Supplementary Information

Laser machining

The machining station consists of an upright microscope, as outlined in reference [1]. The femtosecond laser machining system is located inside an ISO 1000 class clean room. The femtosecond laser consists of a Tsunami oscillator (model 3941, Spectra Physics, Irvine, CA) seeding a Ti-Sapphire RegA 9000 regenerative amplifier (Coherent, Inc., Santa Clara, CA), both of which are pumped by a continuous wave green diode frequency doubled Nd:YVO4 solid-state Verdi-18 laser (Coherent, Inc.). The laser system is operated at a center wavelength of 800 nm and generates amplified 5 μJ femtosecond laser pulses, with a measured pulse width of 160 fs (FWHM), either in single pulse mode or at repetition rates up to 250 kHz. The incoming laser beam is expanded using a pair of lenses so as to fill the entrance pupil of the microscope objective used to focus the laser beam onto the surface of the glass coverslip. The substrate is held in place by an aluminum vacuum chuck attached on top of an Aerotech ANT95-3-V-MP nanopositioner which sits on an Aerotech ANT95-50-XY stage assembly controlled by Aerotech A3200 Npaq MR controller (Aerotech, Pittsburg, PA). The sample positioning assembly has nanometer resolution and moves the substrate under the microscope objective in a user-defined pattern under LabView® control (National Instruments, Austin, TX). For *in vitro* experiments, arrays of 200×200 20μm spaced nanopores arranged in a square lattice were fabricated on the surface of 22×22 mm^2, 500 μm thick UV grade double side polish fused silica by locally ablating the surface of the fused silica with a single 160 fs laser pulse tightly focused by a dry microscope objective (Nikon CF Plan Achromat 79173) with a numerical aperture of 0.85 (Nikon, Melville, NY).

Synthesis of Ac-SDKP

The amino acid sequence SDKP was synthesized on Rink amide-MBHA resin with standard Fmoc-based chemistry [2]. Resin-bound peptide was then acetylated with an excess of acetic anhydride for 4 hours, followed by cleavage with a 90:5:3:2 solution of trifluoroacetic acid/thioanisole/ethanedithiol/anisole for 2 hours. The cleaved peptide was dried, precipitated in cold diethyl ether, and lyophilized to form a white powder. HPLC-MS (data not shown) confirmed that the peptide was greater than 90% acetylated. Fluorescently labeled FITC-SDKP was purchased from GenScript (Piscataway, NJ).

Loading and cleaning methods

Nanopore arrays were loaded by depositing a 50 µl drop of 6 $mg.mL^{-1}$ solution of Ac-SDKP in ultrapure DI water (EMD Millipore, Billerica, MA) on the nanopore array immediately after laser machining and evaporated in a vacuum desiccator. Fluorescently labeled peptides were handled in the dark. This is the equivalent of adding 0.3 mg per substrate for each experiment. After drying, there was peptide residue visible on the surface of the substrates. In order to remove the excess peptide, the substrates were rinsed with ultrapure water and dried under ultrapure nitrogen. This suggests that the actual mass of peptide delivered to the cells in experiments using nanopore or flat substrates was significantly lower than 0.3 mg. One mL of media was used for all experiments, indicating that the maximum concentration possible for nanopore experiments was 0.3 mg/mL. In experiments introducing free FITC-SDKP in culture media, no uptake was seen below 0.5 mg/mL.

Nanopore substrates were cleaned between experiments by immersion in 90% ethanol/deionized water and ultrasonicating overnight (Cole-Palmer, Vernon Hills, IL). After sonication, the substrates were rinsed with isopropanol and dried under ultrapure nitrogen.

Nanopore characterization

Nanopore morphology and distribution were characterized by scanning electron microscopy (SEM). Samples were dried and sputter coated with gold for 30 seconds before imaging on a Hitachi S4200 SEM (Wallingford, CT). Nanopore loading and cleaning was characterized by confocal microscopy. Pore substrates were loaded with FITC-SDKP as previously described and incubated in media for 48 hours prior to imaging. Samples were imaged using an Olympus FV-1000 inverted confocal microscope in laser scanning and DIC modes (Center Valley, PA) using 40x / 1.30 Plan-Neofluar, 60x / 1.45 Plan-Apochromat, and 100x / 1.4 SPlan-UApo oil immersion objectives.

Cell culture and seeding

For *in vitro* studies, RAW 264.7 cells (ATCC, Manassas, VA) were cultured in DMEM supplemented with 10% very low endotoxin fetal bovine serum (Performance Plus, Gibco) and 1% penicillin/streptomycin in 75 cm^2 culture flasks prior to experiments. Fused silica culture substrates were placed in 6-well tissue-culture treated polystyrene plates (Corning, Corning, NY). To constrain the attachment of the cells onto the nanopore area, short segments of 0.25 inch inner diameter polytetrafluoroethylene (PTFE) tubing were cut and fixed to the fused silica substrates with a seal of sterilized vacuum grease. Raw 264.7 cells were removed from the 75cm^2 flask using a cell scraper, spun down, re-suspended at a density of $1x10^4$ cells.mL^{-1}, and 200 µL of cell suspension was seeded into each PTFE tube resulting in a seeding density of $6.3x10^3$ cells.cm^{-2}. Cells were allowed to attach for 15 hours, then the PTFE tubing was removed and the media replaced with 2mL. For LPS treatment, LPS (Sigma-Aldrich, St. Louis, MO) was added to culture medium at a concentration of 1 mg.mL^{-1}. Endpoint analysis was carried out 72 hours after treatment.

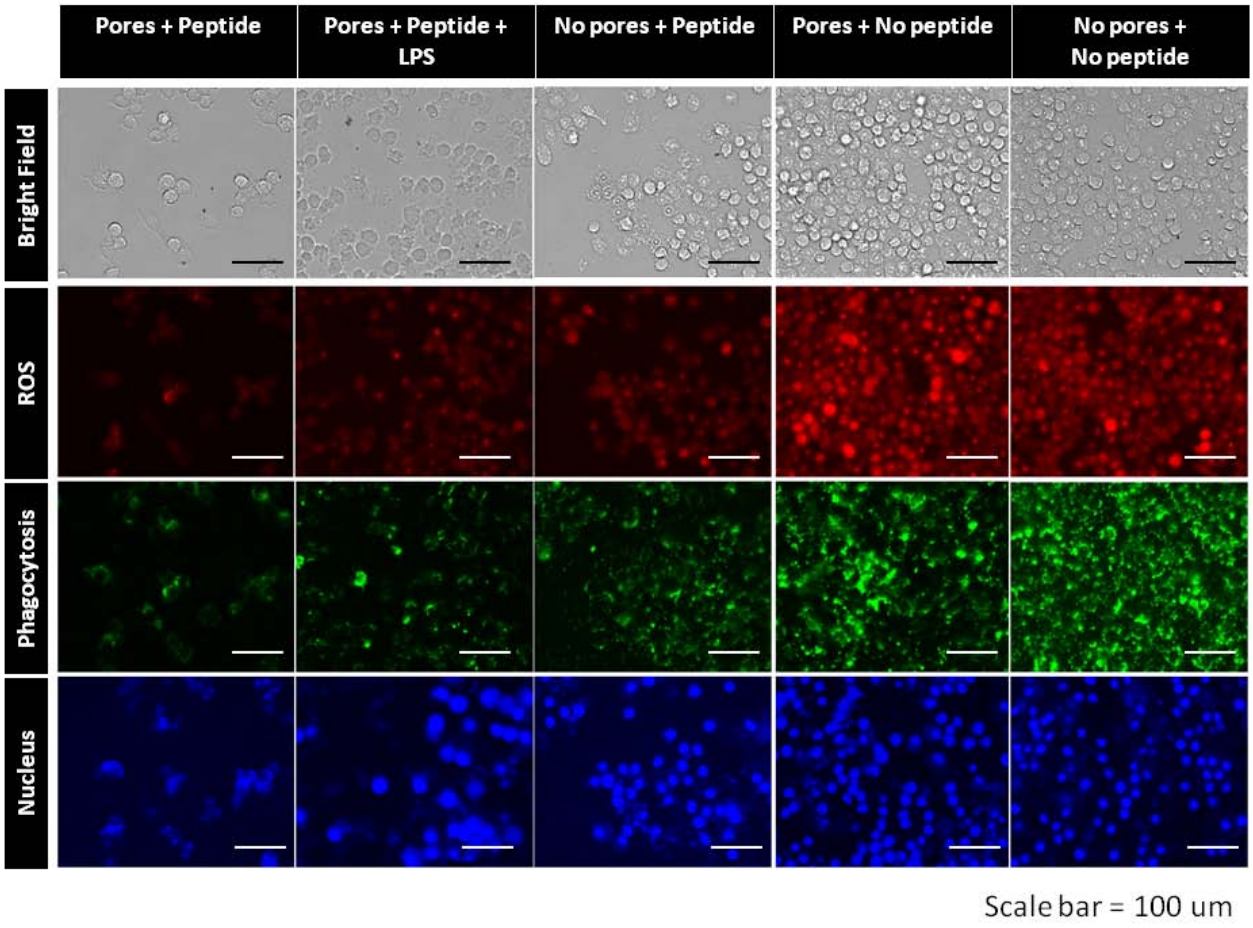
***In vitro* assays**

In vitro studies were imaged using a Nikon Ti inverted microscope with phase and fluorescence capabilities (Nikon, Melville, NY) using 10X and 40X objectives. Phagocytosis was measured using a Vybrant® Phagocytosis Assay Kit (V-6694, Life Technologies, Carlsbad, CA) according to the manufacture's protocol. Briefly, each test condition was treated with green-fluorescent *Escherichia coli* (*E. coli*) particles for 2 hours at 37 ˚C. After incubation, Raw 264.7 cells were washed with trypan blue solution to quench extracellular fluorescence. To measure superoxide production, the cells were then stained with 5 µg/mL dihydroethidium (DHE) and counterstained with 5 ug.mL^{-1} Hoechst for 15 minutes at 37 ˚C and washed with PBS. Fluorescence intensities of *E. coli* particles phagocytized by activated macrophages (Excitation 485nm, Emission 535nm) and the intracellular superoxide measured by DHE (Excitation 340 nm, Emission 590nm) were quantified using a plate reader (Tecan Group, Ltd Männendorf, Switzerland). For plate reader studies, a 900 µm square area was analyzed for all treatment conditions. Phagocytic activity and superoxide production were baseline subtracted and normalized to cell number as measured by Hoechst nuclear staining.

1. Zalloum OHY, Parrish M, Terekhov A, Hofmeister W: **An amplified femtosecond laser system for material micro-/nanostructuring with an integrated Raman microscope.** *Review of Scientific Instruments* 2010, **81**.

2. Yu SS, Koblin RL, Zachman AL, Perrien DS, Hofmeister LH, Giorgio TD, Sung HJ: **Physiologically Relevant Oxidative Degradation of Oligo(proline) Cross-Linked Polymeric Scaffolds.** *Biomacromolecules* 2011.

Supplementary Figure 1



Effects of Ac-SDKP loading to pores on Raw 264.7 cells. Cell number was decreased in response to Ac-SDKP treatment. Intracellular superoxide as measured by DHE fluorescence and phagocytosis as measured by fluorescent E. Coli particle uptake were significantly lower with Ac-SDKP treatments. In addition, treatment with Ac-SDKP loaded pores significantly reduced intracellular superoxide and phagocytosis compared to other conditions of Ac-SDKP treatment.