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SUPPLEMENTARY INFORMATION

Self-Assembled Nanofiber Hydrogels for Mechanoresponsive Therapeutic Anti-TNFα Antibody Delivery

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Detailed Methods

Synthesis of Oleoylamide GNL Gelator

All reagents were purchased from Sigma-Aldrich unless otherwise noted and used as received. Dichloromethane (DCM), ethyl acetate (EtOAc), hexanes, acetonitrile (ACN), tetrahydrofuran (THF), dimethyformamide (DMF), and methanol (MeOH) were purchased from Pharmco-Aaper, Brookfield, CT. Briefly, equimolar oleoyl chloride and propargylamine were combined under N_2 in anhydrous DCM containing 1.5-fold excess of triethylamine. The reaction was stirred overnight to yield N-propargyl oleoylamide ("activated lipid"), which was subsequently purified using liquid chromatography (EtOAc/Hexanes 9:1; 90% yield).

5'-Azido-5'-deoxythymidine ("activated nucleoside") was synthesized starting from thymidine and 1.02 equivalents methansulfonyl chloride and subsequent NaN_3 (excess). Purification via hot crystallization in ACN resulted in white crystals in 60% yield.

The first "click" reaction between lipid and nucleoside was carried out overnight in THF/H_2O (50/50) at 65°C with catalytic amounts of copper (II) sulfate and potassium ascorbate, subsequently cooled, precipitated and dried *en vacuo* for 24 hours. The compound was used in subsequent reactions without purification.

Propargylation of the secondary amine on thymidine of the linked nucleoside-lipid was achieved using a two-fold excess of propargyl bromide and potassium carbonate in anhydrous DMF. The reaction was stirred at RT overnight, and heated *en vacuo* to remove DMF. After workup (DCM/H_2O/Brine washes), the compound was purified using column chromatography (DCM/MeOH 9/1; 83% yield). Finally, this compound was mixed with an equimolar amount of 1-azido-β-(D)-glucopyranoside; reaction conditions were identical to the “click” reaction described above. After column chromatography (EtOAc/MeOH 95/5 increasing to 85/15), the final compound was crystallized from a minimal amount of ethanol (88% yield). **Analysis:** HRMS (ESI $[M + Na]^+$, 852.4596 theoretical; 852.4597 actual). Elemental analysis (57.88% C, 7.65% H theoretical; 57.65% C, 7.80% H actual). ^1H NMR (400 MHz, DMSO-d_6): 0.83 (t, 3H), 1.21 (s, 20H), 1.44-1.46 (m, 2H), 1.86 (s, 3H), 1.95-1.96 (m, 4H), 2.02-2.06 (m, 3H), 2.15-2.20 (m, 2H), 3.18-3.22 (m, 1H), 3.62-3.75 (m, 2H), 4.07-4.12 (m, 1H), 4.25 (d, 2H), 4.60-4.65 (m, 3H), 5.03 (s, 2H), 5.18 (d, 1H), 5.28-5.30 (m, 4H), 5.46 (d, 1H), 5.52 (s, 1H), 6.20 (t, 1H), 7.46 (s, 1H), 7.86 (s, 1H), 8.08 (s, 1H), 8.22 (t, 1H).

Microscopic Characterization

The hydrogel nanostructure was imaged by transmission electron microscopy (Hitachi H 7650) using diluted (1 mg/mL) gel deposited on Ni-carbon coated grids (1% uranyl acetate negative stain). AFM (Asylum Research) was performed on 20 μL hydrogel samples, deposited via syringe on freshly-cleaved mica discs (Ted Pella, Inc.) and rinsed twice with 10 mL PBS. AFM studies were conducted in tapping mode (23 °C) using MLCT-C SiN tips; spring constant = 20 pN/nm (Ted Pella, Inc.).

Rotational Shear Rheometry

Rheometry was performed with a TA Instruments AR1000 stress controlled rheometer using a 40-mm aluminum cone-plate geometry. For oscillatory stress sweeps, temperature and frequency were held constant at 37 °C and 1.0 Hz, respectively, while stress amplitude varied (0.01-100 Pa).

The yield stress of the gels was defined as the stress value resulting in a noticeable change in the value of *delta*. The flow point (gel-sol transition) was interpreted as the point where G” (viscous component) overtook G’ (elastic component). Frequency sweeps (0.1-100 Hz) were conducted at 37 °C and 10.0 Pa stress. Temperature sweeps (1.0 Hz, 10 Pa stress) were performed from 37-58 °C, then down to 37 °C (± °C/min). Reversibility tests were performed by three (3) cycles of the following: shearing at 37 °C and 1 Pa; resting for 2 minutes; shearing at 10 Pa (near yield point); resting for 2 minutes; and shearing at 100 Pa (past the flow point). All measurements were performed on triplicate samples.

FRAP Experiments

Hydrogels (0.7 mL) were loaded with 0.5 mg/mL FITC-dextrans of 19.6, 39, 77, and 167 kDa molecular weight (Sigma-Aldrich, Saint Louis, MO) and imaged at 23 °C on an Olympus FV1000 scanning confocal microscope equipped with a 488 nm excitation laser. A second (405 nm) scan head supplied the 500-ms bleach pulse. Excitation laser power was set to 0.2%, and fluorescence images were collected at 520 nm. Image acquisition rate was varied to obtain 200-300 time series images (including 3 pre-bleach images) for each molecular weight. Diffusion coefficients were calculated by importing images into MATLAB and calculating the average intensity as a function of time within the ROI. The ROI average intensity *versus* time was fit to a Gaussian model (step size = 0.001), according to the equations given by Jain et al[33], to arrive at the diffusivity constant, *D*, in cm^2/sec. Average pixel intensities within the ROI were normalized to percent recovery for graphical illustration. A pilot study was initially performed to determine the time scale for which the experiment would be performed. Because the 19.6 kDa dextran exhibited fast fluorescence recovery, the signal had plateaued before 400 seconds. The larger molecular weight samples took more time to reach a steady state. Note that each curve has the same number of data points.

Shear-Induced Release of Biomacromolecules

The aqueous release environment consisted of 25 mL PBS (37 °C) contained in a 10-cm diameter glass petri dish, whose bottom surface consisted of adherent 60-grit aluminum oxide sandpaper. Hydrogels (0.7 mL) containing 0.5 mg/mL FITC-dextran (167,000 g/mol) or 180 μg rabbit anti-huTNFα IgG (Abcam, Cambridge, MA) were deposited on top of this sandpaper via syringe, the geometry was lowered, and the PBS was then added. Oscillatory shear (10 Pa, 1 Hz), was applied to the samples for 90 minutes, with 3 cycles/timepoint and a 10-second delay time, while non-sheared hydrogels were used as control samples. All experiments were performed with triplicate samples. Aliquots (1 mL) were withdrawn from the PBS bath at predetermined time points to assess for percent release and % neutralization. Percent release of FITC-dextran was calculated from aliquot fluorescence intensity ($\lambda_{ex}/\lambda_{em}$ = 490/513 nm, quartz cuvette, 0.5-mm slits), while release of anti-TNFα was assessed through its neutralization of human TNFα's cytotoxic effect on L929 murine fibroblasts (ATCC, Manassas, VA).

The TNFα cytokine neutralization assay was performed at a final fixed concentration of 1 ng/mL human TNFα full-length protein (Abcam) in assay medium (RPMI 1640 containing 2 v/v% fetal bovine serum). Release aliquots (50 μL) were combined with 50 μL TNFα aliquots and incubated for 2 hours at 37 °C for binding. The mixtures (50 μL) were then fed to L929 cells (3×10^4 cells/well, seeded in 96-well plates 18 hours prior). An additional 50 μL of reduced-serum media containing 2 μg/mL actinomycin D (Sigma-Aldrich) were subsequently fed to the cells. Percent neutralization (% cell viability) was calculated using the colorimetric tetrazolium-based MTS assay by measuring absorbance at 492 nm using a 96-well microplate reader. Assay controls included cells incubated with 1 ng/mL TNFα only and cells incubated with anti-TNFα only. Antibody concentration and percent release were calculated by comparing viability results to a separate TNFα neutralization dose-response experiment of known antibody concentrations.

Statistics

Experimental values such as δ and percent release of macromolecules were assessed for statistical significance using an unpaired, two-tailed *t*-Test, with statistical significance defined by values of $p < 0.05$.

Supplementary Schemes/Figures

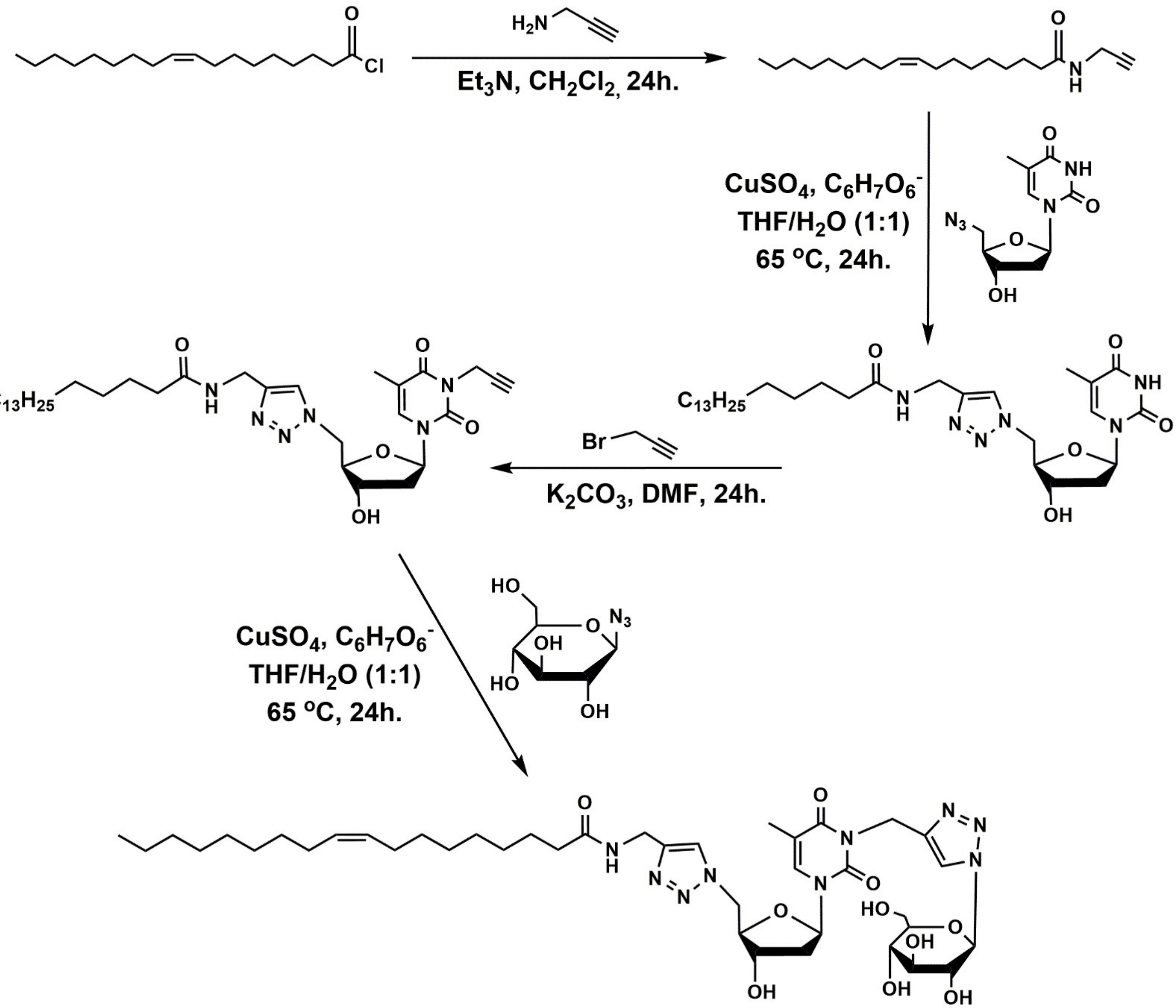

Figure S1. Synthesis of oleoylamide glycosyl-nucleoside-lipid (GNL).

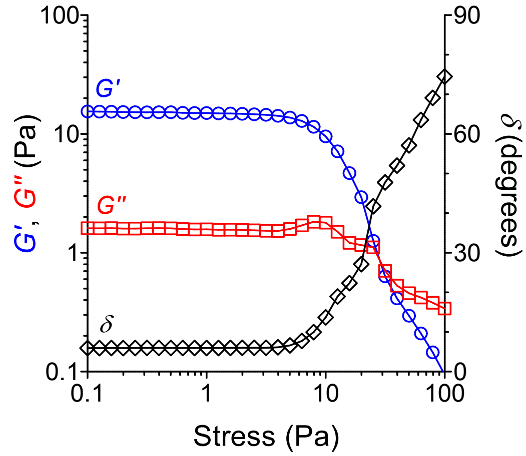

Figure S2. Strength and linear viscoelastic region of 4 wt% GNL hydrogel in PBS.

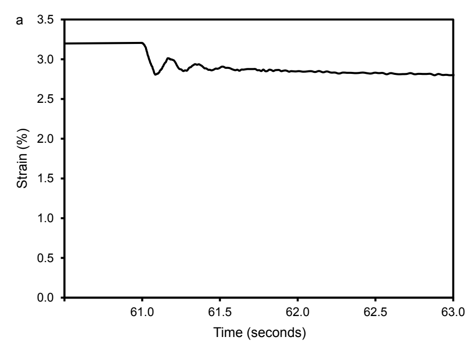

Figure S3. Magnified the time window between 61 and 61.5 seconds for the creep/recovery curve provided in Fig. 2b, showing a decaying sinusoidal (damped oscillation) wave function.

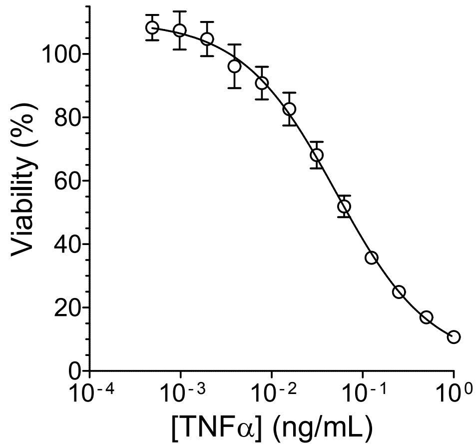

Figure S4. TNFα dose-response curve using L929 murine fibroblasts sensitized with actinomycin D (1 μg/mL).

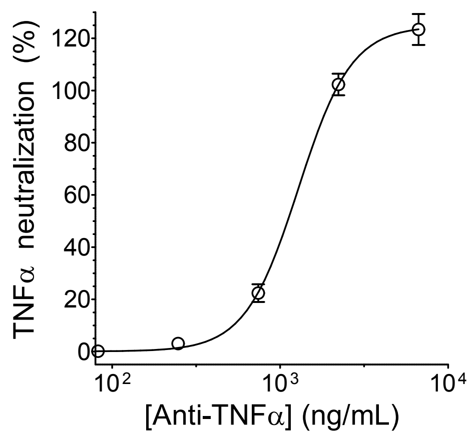

Figure S5. TNFα neutralization curve using L929 cells at fixed final concentrations of TNFα and actinomycin D (1 ng/mL and 1 μg/mL, respectively).