10. Supplementary Information

Supplementary Document S1: This document describes the synthesis method for PIC-$(N_3)_{0.01}$ and PIC-$(DTPA)_{0.01}$ in more detail.

Syntheses and characterization.

Materials. Toluene was distilled over sodium under a nitrogen atmosphere prior to use. The isocyanide monomer 2-(2-(2-methoxyethoxy)ethoxy)ethyl ((*D*)-2-isocyanopropanoyl)-*L*-alaninate **1** (Chiralix b.v., Nijmegen, the Netherlands) was purified by dry column vacuum chromatography with a 0-100% ethyl acetate in heptane gradient as eluent and silica gel (J.T. Baker, Avantor™, Arnhem, the Netherlands) as the stationary phase. The azide monomer 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethyl ((*D*)-2-isocyanopropanoyl)-*L*-alaninate **2** was synthesized according to a literature procedure.[1] All other chemicals were used as received. *S*-2-(4-Aminobenzyl)-diethylenetriamine pentaacetic acid (**aniline-DTPA**) was purchased from Macrocyclics™ (Plano, TX). **DBCO-NHS** was purchased from Click Chemistry Tools LLC. (Scottsdale, AZ). Rheology and viscometry were performed using previously published methods and protocols (see supplementary Figure S1a).[2] Photographs of the PIC-(N_3) and PIC(DTPA) are shown in figure S1b.

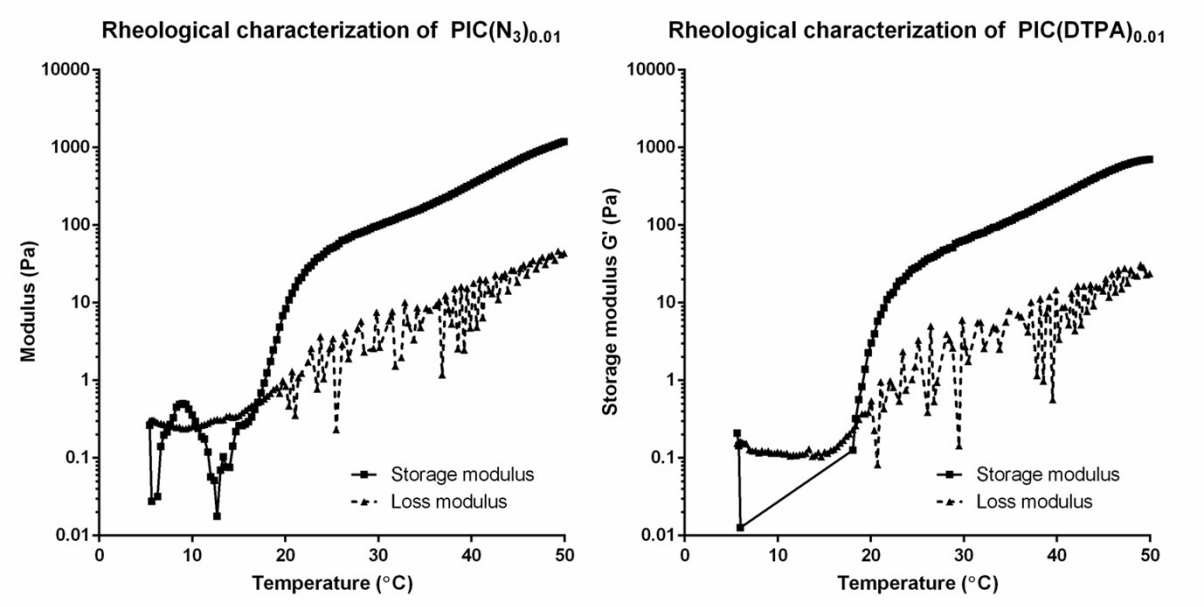


Figure S1a: Rheological characterization of PIC-$(N_3)_{0.01}$ (left) and PIC-$(DTPA)_{0.01}$ (right) displaying a gelation temperature of ~17 and ~18°C, respectively.

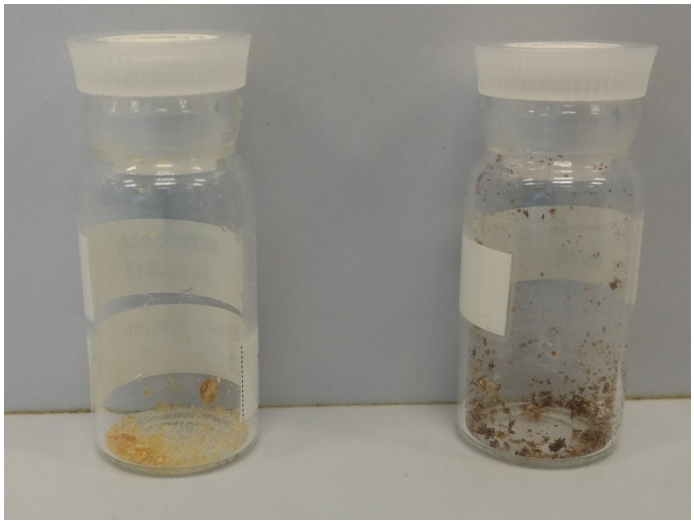


Figure S1b: Photograph showing azide-functionalized PIC (PIC-(N_3)) (left) and DTPA-functionalized PIC (PIC-(DTPA)) (right). The colour change from a yellowish-brown to dark brown is very apparent.

Synthesis of PIC-$(N_3)_{0.01}$. For the polymerization, a modified literature procedure[1] was followed. To a stirred solution of purified monomer **1** (200 mg) and a solution of azide monomer **2** (0.474 mL, 5 mg/mL in toluene, 0.01 eq.) in freshly distilled toluene (3.5 mL) was added $Ni(ClO_4)_2 \cdot 6H_2O$ (Sigma-Aldrich, 79.8 µL of a 36.6 mg solution in toluene/ethanol 80/20, $1.25 \cdot 10^{-4}$ eq.) The reaction mixture was stirred overnight at room temperature for 16 hrs. Subsequently, toluene (2.0 mL) was added and the reaction mixture was stirred for an additional 24 hrs. Then, the reaction mixture was precipitated in diisopropyl ether (40 mL, Acros Organics, Thermo Fisher Scientific, Geel, Belgium) and centrifuged for 5 min at 4000 rpm. The precipitate was dissolved in dichloromethane (Fisher, 4 mL) and subsequently precipitated in diisopropyl ether (40 mL) and centrifuged for 5 min at 4000 rpm. The last step was repeated once more. After drying on air for 20 h, the product was obtained as a yellow glassy solid (178 mg, 88%). Viscometry measurements in acetonitrile (J.T. Baker) gave a molecular weight of 634 kg/mol.

Synthesis of DBCO-DTPA. A solution of **aniline-DTPA** (Macrocyclics, 5 mg) in DMSO-d_6 (Sigma-Aldrich, Zwijndrecht, the Netherlands, 1.0 mL) and **DBCO-NHS** (Click Chemistry Tools, 5 mg/mL in DMSO-d_6, 0.99 mL, 1.0 eq.) was stirred at room temperature for 3 h. Complete conversion was confirmed by ^{1}H-NMR (Bruker, 500MHz, Fig. S1c), based on the shift of the aminobenzyl resonances of the DTPA moiety. The reaction mixture was used in the next step without further purification.

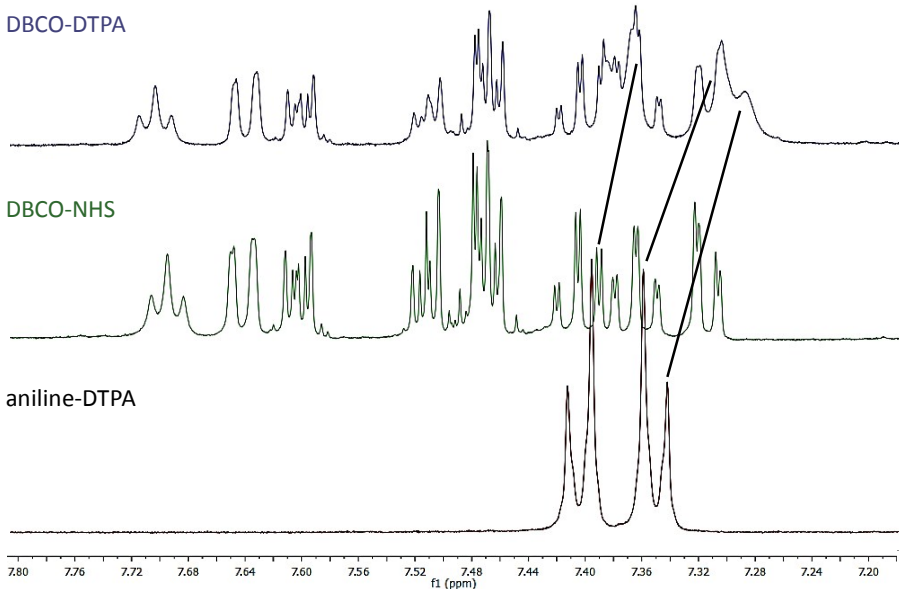


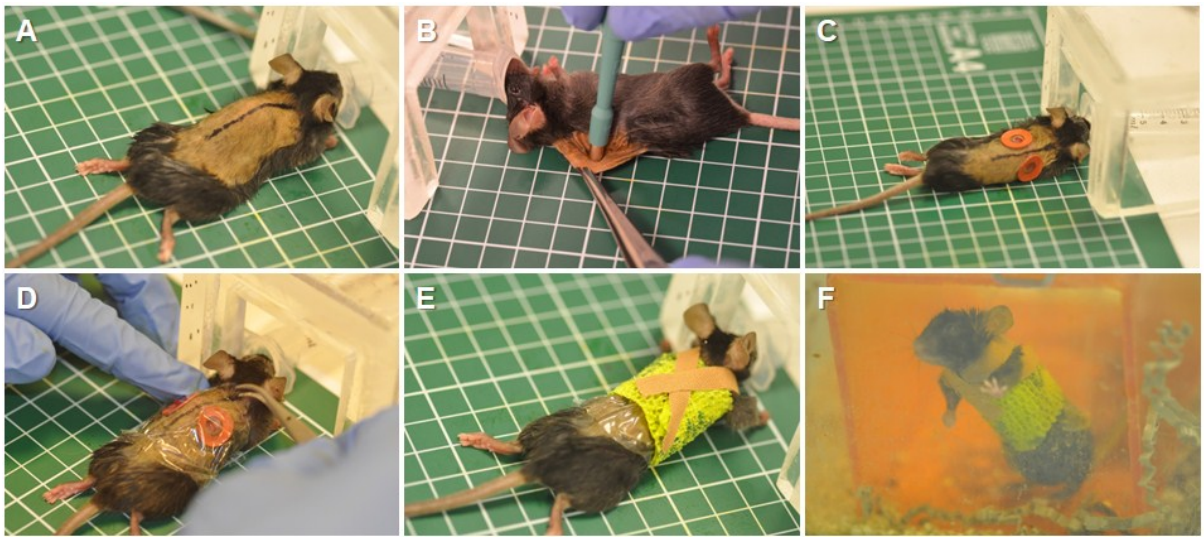
Figure S1c. Zoom of the aromatic part of the ^{1}H NMR spectra of **aniline-DTPA** (bottom), **DBCO-NHS** (middle) and the product **DBCO-DTPA** (top).

Synthesis of PIC-(DTPA)$_{0.01}$. For the highly efficient strain-promoted azide-alkyn coupling (SPAAC) between the PIC-azide and a DBCO-containing molecule, a literature protocol was used.[3] To a stirred solution of **PIC-(N$_3$)$_{0.01}$** (150 mg) in acetonitrile (J.T. Baker, 60 mL), **DBCO-DTPA** (1 eq. with respect to the azide concentration, 1.23 mL of the crude reaction mixture in DMSO-d_6) was added and the reaction mixture was stirred overnight at room temperature. The mixture was then precipitated in diisopropyl ether (600 mL) and the precipitate was filtered off. The solid was air dried overnight to obtain the DTPA-functionalised PIC as a yellow-brown glassy solid (100 mg, 67%). Conversion was not determined, but nearly quantitative for this protocol.[3]

Labelling with indium-111. PIC-(DTPA)$_{0.01}$ (2.4 mg) was dissolved in metal-free 2-(N-morpholino)ethanesulfonic acid (MES, Sigma Aldrich) buffer (369 µl, 0.5 M, pH adjusted to 5.0 using HCl (0.3 to 3.0 M)). From this PIC solution (6.5 mg ml^{-1}) 185 µL was taken and cooled on ice and 55 µl of $^{111}InCl_3$ in HCl (0.814 MBq µl-1, Mallinckrodt, Petten, The Netherlands) was added to it. The labelling with indium-111 occurred in a lead pig for radiation shielding at 4°C during 3 hours. Ethylenediaminetetra-acetic acid (EDTA, 30 µl, 50 mM, Sigma Aldrich) was added post-insertion to complex the unincorporated ^{111}In. The final PIC-(DTPA)$_{0.01}$ solution was diluted further to 4 mg ml^{-1} using 30 µl PBS (0.25 M, Sigma Aldrich).

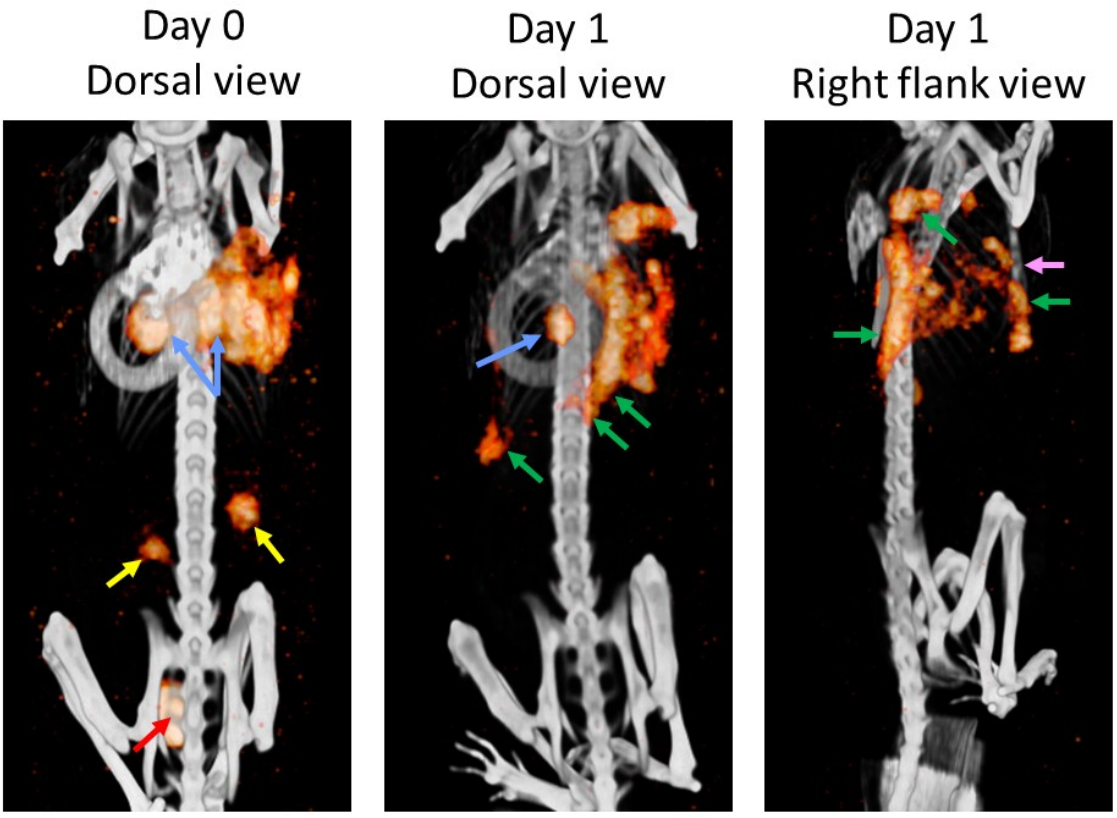
References

1. Mandal S, Eksteen-Akeroyd ZH, Jacobs MJ, Hammink R, Koepf M, Lambeck AJA, et al. Therapeutic nanoworms: towards novel synthetic dendritic cells for immunotherapy. Chem Sci. 2013;4(11):4168-74.
2. Jaspers M, Dennison M, Mabesoone MFJ, MacKintosh FC, Rowan AE, Kouwer PHJ. Ultra-responsive soft matter from strain-stiffening hydrogels. Nat Commun. 2014;5:5808.
3. Das RK, Gocheva V, Hammink R, Zouani OF, Rowan AE. Stress-stiffening-mediated stem-cell commitment switch in soft responsive hydrogels. Nat Mater. 2016;15(3):318-25.

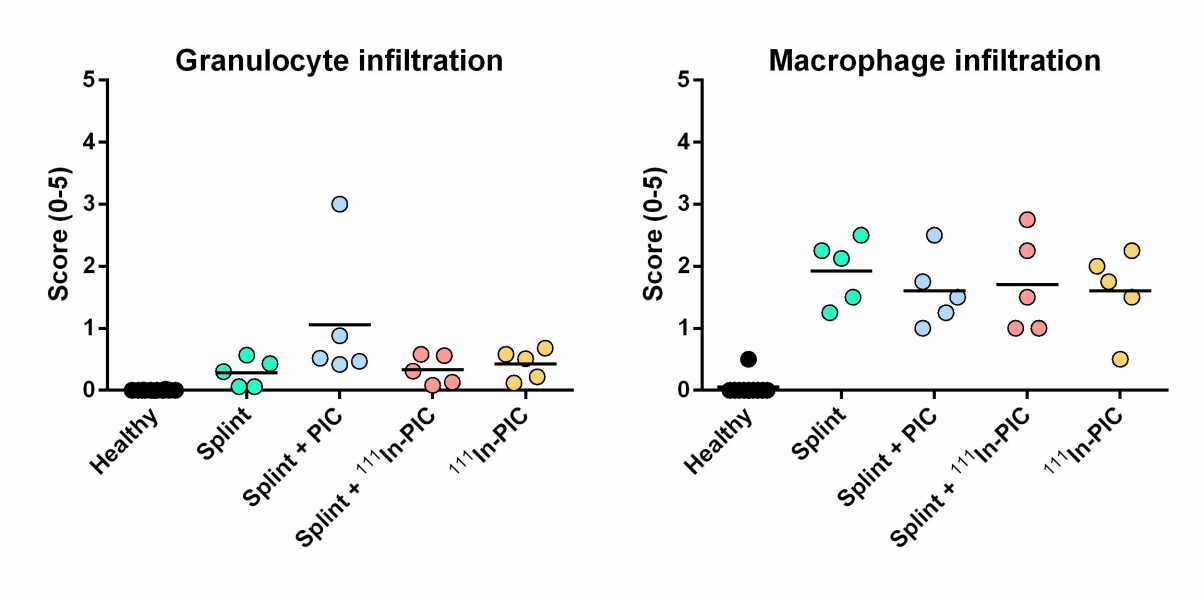


Supplementary Figure S2: Overview of the surgical procedure and wound dressing method. (A) The mouse is anesthetized and shaven. (B) The dorsal skin is stretched and two full-thickness wounds are created by a single press with a biopsy punch through the folded skin. (C) Silicone ring splints are glued to the skin surrounding the wounds. (D) A layer of Tegaderm™ is applied and secured tightly to the skin and splint, but not the wound. (E) The mouse's mid-section is wrapped in a single layer of Petflex® bandage, which is secured in place with leucoplast tape. (F) At 7 days post-surgery, the secondary dressings are still in place and the mouse has a decent level of mobility.

Supplementary Videos S3: These videos available in the online version of this article display a 3D rotation of the SPECT/CT scans of two mice, one from group A (both wounds splinted, left with PIC and right with ^{111}In-PIC) and one from the group B (left wound untreated with splint, right non-splinted with ^{111}In-PIC).



Supplemental Figure S4: An example of a mouse from group B (left wound without gel but with splint and right wound with ^{111}In-PIC gel but no splint) in which gel can be observed to relocate both below and over the skin. Arrows illustrate the points of interest in this figure. Yellow arrows are the kidneys and the red arrow is the bladder. Blue arrows demonstrate gel moving underneath the skin; this is made evident by the SPECT signal that can be observed to be deeper than the splint but shallower than the spine. This indicates this signal is present inside the body (i.e. below the skin) and has moved below surface from the right wound into the left wound. Green arrows indicate gel that has left the wound over the skin: in the middle panel it can be observed how this gel has 'trailed' towards a distant location. In the right panel the green arrows indicate gel right next to the splint and right next to a piece of Leukoplast® tape (pink arrow) that was used to secure the bandage dressing. The position and height of the signal indicated by green arrows relative to the CT signal from the splint and tape reveal this gel is on top of the skin and not below it.



Supplementary Figure S5: Scoring of immuno-histological stainings for granulocyte (GR-1 marker) and macrophage (F4/80 marker) infiltration, each dot represents one wound or healthy skin section. The expression of the markers was scored on a scale of 0 (no expression) to 5 (almost exclusive). Both granulocyte and macrophage levels were comparable in all groups, although one wound in the splinted PIC gel treated group showed clearly elevated granulocytes possibly indicating an infection. No statistical analysis could be performed due to cross-contamination of control wounds with PIC gel.