination) therapy, may produce a largely avascular and non-inflamed integration site, which may be critical towards achieving a scar-free long-term integration. Given their proven safety profile in clinical application, where they are generally tolerated well with no long-term adverse effects on the immune system, individual or combined administration of bisphosphonates and anti-angiogenesis regimens represent promising systemic, or local, topical strategies to improve long-term integration and functionality of material-based grafts.

Methods
Scaffold design and fabrication. mPCL-CaP scaffolds were prepared by melt electrospinning (MEW) to 3D porous mPCL-CaP scaffolds and the related cell and tissue dynamics. We identified the connection between giant cells and VEGF-induced neovessels as the central pathogenic axis driving the FBR and late-stage fibrosis. In a reciprocal process, implant-associated myeloid cells, including giant cells, locally release VEGF-A, which is required to induce neovessels that, in turn, maintain additional cell recruitment and enhance myeloid inflammation and local encapsulation of the graft site by collagen fibres. Continuous arrows represent migratory events or release; dashed arrows represent induction of a phenomenon; and 1 and 2 highlight steps of interference with macrophage recruitment and VEGF functions, respectively.

Discussion
In summary, by implementing nonlinear intravital microscopy to monitor 3D porous mPCL-CaP scaffolds and the related cell and tissue dynamics, we identified the connection between giant cells and VEGF-induced neovessels as the central pathogenic axis driving the FBR and late-stage fibrosis. In a reciprocal process, implant-associated myeloid cells, including giant cells, locally release VEGF-A, which is required to induce neovessels that, in turn, maintain additional cell recruitment and enhance myeloid inflammation and local encapsulation of the graft site by collagen fibres (Fig. 7). The results suggest that an immature neovessel network aggravates the FBR, similar to the way that tumour-associated neovessels support inflammation, metabolic deregulation and desmoplastic remodelling of the tumour stroma. Because macrophage recruitment, giant-cell development and emerging neovascularization cooperate in series to entertain the fibrosis response, therapeutic interference with either process is suited to limit tissue remodelling and scar formation.

A rich set of cytokines is released during a FBR, including IL-1β, IL-4, IL-6, IL-8, IL-10, IL-13, MCP-1, TGF-β and VEGF. Depleting myeloid cells and neovascularization using targeted (combination) therapy, may produce a largely avascular and non-inflamed integration site, which may be critical towards achieving a scar-free long-term integration. Given their proven safety profile in clinical application, where they are generally tolerated well with no long-term adverse effects on the immune system, individual or combined administration of bisphosphonates and anti-angiogenesis regimens represent promising systemic, or local, topical strategies to improve long-term integration and functionality of material-based grafts.
of the total area. Four to eight independent fields were averaged per implant from at least four mice per condition. The experiment was repeated three times.

For topography-controlled analysis of fibrillar collagen density in association with scaffold fibres, the SHG intensity of 40 × 40 μm regions were quantified as an average from two adjacent slices preceding the appearance of the PCL fibre, as identified by the THG signal (three to five PCL fibres per sample; three mice per group). Each region was masked, thresholded (Li algorithm) and quantified for the signal-positive area fraction. Single cell velocities from time-lapse sequences were obtained by computer-assisted cell tracking (Autocell). For giant cell counts, any cell displaying ≥2 nuclei was counted as multinucleated. The mean number of nuclei per giant cell was 5.95 ± 2, quantified from 60 random giant cells; only a minority of giant cells retained only two nuclei.

**Therapeutic intervention.** Mice bearing mPCL-Cap scaffold in the subcutaneous tissue received VEGF Trap (500 ng per week, once per week) starting four days after scaffold implantation. VEGF Trap is a recombinant fusion protein with VEGF-A neutralizing activity combining the binding portions of VEGF receptors 1 and 2, with the Fc region of the human IgG1. Clodronate liposomes (200 μl per mouse) were administered every two to three days, starting three days before scaffold implantation to deplete macrophages by the day of implantation. For combination therapy, both regimens were combined. For intravital microscopy, three independent fields were averaged per implant, with three mice per group. The experiment was repeated twice.

**Histological analysis.** Mice were euthanized 14, 28, 35 or 49 days after implantation of the scaffold. Scaffold-bearing skin was excised, fixed (4% buffered paraformaldehyde) and embedded in paraffin for hematoxylin and eosin (H & E) or Masson’s Trichrome staining (five sections per sample, 5 μm thick, three samples per treatment). The experiment was repeated twice.

**Flow cytometry.** Infiltrate cells associated with the FBR in vivo were isolated and phenotyped by flow cytometry (LSRII FACS, Becton-Dickinson; Diva). To reach sufficient cell numbers, 20 scaffolds were recovered 14 days post implantation, mechanically disaggregated to generate a single-cell suspension and pooled. Phenotyping was performed using the following rat monoclonal antibodies (BD Pharmingen): PE-Cy 7 anti-CD45; PerCP-Cy 5.5 anti-CD11b; and APC-Cy7 conjugated anti-F4/80 and anti-CD86. Subset gating and analysis were performed using FlowJo10.2.

**Data availability.** All the relevant data supporting the findings of this study are available within the paper, its Supplementary Information and from the corresponding authors upon reasonable request.

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E.D., B.M.H., S.A., D.W.H. and P.F. designed the research. E.D., B.M.H., S.A., S.F. and D.W.H. performed the research. E.D., S.F., D.W.H. and P.F. analysed the data. E.D., B.M.H., D.W.H. and P.F. wrote the paper.

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Competing interests
The authors declare no competing financial interests.