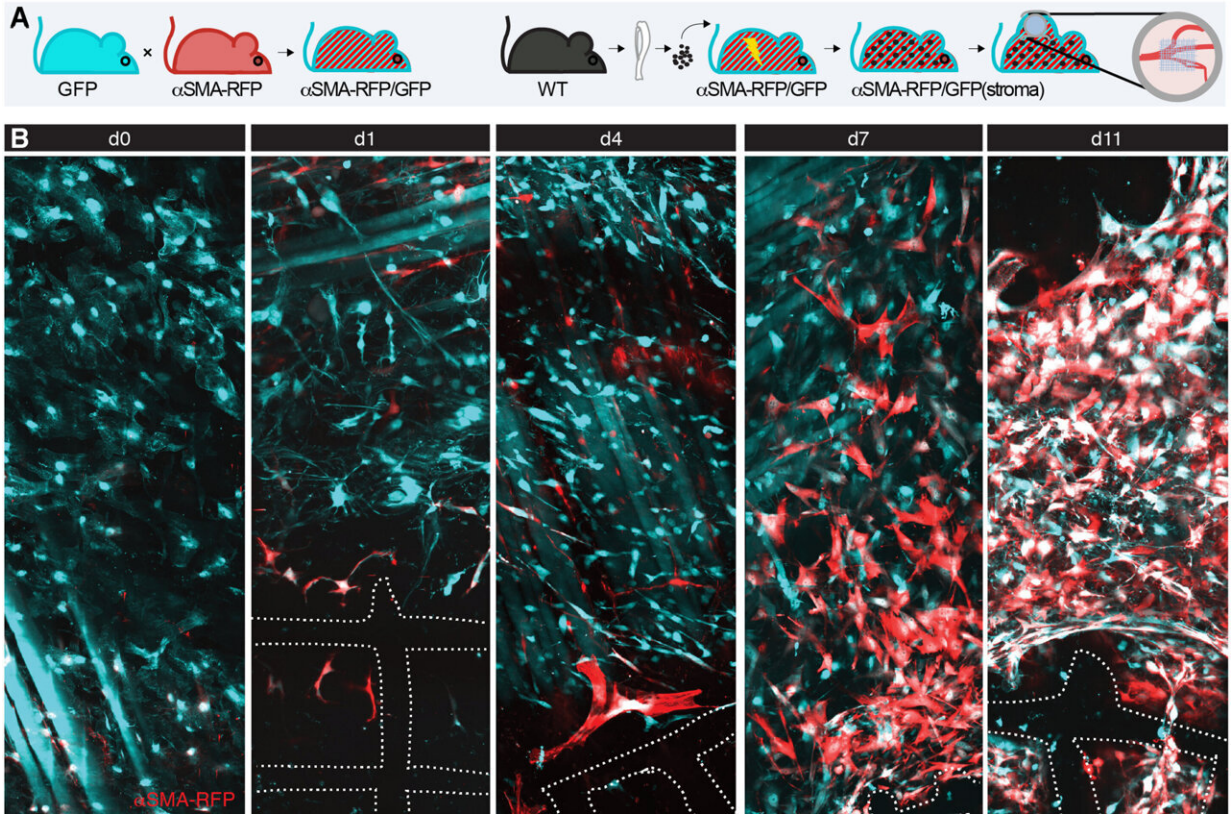
Understanding the alpha smooth muscle actin–driven foreign body response during wound healing

January 16 2023, by Thamarasee Jeewandara



Monitoring fibroblast activation and dynamics upon material implantation by longitudinal iMPM. (A) Schematic representation of the model. An αSMA-RFP/GFP model is generated by breeding followed by bone marrow transplant, resulting in the αSMA-RFP/GFP(stroma) reporter mouse, which is implanted with a dorsal skinfold chamber (DSFC) and a PCL scaffold. WT, wild type. (B) Longitudinal intravital imaging of the fibroblast recruitment on the day of the

scaffold implantation and after 1, 4, 7, and 11 days. Dotted lines, scaffold position; GFP+ cells, cyan; αSMA-RFP+ cells, red. Scale bar, 50 μm. (C) Morphology of representative quiescent and activated fibroblast over time (days 0, 1, 4, 7, and 11 after the scaffold implantation). GFP+ cells, cyan; αSMA-RFP+ cells, red. Scale bar, 50 μm. (D) Average number of fibroblasts counted over time (360 μm by 360 μm by 50 μm, three mice per time point). (E) Percentage of αSMA− (cyan) and αSMA+ (red) fibroblasts over time (360 μm by 360 μm by 50 μm, three mice per time point). (F) Cell size over time with frequency distribution over time; n = 3 mice, 50 cells per time point. (G) Sequential frames obtained at different time points of representative αSMA− cell before scaffold implantation, an αSMA− cell and an αSMA+ cell 4 days after the scaffold implantation. The dynamics of αSMA− and αSMA+ cells at different time points were monitored by time-lapse intravital microscopy and analyzed by single-cell tracking. Cell speed over time is shown, n = 25 to 40 cells in three mice per time point. Scale bar, 10 μm. (H) Heatmaps of the speed from seven representative cells per time points are shown. *P Science Advances, DOI: 10.1126/sciadv.add0014

The foreign body response is a clinically relevant process that can lead to issues with biocompatibility in implanted medical devices due to fibrosis. While the inflammatory nature of the foreign body response is already established, bioengineers still seek to understand underlying fibroblast-dependent mechanisms during wound healing.

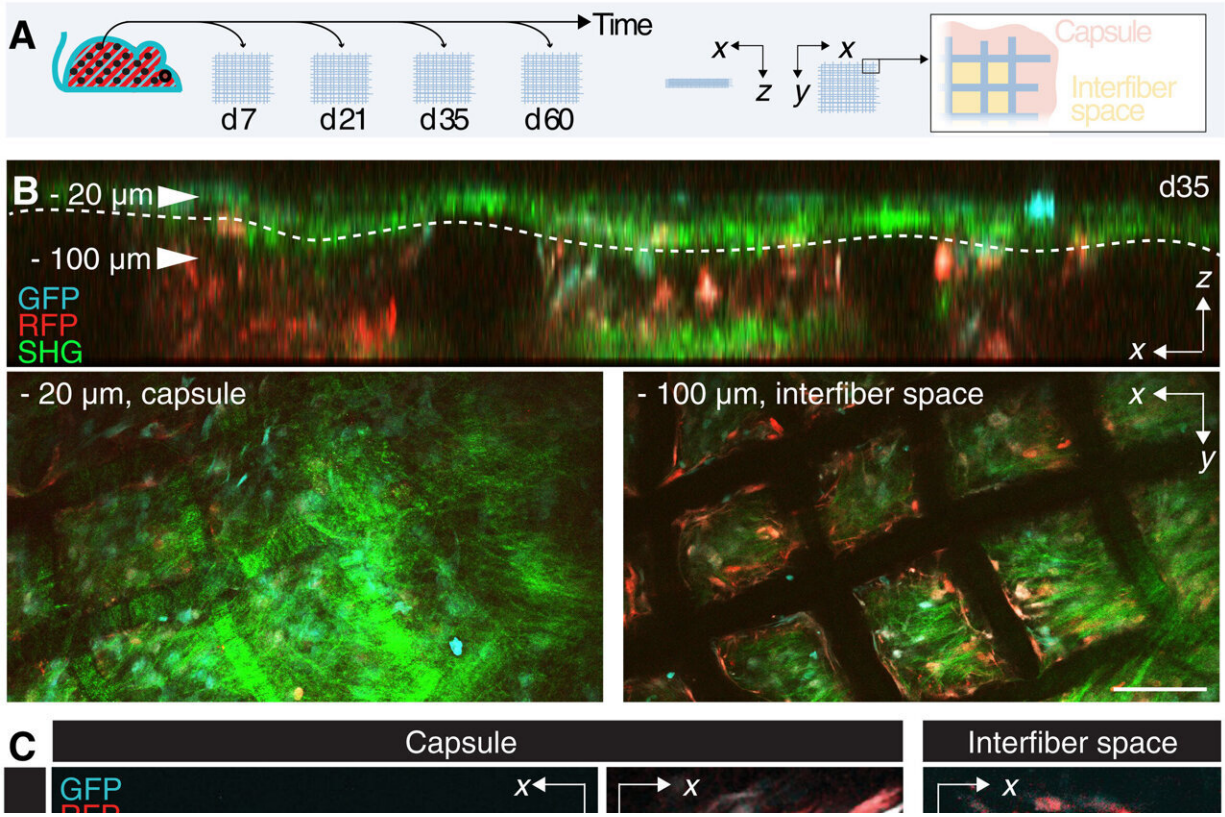
In a new report now published in *Science Advances*, Maria Parlani and a team of scientists in oncology and bioengineering in the U.S., and Netherlands, combined multiphoton microscopy with animal models expressing a modified smooth muscle alpha actin (αSMA) protein to investigate the dynamics of fibroblasts relative to their activation and fibrotic encapsulation of polymer materials.

During the experiments, they noted the invasion of fibroblasts as individual cells that developed a multicellular network with a two-

component fibrotic response to display an external cold capsule of smooth muscle cells and a relatively hotter and long-lasting inner alpha smooth muscle actin environment. Parlani and colleagues noted how the recruitment of fibroblasts and the extent of fibrosis inhibited after macrophage depletion. These outcomes implied the co-existence of macrophage-dependent and macrophage-independent mediators. The results highlighted the foreign body response as a conserved and self-organizing process that is partially independent of macrophages; specialized cells involved in enabling an immune response in vivo.

The foreign body response during the process of fibrosis

The foreign body response is an ultimate outcome of inflammation and wound healing after biomaterial implantation in a biological environment. This pathophysiological process has increasingly received clinical attention due to its role in inflammation and fibrotic encapsulation of patients' medical implants that can compromise the long-term integration and functional viability of biological implants. The step-wise process of the foreign body response begins with vascular damage and plasma protein engagement on an implant surface, followed by neutrophilic inflammation, monocyte recruitment that leads to macrophage activation as well as the formation of foreign body giant cells.



Longitudinal analysis of fibroblast activation. (A) Schematic representation of the model; PCL scaffolds were implanted subcutaneously in the back of αSMA-RFP/GFP(stroma) reporter mice (n = 4 per group); explanted at days 7, 21, 35, and 60; and analyzed by ex vivo MPM. (B) Orthogonal view and details of the capsule and interfiber space. Scale bar, 100 µm. (C) Representative images of the fibroblast activation in the external capsule (overviews shown as xy and xz sections) and inside the scaffold pores (XY sections, interfiber space), at days 7, 21, 35, and 60. Dot plots (ratio between the area covered by GFP and RFP signal) in the capsule and the interfiber space are shown over time. GFP+ cells, cyan; αSMA-RFP+ cells, red. Scale bar, 50 µm. (D) Details of blood vessels covered with RFP+ pericytes. Scale bar, 50 µm. GFP+ cells, cyan; αSMA-RFP+ cells, red; collagen, SHG, green. ***P Science Advances, DOI: 10.1126/sciadv.add0014

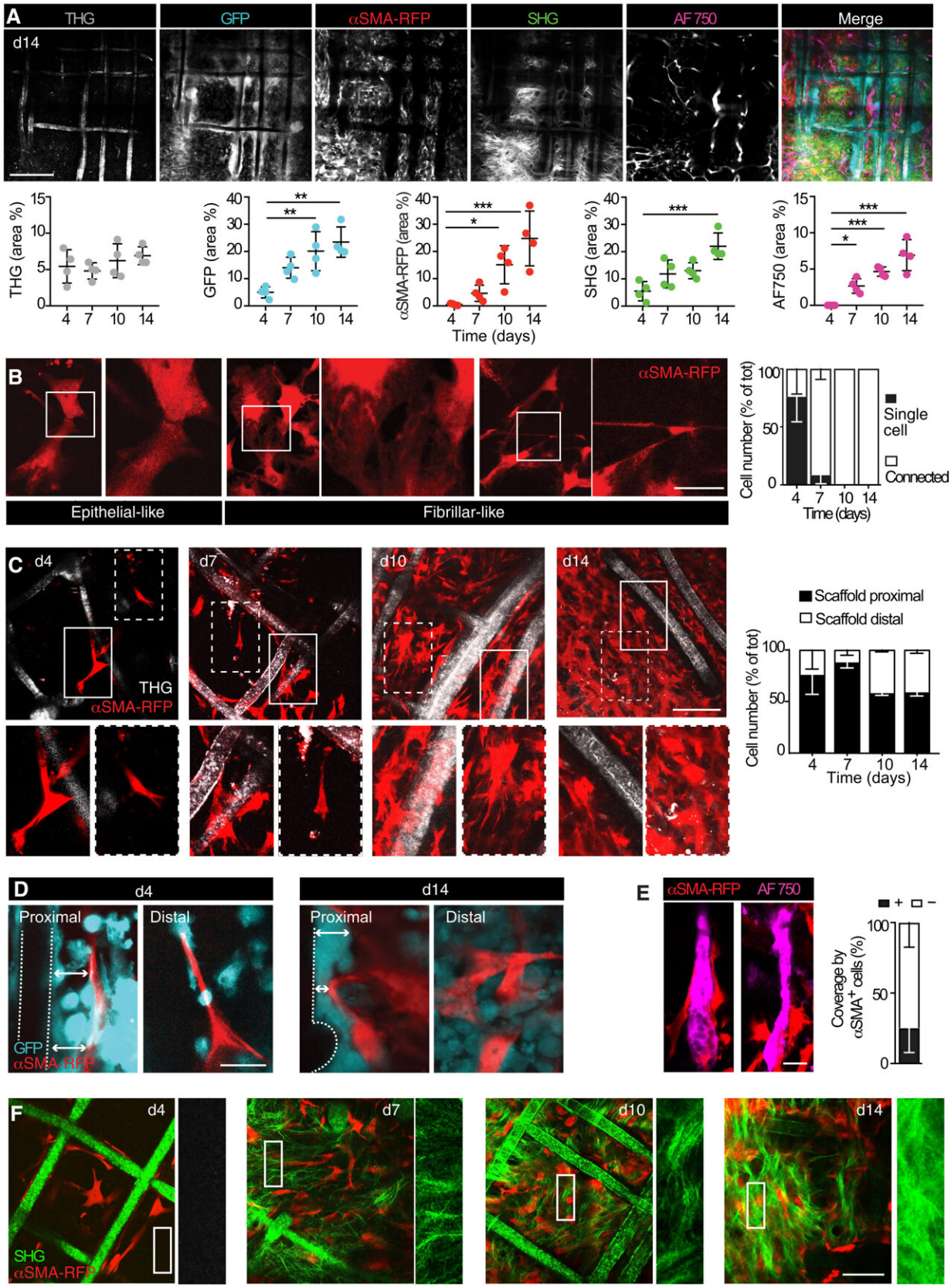
Interstitial fibroblasts are central effectors of tissue homeostasis and are

central to tissue remodeling and wound healing. The progression of wound healing is a transient fibrotic process in which myofibroblasts undergo elimination via apoptosis. The principles governing fibroblast engagement during the foreign body response have yet to be systematically investigated. The researchers therefore sought to understand the impact of macrophages and materials composition on fibroblast activation and on the outcome of fibrosis.

Fibroblast activation after biomaterial implantation

The research team monitored the engagement and fate of fibroblasts during the foreign body response within the deep dermis in a mouse model implanted with a fibrous polymer scaffold. They noted how deeper skin layers of the animal model contained adipocytes, nerves and muscle fibers. The team observed fibroblast activation and alpha smooth muscle actin upregulation within 24-hours of implanting the biomaterial.

The scientists then identified the impact of alpha smooth muscle actin upregulation in mice implanted with scaffolds at diverse timepoints. They noted two regions of interest: a fibrotic capsule containing fibroblasts and bundled collagen connected to the surrounding interstitial tissue, and an inner core with cells and collagen molecules filling up the space between implants. These outcomes highlighted the foreign body response as a two-compartment process.


A
THG
GFP
αSMA-RFP
SHG
AF750
Merge
d14
THG (area %)
GFP (area %)
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SHG (area %)
AF750 (area %)
Time (days)
B
αSMA-RFP
Epithelial-like
Fibrillar-like
Cell number (% of tot)
Single cell
Connected
Time (days)
C
d4
d7
d10
d14
THG
αSMA-RFP
Scaffold proximal
Scaffold distal
Cell number (% of tot)
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Coverage by αSMA+ cells (%)
F
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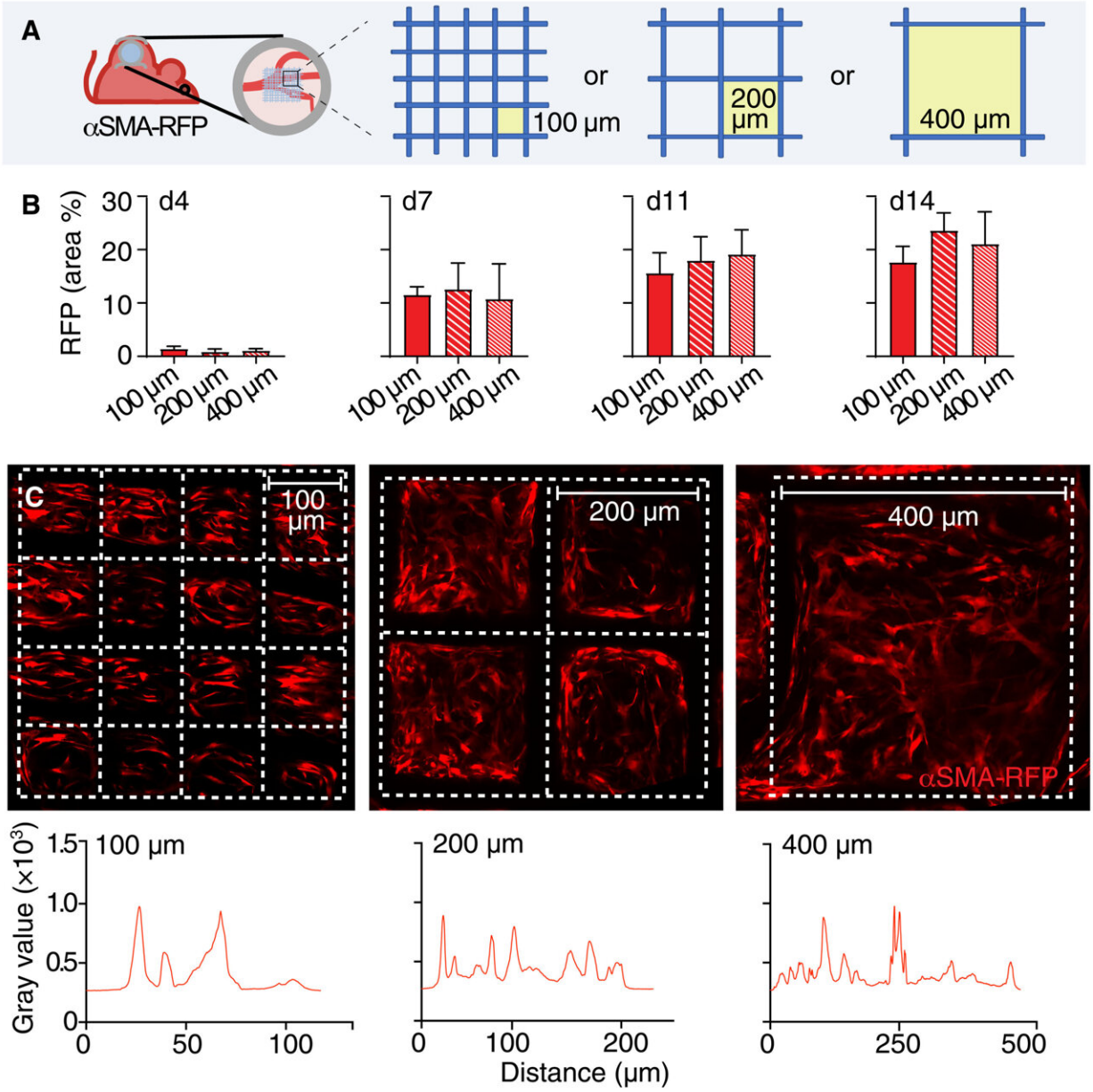
Longitudinal iMPM imaging of αSMA+ fibroblast interaction with partner elements of the FBR. (A) FBR at day 14 after implantation, single channels, and merge. Scale bar, 100 μm. A quantification is shown; AF750, Alexa Fluor 750 ; means ± SD, n = 4 mice per implant; four independent fields per implant were averaged. *P Science Advances, DOI: 10.1126/sciadv.add0014

Alpha smooth muscle actin (αSMA) fibroblast self-organization and interaction with the implant

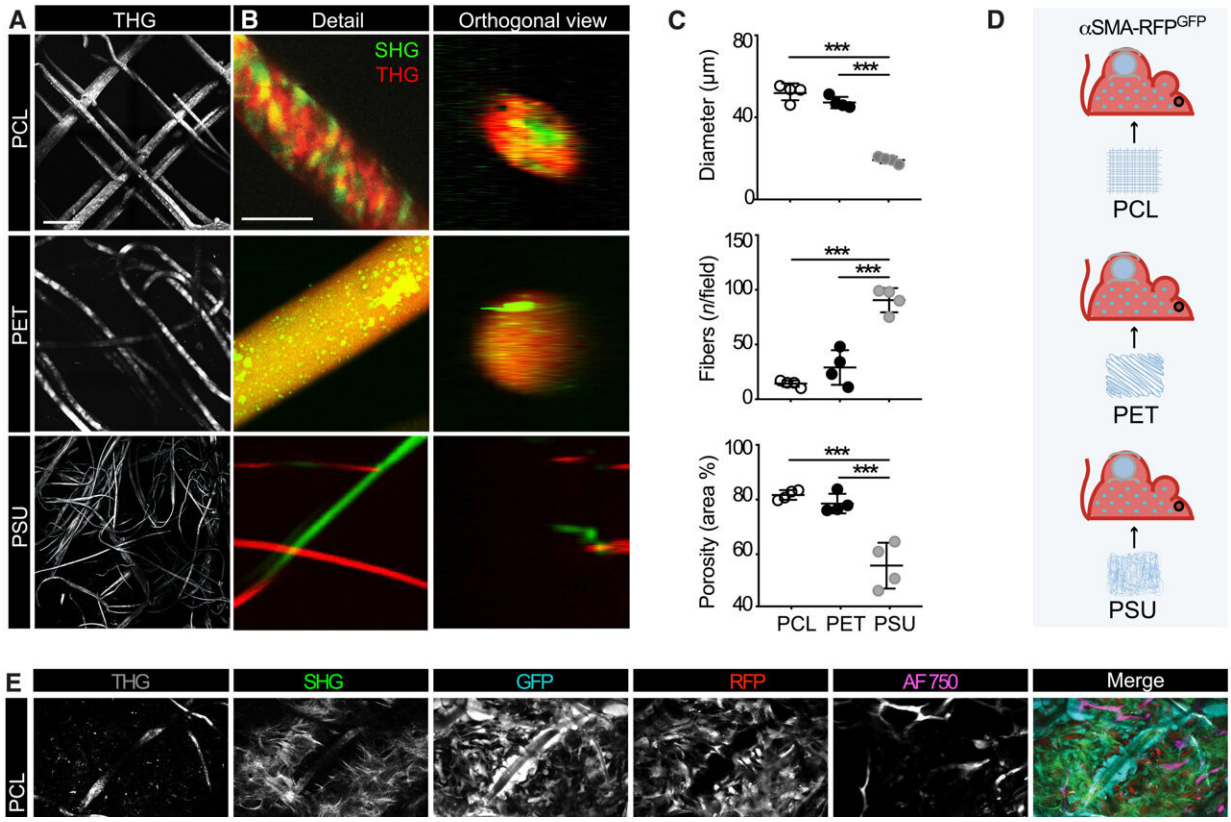
Upon biomaterial implantation, the team gained insights to the organization of alpha smooth muscle actin cells during biomaterial encapsulation by using nonlinear intravital multiphoton microscopy. The αSMA fibroblasts first populated the implant site without directly engaging with the material to establish a multicellular network. This included the process of collagen secretion. The researchers then identified the presence of sub-components that enriched the specialized foreign body giant cells to affect the recruitment process and the positioning of alpha smooth muscle actin after scaffold implantation.



Analysis of αSMA-RFP+ cell recruitment by scaffolds of different grid sizes. (A) Schematic representation of the model; scaffolds of three different grid sizes (pore sizes of 100 × 100, 200 × 200, and 400 × 400 µm) were implanted inside the DSFC. (B) Total amount of αSMA-RFP+ cells recruited measured as % area occupied, over time (days 4, 7, 11, and 14). (C) Overviews of the fibroblast distribution in the three different scaffold types. Dotted lines, scaffold position. XY intensity profile along single pores for each of the three scaffold geometries is shown. Credit: *Science Advances*, DOI: 10.1126/sciadv.add0014

The team noted how the implantation of a polymer scaffold induced recruitment, activation, and redistribution of the αSMA cells. Additionally, since the macrophages and foreign body giant cells are two key regulators of the foreign body response during fibrotic encapsulation of an implant, the researchers reduced the macrophage lineage in scaffold-implanted mice.

They accomplished this by using a chemical that caused apoptosis to ablate macrophages and the giant cells and thereby markedly reduce collagen deposition and fibrotic encapsulation around the implant. While these experiments reduced the recruitment of infiltrating immune cells and decreased the αSMA cell count, they did not impair their activation.



Longitudinal intravital imaging of the FBR in the three different biomaterials. (A) 3D reconstruction of PCL, PSU, and PET scaffolds by THG, macroscopic

overview; scale bar, 100 μm. (B) High-resolution SHG and THG projection of a single fiber of each scaffold in the horizontal (xy) and orthogonal (xz) directions. Scale bar, 50 μm. (C) Quantification of fibers diameter, number of fibers per field, and porosity of each scaffold (image size, 360 × 360 μm) are shown, means ± SD.***P Science Advances, DOI: 10.1126/sciadv.add0014

The engagement of αSMA in response to the material composition

The researchers noted how the properties of biomaterials, including their composition, charge and porosity regulated the severity of the foreign body response. The scientists modulated the material type and geometry of the implant to affect the process of recruitment and self-organization of the αSMA cells. They examined this using two different, clinically relevant polymer biomaterials. Based on nonlinear intravital multiphoton microscopy, the team observed the gradual infiltration of the fluorescently labeled smooth muscle alpha actin cells, followed by the deposition of collagen and the growth of neovessels.

Their work further sought to establish if the materials promoted the polarization of macrophages to types M1 and M2, which inhibit cell proliferation during tissue damage while promoting cell proliferation during wound healing, respectively.

Outlook

In this way, Maria Parlani and colleagues noted how fibroblasts are known effectors of fibrosis during the foreign body response, and formed a step-wise response during wound healing. Nevertheless, their process of recruitment and activation has remained relatively unknown.

Using the nonlinear intravital multiphoton microscopy technique, the

scientists closed a knowledge gap to dissect the contributions of fibroblasts to promote the foreign body response, and identify the step-wise organization and activation of a biologically conserved process. The work outlines the alpha smooth muscle actin expressing fibroblasts to be a persistent element of the foreign body response. The recruitment activation and self-organization of cells around the porous implant material resulted in a biologically conserved two-part process.

More information: Maria Parlani et al, Dissecting the recruitment and self-organization of αSMA-positive fibroblasts in the foreign body response, *Science Advances* (2022). DOI: 10.1126/sciadv.add0014

Arturo J Vegas et al, Combinatorial hydrogel library enables identification of materials that mitigate the foreign body response in primates, *Nature Biotechnology* (2016). DOI: 10.1038/nbt.3462

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