

Supporting information

Thermosensitive nanogels based on dendritic polyglycerol and N-isopropylacrylamide for biomedical applications

Julio C. Cuggino,^a Cecilia I. Alvarez I.,^a Miriam C. Strumia,^a Pia Welker,^b Kai Licha,^b Dirk Steinhilber,^c
Radu-Cristian Mutihac,^c Marcelo Calderón^{c,*}

^a IMBIV-CONICET. Departamento de Química Orgánica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba. Haya de la Torre y Medina Allende, Edificio de Ciencias II, Ciudad Universitaria, Córdoba, Argentina.

^b mivenion GmbH Robert-Koch-Platz 4, Berlin 10115, Germany.

^c Institut für Chemie und Biochemie, Freie Universität Berlin, Takustrasse 3, Berlin 14195, Germany. Fax: (+49) 30-838-53357. E-mail: calderonmarcelo@yahoo.es. Homepage: <http://www.polytree.de>

S1. Additional information on the nanogels characterization

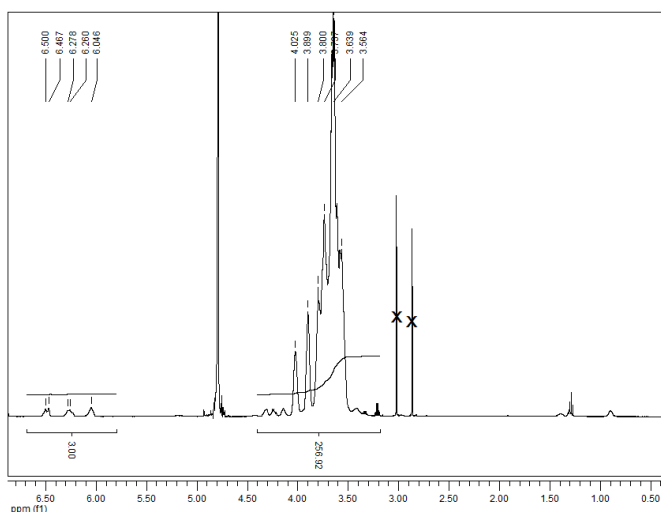
Table S1

Product	26 °C		37 °C		LCST (°C)
	Size (nm)	Dispersity ^(a)	Size (nm)	Dispersity ^(a)	
0% HPG	220	0.236	Precipitation		32.5
22% HPG	158	0.271	83	0.014	33.0
33% HPG-A	78	0.276	62	0.019	33.3
33% HPG-B	137	0.116	72	0.015	33.1
33% HPG-C	101	0.172	60	0.023	33.4
50% HPG	95	0.144	60	0.025	34.6
60% HPG	75	0.148	58	0.102	34.4

(a) Dispersity (PDI) according to DLS measurements (volume distribution curve).

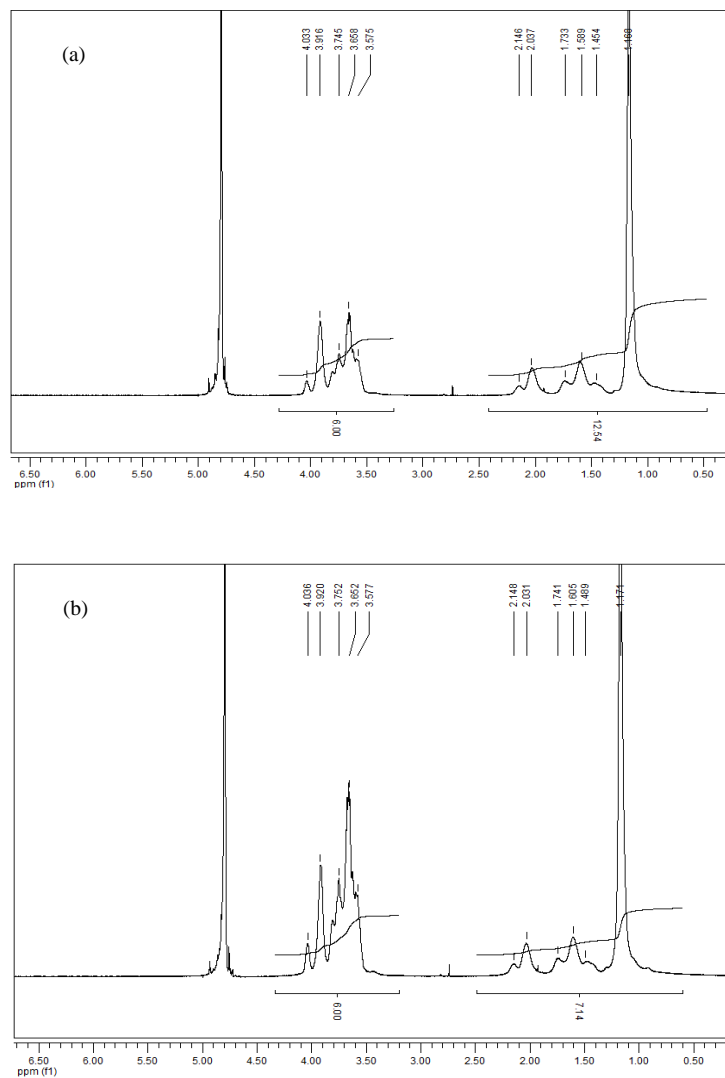
S2. ¹H-NMR spectrum of 5% Ac-HPG

The integral of the signals of core glycerol protons (HPG) 3.2–4.6 (m, 5H) respect the signals of the 3 alquene protons 5.76–5.90 (m, 1H), 6.02–6.18 (m, 1H), 6.30–6.48 (m, 1H) were used to determine the percentage of modification of OH into acryloyl groups (5% modification).



S3. ¹H-NMR spectra of two nanogels

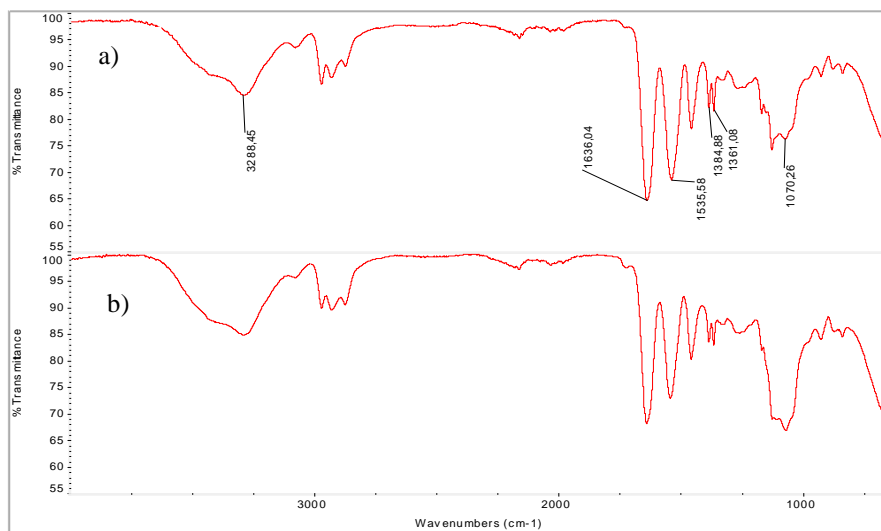
The integral of the signal at 3.2–4.4 (5H, HPG + 1H, NIPAm) respect to the signals of polymer backbone 2.03 (1H, polymer backbone), 1.47 (2H, polymer backbone), 1.16 (6H, isopropyl groups of NIPAm) were used to determine the ratio between HPG and NIPAm. a) Nanogel 33% HPG-B b) Nanogel 50% HPG.



S4. FT-IR Spectrum of nanogels

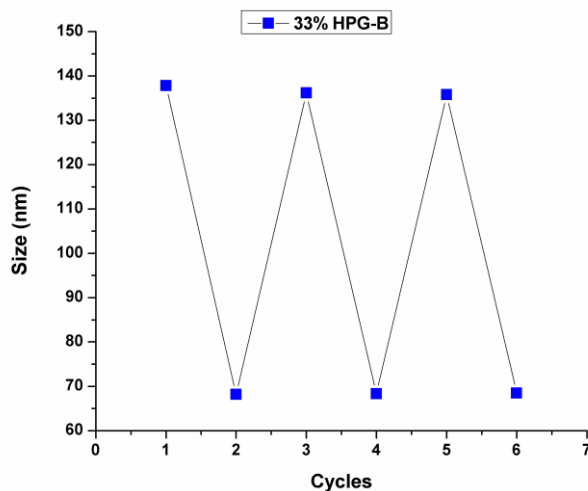
As expected the intensity of C-O signal at 1070 cm⁻¹ of HPG increase respect to the intensity of NIPAm band at 1636 (CO amide) when the amount of HPG in the synthesis is

higher. **a)** Nanogel 33% HPG-B **b)** Nanogel 50% HPG. IR (cm^{-1}): 3100-3500 (OH, HPG), 1636 (CO amide NIPAm), 1536, 1386 (CH isopropyl group NIPAm), 1366 (CH isopropyl group NIPAm), 1070 (C-O, HPG).

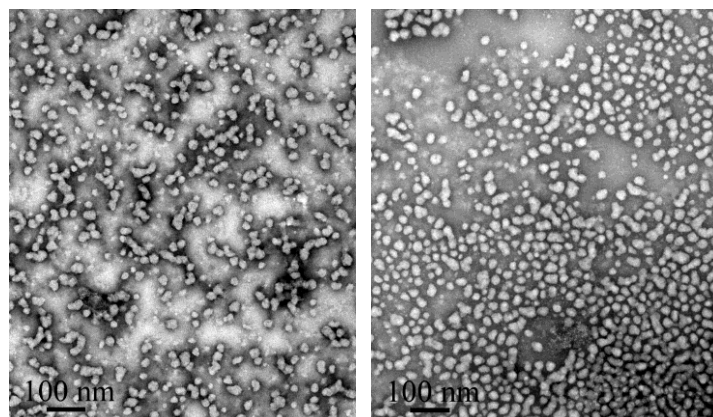


S5. Thermoresponsive reversibility

Thermoresponsive reversibility was shown by DLS. The size of 1 mg mL^{-1} solution of 30 % HPG-B in phosphate buffer pH= 7.4 was the same after 3 cycles of measuring at $26 \text{ }^\circ\text{C}$ (cycle 1, 3, 5) and $37 \text{ }^\circ\text{C}$ (cycle 2, 4, 6).

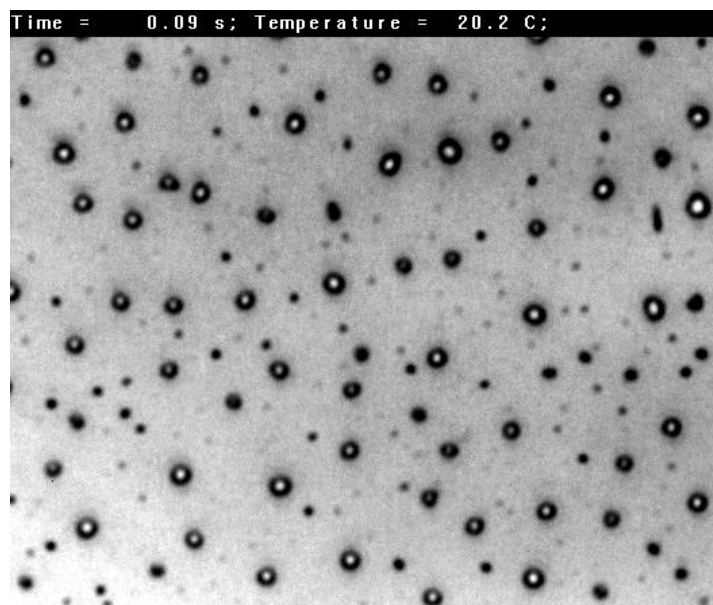


S6. TEM images for 50% HPG nanogel incubated at 25 °C and 37 °C respectively



S7. Interference enhanced reflection light microscopy video

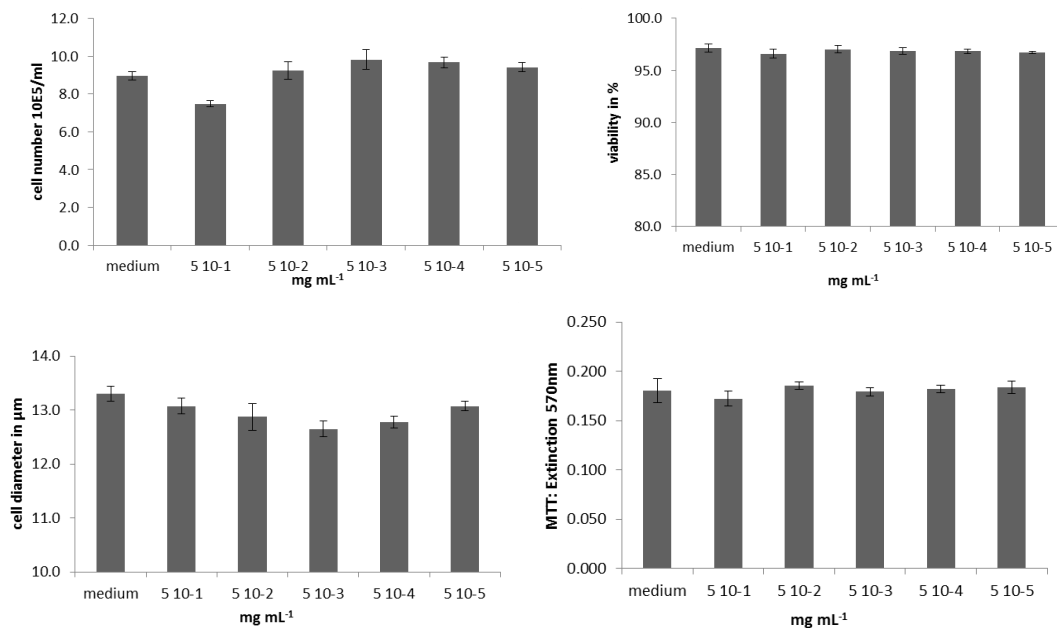
A movie with the changes induced in the thermoresponsive 33% HPG-B by a continuous increase/decrease of temperature between 20 °C and 40 °C, at a rate of 20 °C per minute, is linked to the following figure.



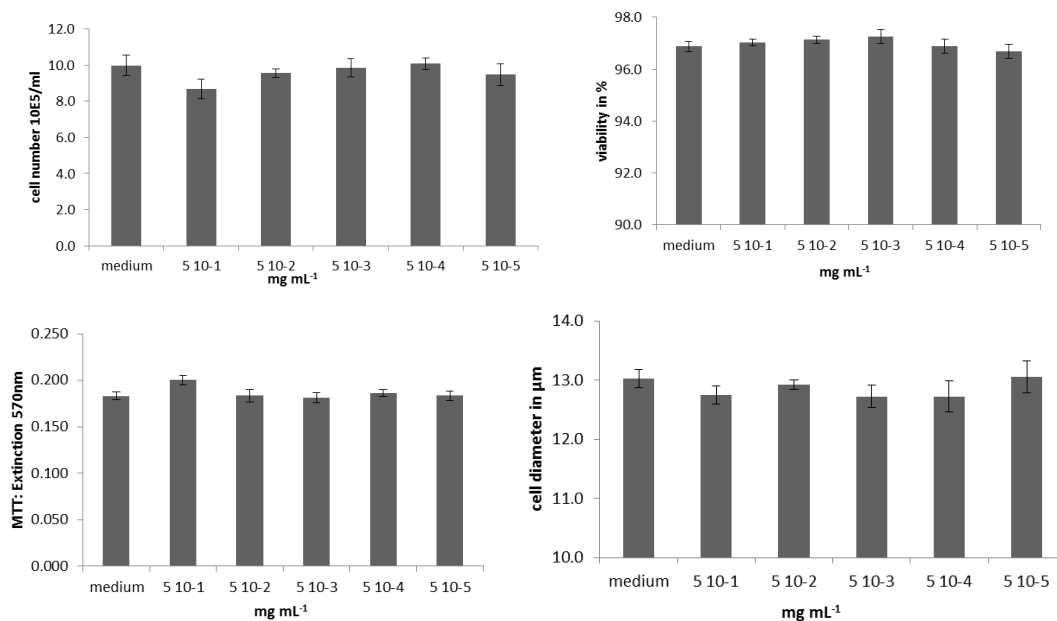
S8. Cytotoxicity assays

Human hematopoietic U-937 cell proliferation (cell number), viability, cell diameter, and metabolic activity (MTT) tests in a culture with medium or different concentrations of HPG nanogels.

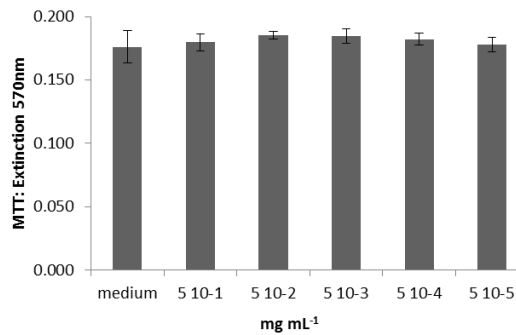
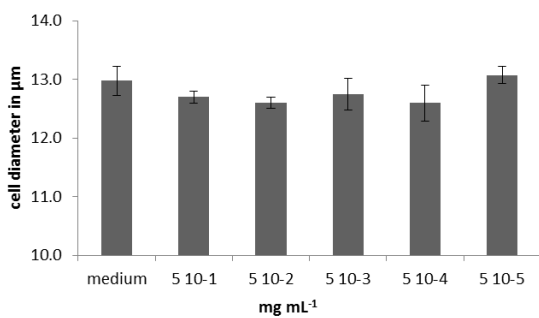
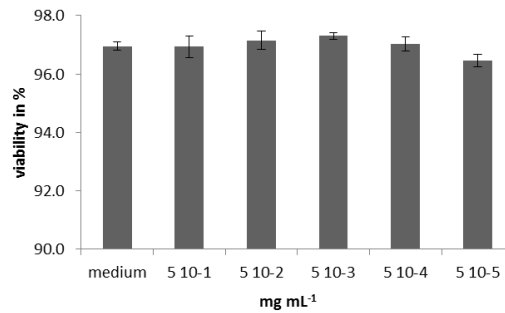
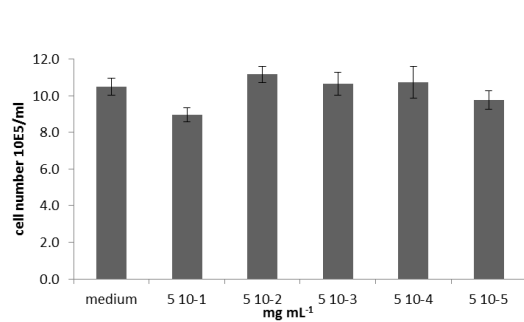
S8.A. 22% HPG



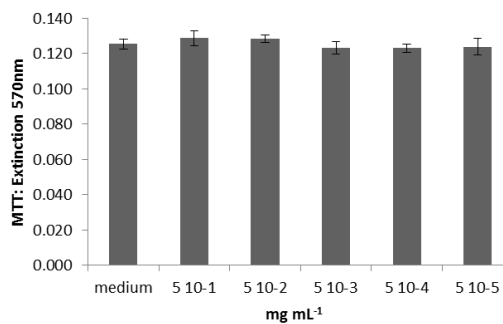
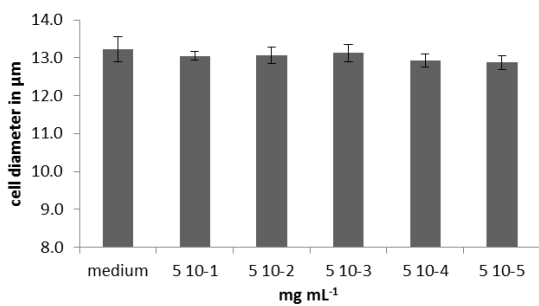
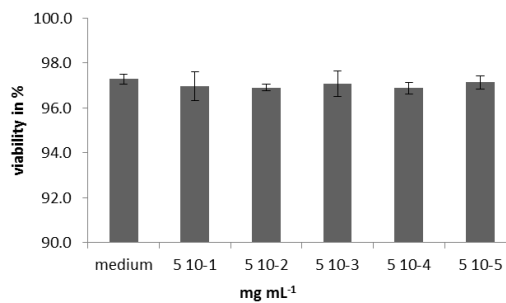
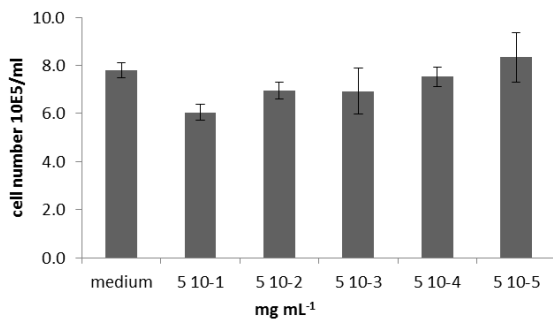
S8.B. 33% HPG-A



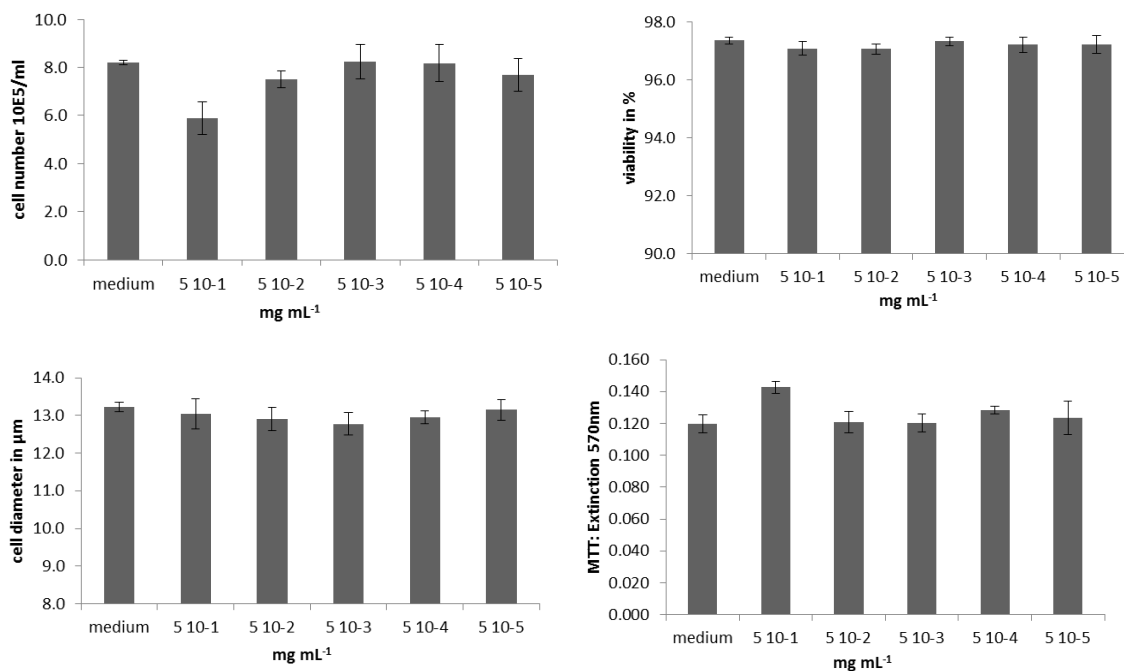
S8.C. 33% HPG-B



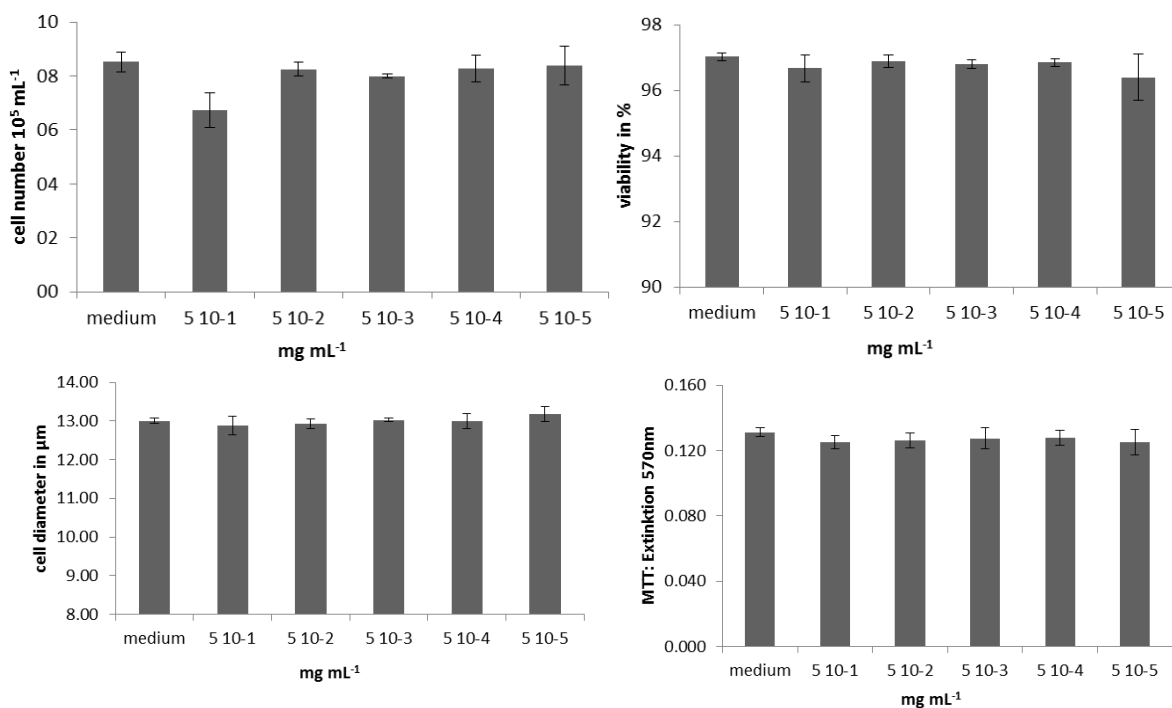
S8.D. 33% HPG-C



S8.E. 50% HPG

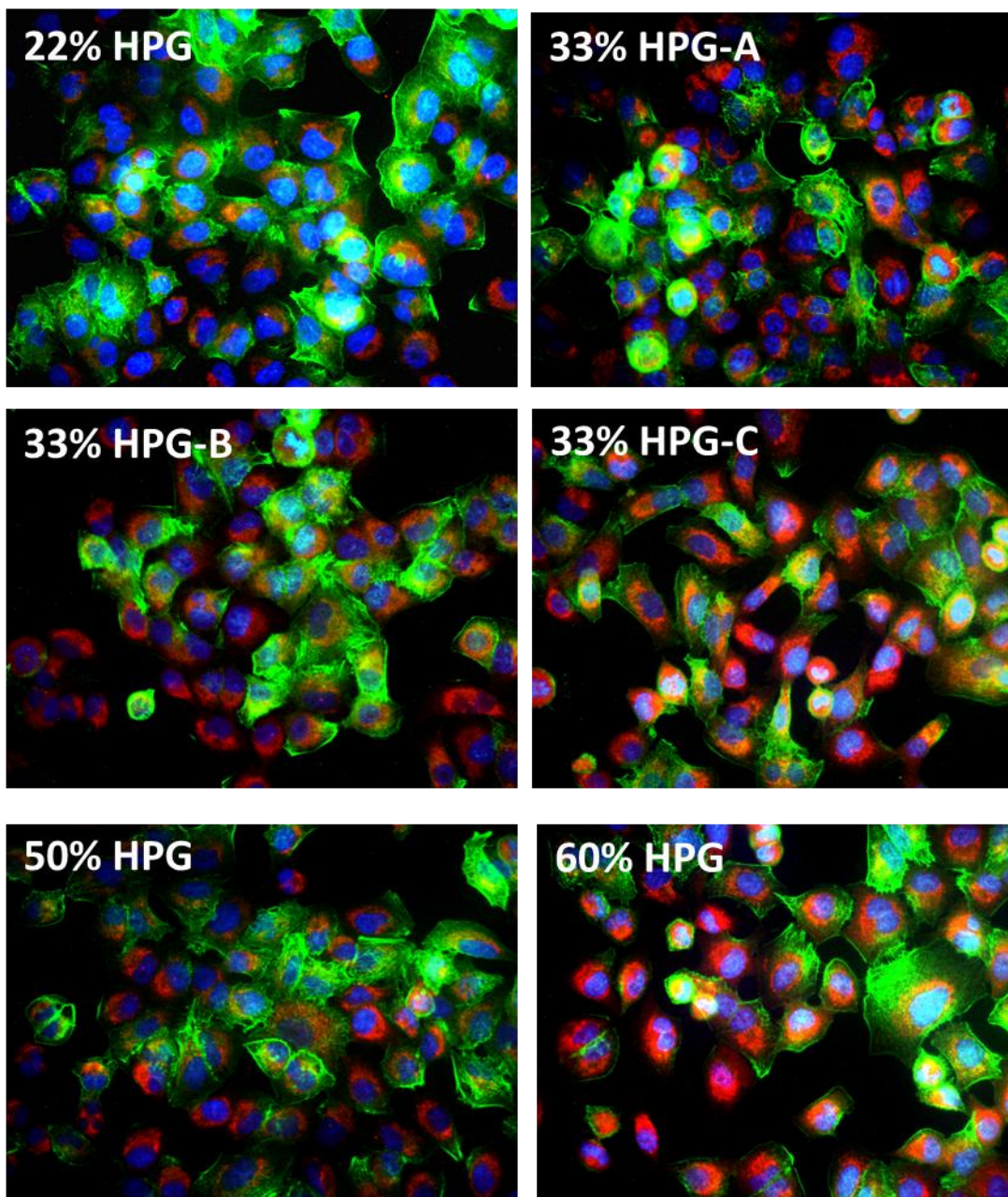


S8.F. 60% HPG



S9. Cellular uptake analysis

A549 lung cancer cells were cultured for 4 h at 37°C with different HPG-RodB nanogels (red). Cytoskeleton was stained with Phalloidin-Alexa488 (green) and the nucleus with DAPI (blue).



Likewise, human epidermoid cancer cells were cultured and analyzed regarding their nanogel uptake profile. The following figure compares qualitatively the uptake of HPG nanogels (rhodamin B labeled, red) in A549 lung cancer cells and A431 human epidermoid cancer cells at 37 °C. Nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI, blue).

