Electronic Supplementary Information

Effect of Mechanical Properties of Hydrogel Nanoparticles on Macrophage Cell Uptake

Xavier Banquy, Fernando Suarez, Anteneh Argaw, Jean-Michel Rabanel, Peter Grutter, Jean-François Bouchard, Patrice Hildgen and Suzanne Giasson

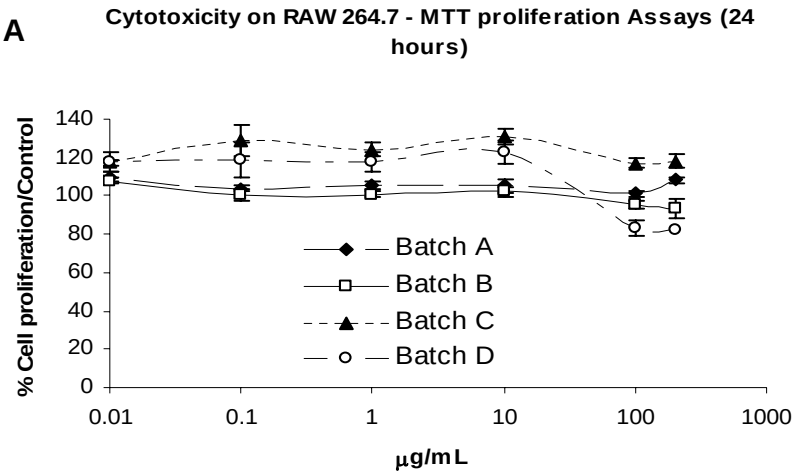
NPs Cytotoxicity. NPs cytotoxicity was assessed using MTT and LDH assays. Raw 264.7 cells were diluted in complete medium at a final concentration of 5 × 105 cells/mL and plated (100 µL/well) on a 96 well-flat bottom microplate (Corning, NY, USA). NPs suspended in 10 µL PBS were added in the wells in triplicate for each different NP concentrations (0 et 100 µg/ml. The plates were incubated for 24 h after which cellular proliferation was assessed by MTT assay. Cell medium was removed and the cell monolayer was washed with PBS. Then fresh medium 90 µL and 10 µL of thiazolyl blue tetrazolium bromide dissolved in PBS (10 mM, pH 7.4) at a concentration of 5 mg/mL and filtered on 0.22 µm sterile filter (Millipore, Bedford NMA USA) were added to each well. After 3 h incubation time at 37 °C in 5 % CO_2 atmosphere, 50 µL of solubilising solution (Isopropanol, 10 % (w/v) Triton-X 100, 0.1 N HCl) was added to each well to dissolve the dark blue formazan crystals. Absorbance was measured at a wavelength of 570 nm using a microplate reader (SAFIRE, Tecan, Austria).

The presence of lactate deshydrogenase (LDH) in the supernatant obtained from proliferation assays was used as an indicator of cell lysis. It was determined using a commercial dosing kit (Sigma, Oakville, ON, Canada). Briefly, after incubation of Raw 264.7 cells with the NPs, 5 µl of cell supernatant were transferred to a new microplate and incubated in the dark with the reaction mixture from the dosing kit for 30 minutes. The reaction was stopped with 0.1 N HCl. Microplates were analyzed using a microplate reader at a wavelength of 450 nm (reference wavelength at 690 nm). Results are compared with positive control wells consisting of 100 % Triton-X 100 lysed cells.

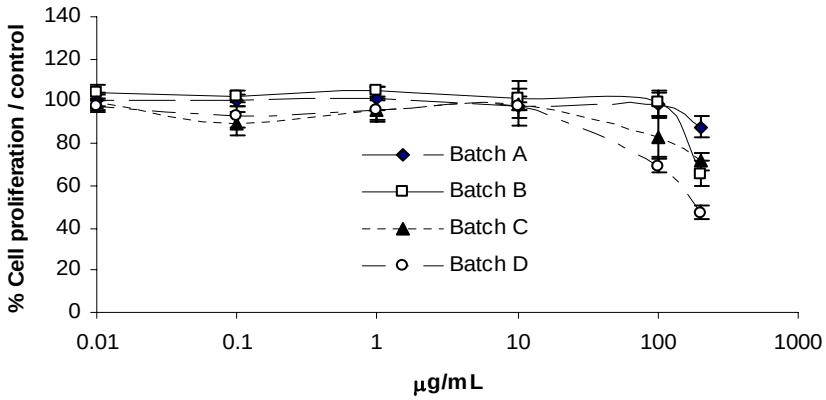
No cytotoxicity, assessed by both assays, could be evidenced for 24 (Fig. a and c) and 72 h (Fig. b and d) for NPs from the 4 batches for concentrations lower than 100 µg NPs/mL.

At a concentration of 100 µg NPs/mL, the cell proliferation remained relatively large for batches A, B, C, i.e.> 85 %, and decreased to 75% for D (Fig. a and b). However, the cell lysis remained relatively low for all batches (Fig. b and d). Therefore, the NPs from all batches are not considered as cytotoxic. No changes in cell morphology were observed during the experiment which indicates that the presence of NPs did not activate the cells.

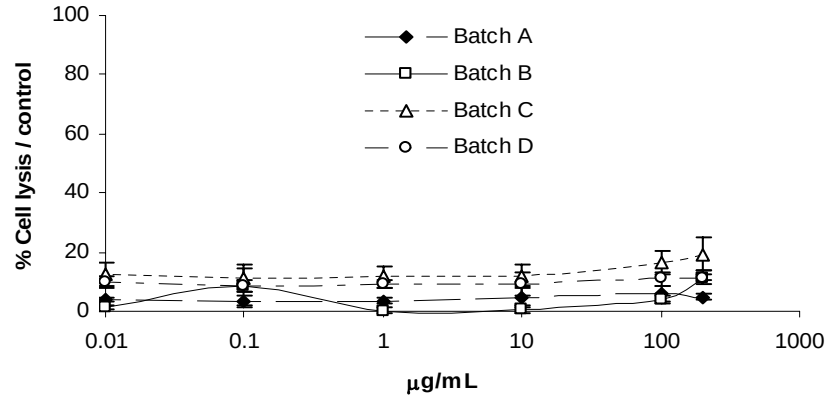
A


Cytotoxicity on RAW 264.7 - MTT proliferation Assays (24 hours)
% Cell proliferation/Control
140
120
100
80
60
40
20
0
0.01
0.1
1
10
100
1000
µg/mL
Batch A
Batch B
Batch C
Batch D

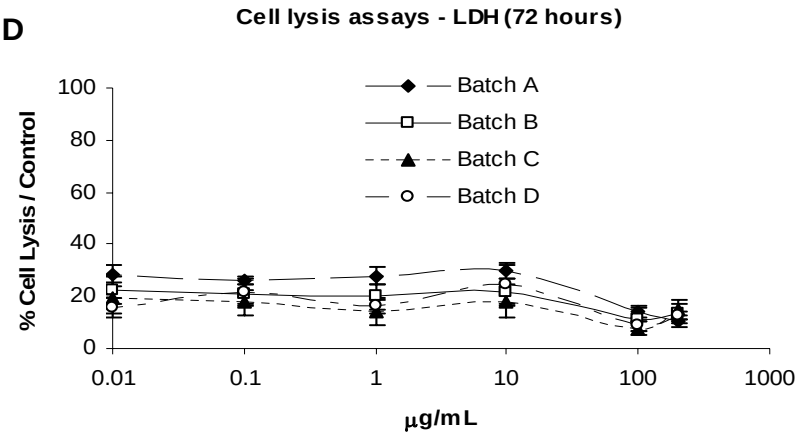
B


Cytotoxicity on RAW 264.7 - MTT proliferation Assays (72 hours)
% Cell proliferation / control
140
120
100
80
60
40
20
0
0.01
0.1
1
10
100
1000
µg/mL
Batch A
Batch B
Batch C
Batch D

C


Cell lysis assays - LDH (24 hours)
% Cell lysis / control
100
80
60
40
20
0
0.01
0.1
1
10
100
1000
µg/mL
Batch A
Batch B
Batch C
Batch D

D


Cell lysis assays - LDH (72 hours)
Batch A
Batch B
Batch C
Batch D
% Cell Lysis / Control
100
80
60
40
20
0
0.01
0.1
1
10
100
1000
µg/mL