Supporting Information for

De Novo Chemoattractants Form Supramolecular Hydrogels for Immunomodulating Neutrophils *In Vivo*

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Content

Synthesis: Chemical reagents and solvents were used as received from commercial sources. The peptide synthesis was described previously in details.[1] Briefly, the peptides were synthesized by standard Fmoc solid-phase peptide synthesis and formylated by 2,2,2-trifluoroethyl formate.[2] The peptides were purified by a Waters Delta600 HPLC system equipped with an in-line diode array UV detector using a XTerra C18 RP column with CH3CN (0.1% of trifluoroacetic acid) and water (0.1% of trifluoroacetic acid) as the eluent. ^{1}H NMR and ^{13}C NMR spectra were obtained on Varian Unity Inova 400 (Varian), LC-MS on Waters Acouity UPLC with Waters MICROMASS detector (Waters).

Hydrogel preparation: The supramolecular hydrogels were prepared by adding the determined amount of peptides into the DPBS buffer (pH=7.4, Life Technologies), except that the hydrogels for rheology test and controlled release experiment were all prepared in PBS buffer (pH=8.0). The vial contained peptides and the buffer was heated to 100 °C for 1 min to completely dissolve the peptides and the vial was left at room temperature for hydrogel formation. The stability of the formyl peptides were demonstrated by no change of LC-MS after boiling for 30 mins (Figure S19 A-D of Ref 3). And minimum gelation concentration was determined by changing the concentration every 0.025 w/v %.

TEM: TEM micrographs were obtained using negatively stained samples on a Morgagni 268 electron microscope (FEI) with a 1k CCD camera (GATAN). The detailed negative staining procedure is available in the methods of Ref 1.

Rheology test: It was carried out on TA ARES-G2 with the parallel plate (diameter = 25 mm) (TA Instruments). The hydrogels of **1**, **2**, **3** and **4** were prepared in PBS buffer (pH=8) at their minimum gelation concentrations.

Proteolytic stability assay: It was carried out in 3.5 mL HEPES buffer solution of 1.4 mg (0.4 mg/mL) by adding 2.8 μL of proteinase K solution (>0.8 U/μL, P4850, Sigma) in 37°C water bath. At the indicated time points, 100 μL was obtained from the digestion soup and quenched by adding 10 μL 1M HCl solution and 90 μL acetonitrile. And 25 μL were injected into the analytic HPLC (the same HPLC system for compound purification) for quantification.

Release experiment: The 0.6 mL hydrogels (0.4% w/v) of were formed in in PBS buffer (pH = 8). The hydrogel was immersed into 0.6 mL DPBS buffer (pH = 7.4, Life Technologies) and it was in 37 °C water bath. Every 2 hours the buffer was changed and the release amount was quantified by analytic HPLC.

Purification of murine neutrophils: Murine bone-marrow derived neutrophils were purified from the bone marrow of the wild type C57BL/6 mice by the EasySep® mouse neutrophil enrichment kit (STEMCELL Technologies). We exactly followed the instructions from the kit.

Purification of human neutrophils: We isolated human primary neutrophils from discarded white blood cell filters (WBF2 filter; Pall Corporation) from healthy donors, which were provided by the blood bank at Children's Hospital Boston. The gradient separation method is a standard protocol and has been described in details.[4]

Neutrophils chemotaxis assay: The chemotaxis assay and the analysis have been previously described in details.[5] The chemotaxis assay was carried out on the EZ-taxiscan (Effector Cell Institute, Tokyo, Japan). Purified neutrophils (1 μL, $3x10^6$ /mL) were added to the lower reservoir of the chamber and 1 μL of the chemoattractant was then added to the upper reservoir, with the final concentrations indicated in the manuscript. And neutrophil migration (at 37 °C) in each of the channels was recorded sequentially every 30 secs for 20 mins. The analysis of the recorded sequential images using DIAS imaging software (Solltech) gave the traces of the migrating neutrophils. And the migration speed, directionality and upward directionality were calculated from the (x,y) coordinates from the traces. The minimum effective concentrations were determined by 10-fold dilutions.

Neutrophils ROS production assay: It was described previously in details.[6] Briefly, the 0.4 $x10^6$ mouse or human neutrophils were suspended in HBSS containing 4 U/ml HRP, 5.5 μM isoluminol, and 0.2% BSA and the total volume is 180 μL where transferred to the plates. After adding the indicated amount of peptides in 20 μL, the recording of the chemiluminescence starts on a TriStar LB941 microplate luminometer (Berthold Technologies USA)

Mouse peritonitis model: It was described previously in details except that the gel or solution of formyl-peptides were used instead of *E.coli.*[5] Peritonitis was induced by intraperitoneally injecting the indicated amount of gel or solution into the wild type C57BL/6 mice. At the indicated time points, mice were sacrificed and peritoneal exudates were harvested in 3 successive washes with 3 mL of DPBS buffer containing 5 mM EDTA. The cells in the lavage were collected and stained by APC-Gr-1 (APC: Allophycocyanin; eBioscience) and PE-CD11b antibodies (PE: Phycoerythrin; eBioscience) and analyzed by FACS Canto II flow cytometer and FACSDiva software (BD Biosciences).

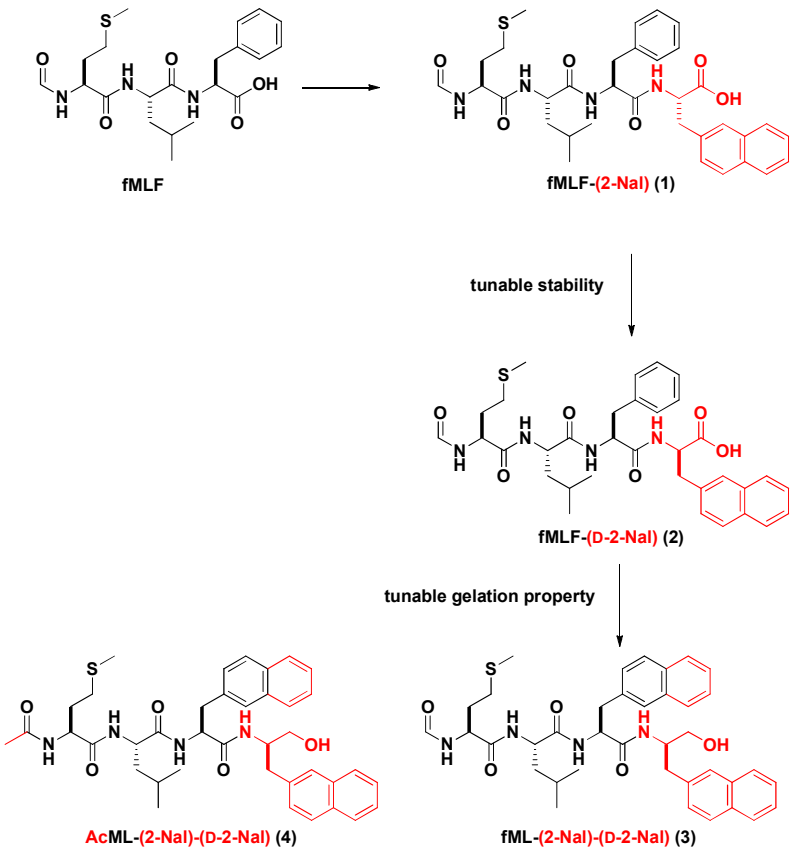

Figure S1. The chemical structures of the fMLF-based hydrogelators (**1**, **2**, and **3**) and a control (**4**).

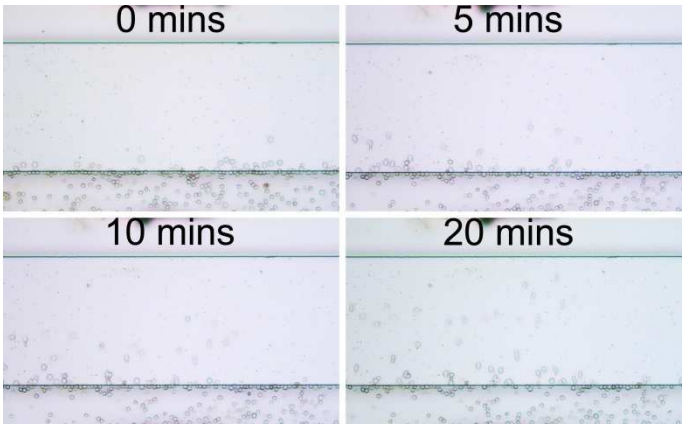

Figure S2. The snapshots of wild type murine neutrophils at 0, 5, 10 and 20 minutes in the gradient of **4** starting at 113 μM performed on EZ-TAXIScan.

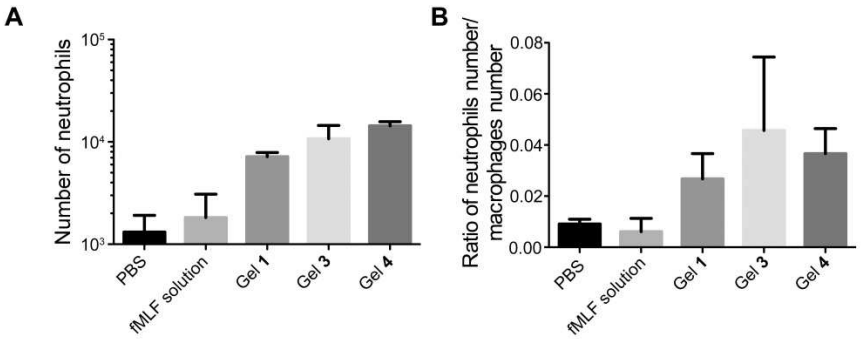

Figure S3. (A) The number of neutrophils and (B) the ratio of the number of neutrophils vs the number of macrophages from the FACS quantification of the cells collected from the peritoneal lavage of wild-type mice 48 hr. after receiving the IP injections of 500 μL of PBS (as the control), the solution of fMLF and the hydrogels of 1, 3 and 4 containing 0.935 μmole of peptides.

1 Gao, Y., Shi, J., Yuan, D. & Xu, B. Imaging enzyme-triggered self-assembly of small molecules inside live cells. *Nat Commun* **3**, 1033 (2012).

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3 Chiu, I. M. *et al.* Direct activation of sensory neurons by bacteria mediates pain and inflammation. *Nature*, **501**, 52-57 (2013).

4 Zhu, D. *et al.* Deactivation of phosphatidylinositol 3,4,5-trisphosphate/Akt signaling mediates neutrophil spontaneous death. *Proc. Natl. Acad. Sci. USA* **103**, 14836-14841 (2006).

5 Sakai, J. *et al.* Reactive Oxygen Species-Induced Actin Glutathionylation Controls Actin Dynamics in Neutrophils. *Immunity* **37**, 1037-1049 (2012).

6 Prasad, A. *et al.* Inositol hexakisphosphate kinase 1 regulates neutrophil function in innate immunity by inhibiting phosphatidylinositol-(3,4,5)-trisphosphate signaling. *Nat. Immunol.* **12**, 752-760 (2011).