Supplementary Materials

Nanoporous Microsponge Particles (NMP) of Polysaccharides as Universal Carriers for Biomolecules Delivery

Maria Federica Caso [1,†], Felicia Carotenuto [2,3,†], Paolo Di Nardo [2,3,4], Alberto Migliore [5], Ana Aguilera [6], Cruz Matilde Lopez [6], Mariano Venanzi [7], Francesca Cavalieri [7,*] and Antonio Rinaldi [1,8,*]

[1] NANOFABER srl, 00123 Rome, Italy; maria.federica.caso@gmail.com
[2] Center of Regenerative Medicine, University of Rome "Tor Vergata", 00133 Rome, Italy; carotenuto@med.uniroma2.it (F.C.); dinardo@uniroma2.it (P.D.N.)
[3] Department Clinical Sciences and Translational Medicine, University of Rome "Tor Vergata", 00133 Rome, Italy
[4] I.M. Sechenov First Moscow State Medical University, 119991 Moscow, Russia
[5] Rheumatology Department, San Pietro Hospital Fatebenefratelli, 00189 Rome, Italy; migliore.alberto60@gmail.com
[6] Center for Genetic Engineering and Biotechnology, 10600 Havana, Cuba; ana.aguilera@cigb.edu.cu (A.A.); matilde.lopez@cigb.edu.cu (C.M.L.)
[7] Department of Chemical Science and Technologies, University of Rome "Tor Vergata", 00133 Rome, Italy; venanzi@uniroma2.it
[8] PROMAS-MATPRO Laboratory, Sustainability Department, ENEA, 00123, Rome, Italy
* Correspondence: francesca.cavalieri@uniroma2.it (F.C.); antonio.rinaldi@nanofaber.com (A.R.); +39-06-7259-4461 (F.C.); Tel.: +39-06-3048-3393 (A.R)
† These authors contributed equally to this work.

Received: 9 May 2020; Accepted: 23 May 2020; Published: date

1. Synthesis and characterization of cross-linker

Cross-linker (CL) dissolved in DMSO was examined by NMR. Spectra were recorded on NMR spectrometer operating at 400 MHz (Bruker DRX, Bruker AVANCE) and mass spectrometry.

Purified product.

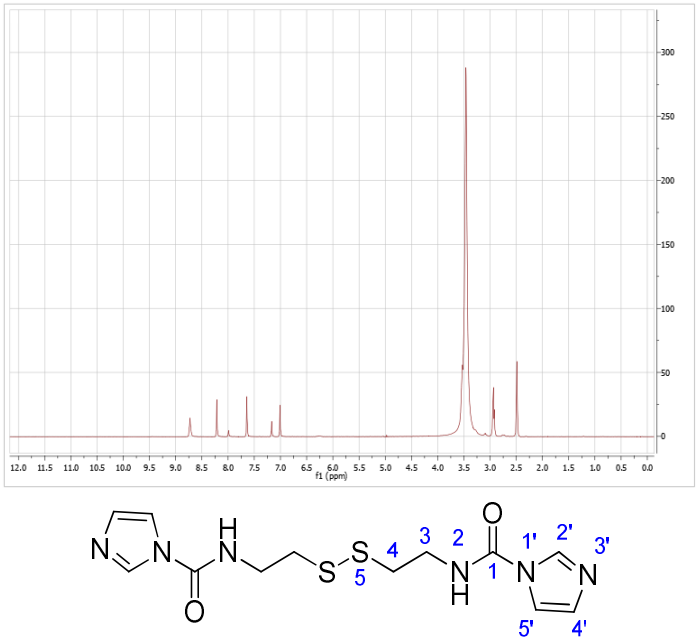


Figure S1. Spectrum ^{1}H, 400 MHz, DMSO-d_6. δ: 2.49 (t, J = 1.4 Hz, 4H, H-4), 2.93 (t, J = 8.8 Hz, 4H, H-3), 7.01 (br s, 2H, H-5'), 7.17 (br s, 1H, ?), 7.64 (br s, 2H, H-4'), 7.99 (br s, ?), 8.22 (br s, 2H, H-2'), 8.73 (t, J = 4.8 Hz, 2H, H-2). NOTE: Peaks at 7.17, 7.99 and 7.01 do not belong to product but to imidazole, byproduct obtained with degradation in water of CDI.

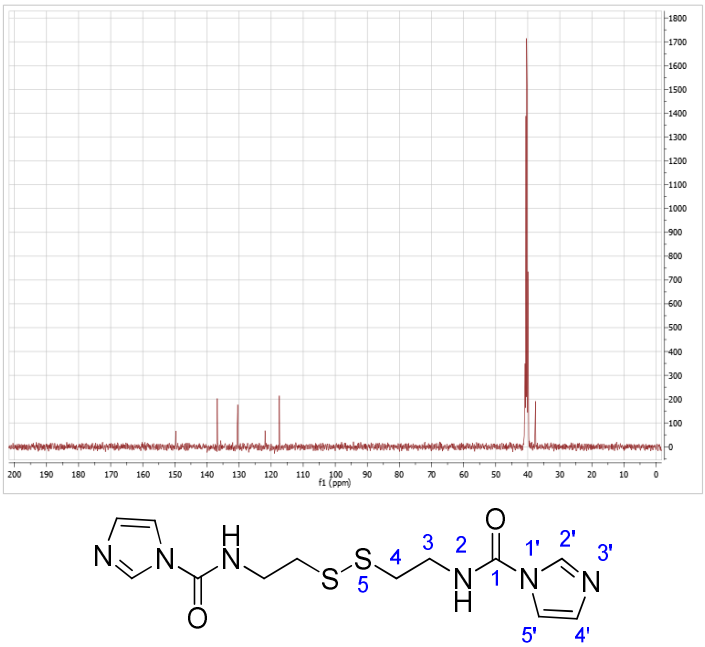


Figure S2. Spectrum 13C, 100 MHz, DMSO-d$_6$. δ: 37.6 (C-4), 41.0 (C-3), 117.4 (C-5'), 121.9 (starting product), 130.4 (C-4'), 136.8 (C-2'), 149.7 (C-1). NOTE: Peak of C-3 is under DMSO signal, while 121 and 136 belong to imidazole.

Crude product

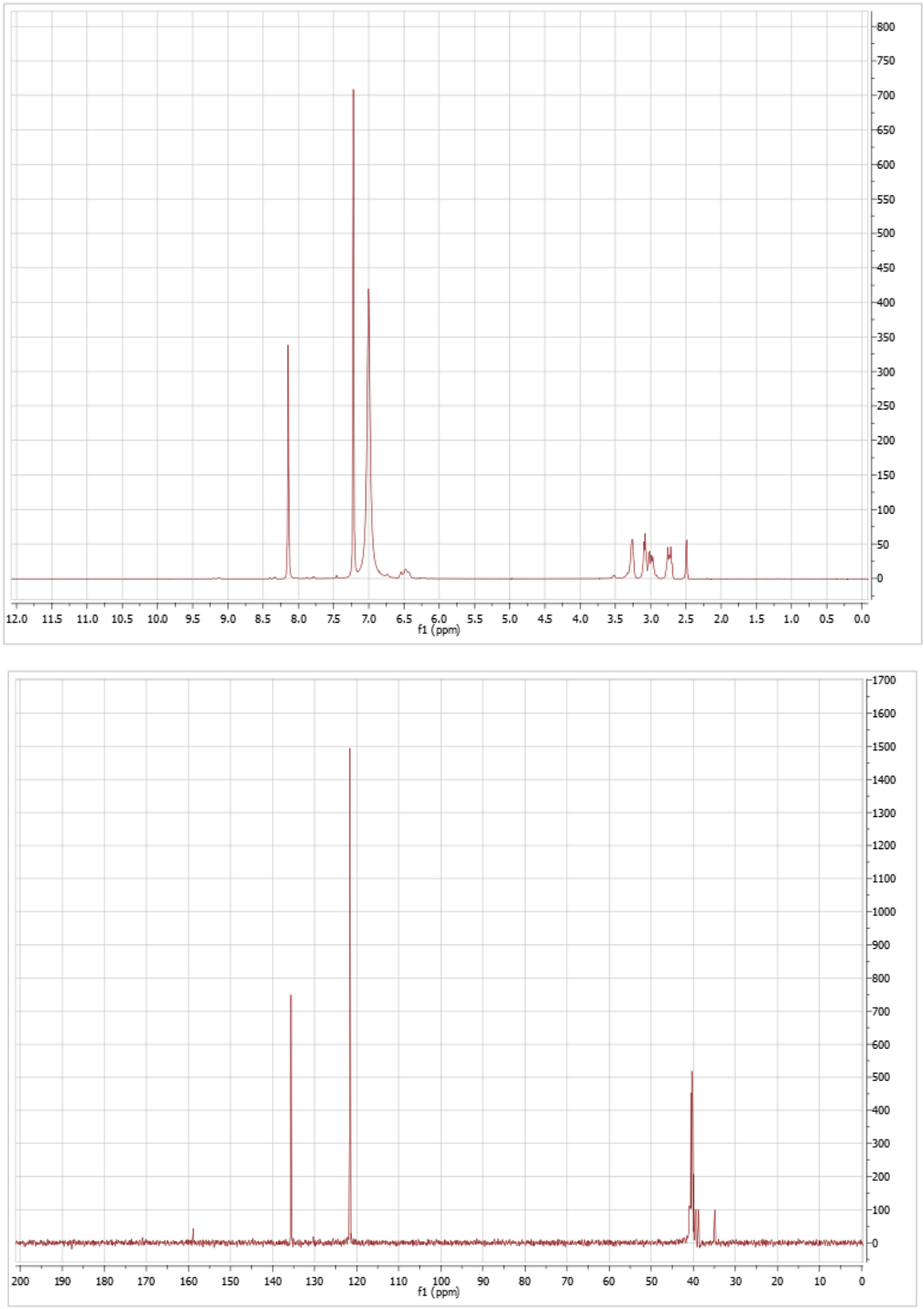
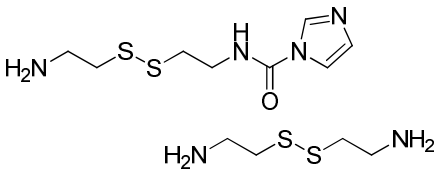


Figure S3. Peaks from 30 to 40 ppm indicate the presence of the following molecules (NOTE: Peaks 7.01, 7.22, 8.14 in 1H and 121.7, 135.6 in 13C belong to imidazole).



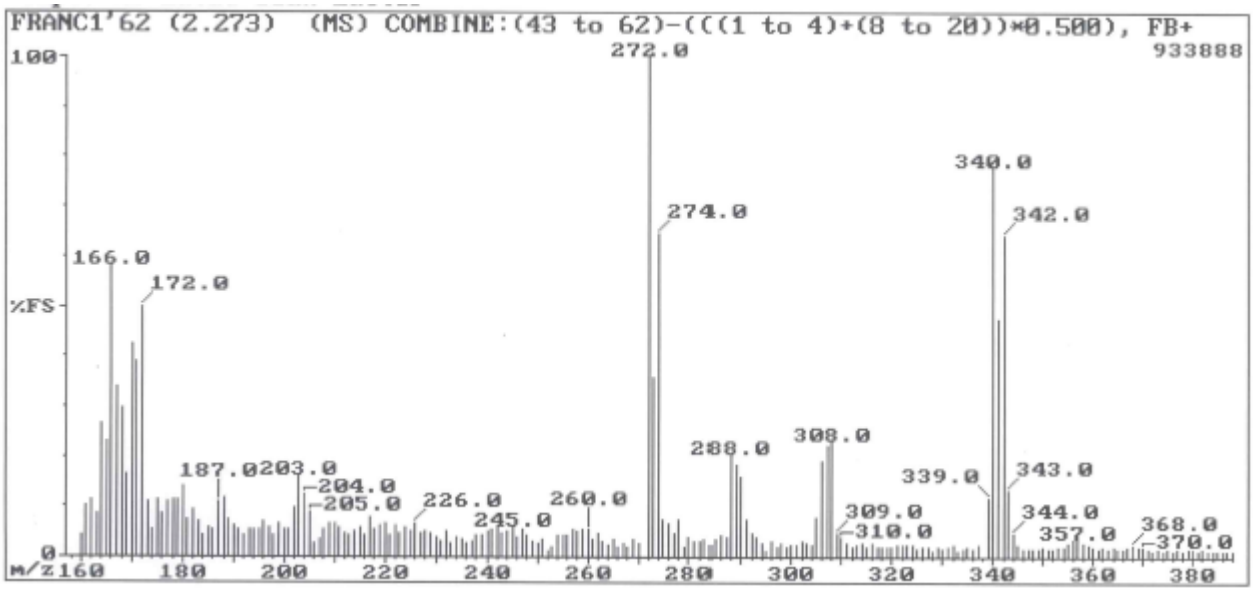
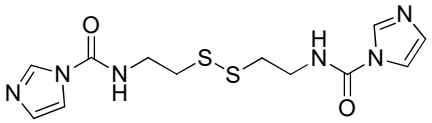
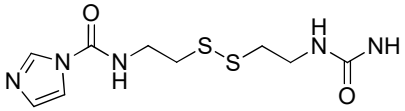


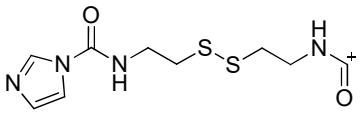
Figure S4. FAB mass spectra were obtained with a VG-Quattro spectrometer in the positive-ion mode by 3-nitrobenzylalcohol (peak 306, Sigma Aldrich) as the matrix. Resulting peaks derived from fragments:



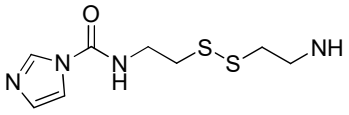
Molecular Weight: 340,42



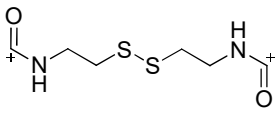
Molecular Weight: 288,37



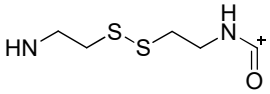
Molecular Weight: 273,35



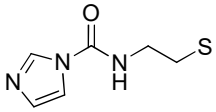
Molecular Weight: 245,35



Molecular Weight: 206,28



Molecular Weight: 178,28



Molecular Weight: 170,21

2. DTT Degradation Kinetics of NMPs

50 μL of suspensions containing labeled microparticles (carbohydrate concentrations: 1.51 g/L) were added to 1 mL of a solution 0.01 M of DL-Dithiothreitol (DTT, Sigma-Aldrich, 99%) in PBS. At variable time intervals, the suspension was centrifuged and the supernatant was analyzed with UV-vis spectra. The degradation study was carried out for 24 h, when the degradation resulted complete, as shown in Tables S1,2.

Table S1. DMSO-Particles degradation by DTT.

Polymer	Yield 1h (%)	Yield 2h (%)	Yield 3h (%)	Yield 4h (%)	Yield 6h (%)	Yield 9h (%)	Yield 24h (%)
CM-Dextran	31.9	59.8	66.7	72.4	79.3	89.7	99.3
HA low MW	21.0	60.9	70.7	81.5	88.4	92.5	98.8
Alginate	25.0	53.2	68.5	81.8	85.1	91.2	99.3
Dextran	40.0	60.5	66.0	76.6	85.3	88.3	99.6

Table S2. H_2O-Particles degradation by DTT.

Polymer	Yield 1 h (%)	Yield 2 h (%)	Yield 3 h (%)	Yield 4 h (%)	Yield 6 h (%)	Yield 9 h (%)	Yield 24 h (%)
CM-Dextran	27.1	51.3	72.3	84.8	94.8	97.9	99.8
HA low MW	10.2	40.4	63.9	77.6	88.8	95.3	99.2
Alginate	11.1	42.9	63.7	71.4	89.1	92.6	99.7
Dextran	27.5	54.1	75.0	85.2	96.4	99.9	100.0

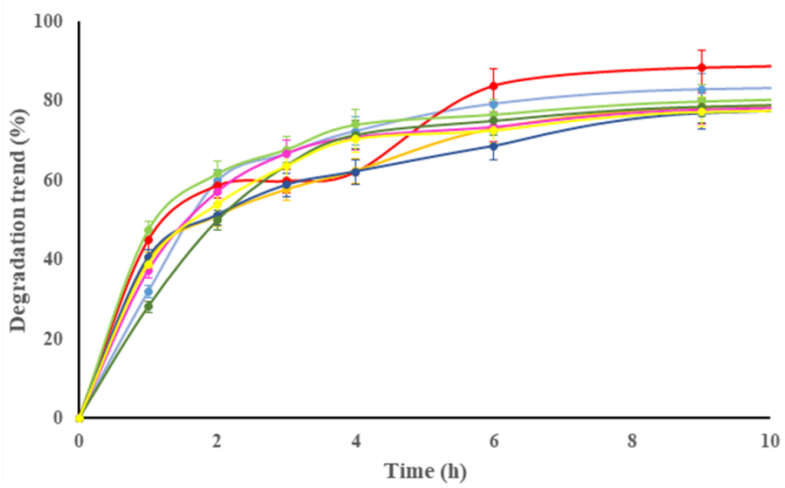


Figure S5. Particles degradation trend. Light blue: CM-dextran DMSO-particles; red: hyaluronic acid DMSO-particles; light green: alginate DMSO-particles; orange: dextran DMSO-particles; blue: CM-dextran H_2O-particles; pink: hyaluronic acid H_2O-particles; dark green: alginate H_2O-particles; yellow: dextran H_2O-particles.