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SUPPLEMENTARY INFORMATION

Poly (ε-caprolactone) microspheres for prolonged release of selenium nanoparticles

Nenad Filipović[1], Ljiljana Veselinović[1], Slavica Razić[2], Sanja Jeremić[3], Metka Filipič[4], Bojana Žegura[4], Sergej Tomić[5], Miodrag Čolić[5,6], Magdalena Stevanović[1]1

[1]Institute of Technical Sciences of the Serbian Academy of Sciences and Arts, Knez Mihailova 35/IV, 11000 Belgrade, Serbia

[2]Faculty of Pharmacy - Department of Analytical Chemistry, University of Belgrade, 11000 Belgrade, Serbia

[3]Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, 11000 Belgrade, Serbia

[4]Department of Genetic Toxicology and Cancer Biology, National Institute of Biology, Večna pot 111, 1000 Ljubljana, Slovenia

[5]Institute for the Application of Nuclear Energy, University of Belgrade, 11000 Belgrade, Serbia

[6]Medical Faculty of the Military Medical Academy, University of Defence, 11000 Belgrade, Serbia

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[1] Corresponding author: Magdalena Stevanović; Tel.: +381-11-2636-994; Fax:+381-11-2185-263

Institute of Technical Sciences of the Serbian Academy of Sciences and Arts

Knez Mihailova 35/IV, 11000 Belgrade, Serbia

E-mail address :magdalena.stevanovic@itn.sanu.ac.rs; magir@eunet.rs

nuclear stain. PCL/SeNPs were detected as brightly scattering particles sized about 1-4 μm after 546nm laser excitation either intracellularly within granulocytes (A) or extracellularly (B). Note that some cells expressed strongly CD45 on the membrane and the cytoplasm, whereas others displayed a weak membrane expression and a strong expression in the granular ER at the nucleus level.

Table 1. Melting temperatures Tm and corresponding enthalpies (heat) of fusion ΔHf of PCL/SeNPs samples taken after different time from different degradation mediums

Table 2. Melting temperatures and corresponding enthalpies of PCL/SeNPs samples taken after different degradation periods from P. aeruginosa CFE medium

1. Experimental deteails for ICP-OES measurments

1.1. Instrumental and operating conditions

A ICP-OES Thermo Scientific iCap 6500 Duo instrument, equipped with a concetric glass nebulizer was used, with an input power of 1150 W and a frequency of 27 MHz, the argon flow rates were: 12 L/min (outer gas), 0.5 L/min (intermediate gas) and 0.4 L/min (carrier gas). The sample uptake rate of 1.2 mL/min, was controlled by a peristaltic pump. For microwave acid assisted digestion, close-vessel high-pressure microwave digesters - CEM MDS-2100 was used. For accurately weighing of samples analitical balance – METTLER AE 166 was used. The intensity of emission was measured at λ= 196.26 nm

1.2.Solutions and Reagents

Analytical grade chemicals and Milli-Q (18 MΩ) water were used. Nitric acid (65%, v/v) was provided by Lachner (Czech Republic) and solution of H_2O_2 (30%, v/v) by Centrohem (Serbia). Single standard, Se, c = 1000 μg/ml Accu Trace AA51N-1 and multi standard MES 21-1 ACcu Trace were used.

1.3. Microwave assisted acid digestion

Accurately weighed (±0.0001 g) samples were subjected to microwave assisted acid digestion with 7 mL of HNO_3 (65%, v/v) and 2 mL of H_2O_2 (30%, v/v). After waiting for 10 min to avoid the first vigorous chemical reaction the digestion was performed according to the temperature programmes described elsewhere (*S. Ražić et al., J. Serbian Chem. Soc. 71 (2006) 1095–1105*). After cooling, the content was filtrated through a Millipore 0.45μm filter, and the solution was transferred quantitatively into 25 mL calibrated flask and diluted to volume with Milli-Q water. At least one sample blank, containing the same amounts of acid and oxidant, was processed along with each set of samples.

1.4. Calibration curve

External calibration was used for determination of Se. Correlation coefficient, corresponding calibration curve and limit of detection were as folows:

R = 0.9997, y= 8.11 + 931.45x

LOD = 0,0017 mg/l

2. Experimental deteails for biocompatibility investigations of PCL/SeNPs

2.1. Cell culture

The HepG2 cell line was obtained from American Type Culture Collection (Manassas, VA, USA). The cells were grown at 37 °C and 5% CO2 in humidified atmosphere in Minimal Essential Medium Eagle, (MEM; Gibco, Paisley, UK) medium supplemented with 10% foetal bovine serum (Gibco, Paisley, UK), 2 mM L-glutamine (Sigma, Steinheim, Germany 1% non-essential aminoacids (Sigma, 100 IU/ml penicillin/ streptomycin (Sigma, Steinheim, Germany). Cells were routinely checked for mycoplasma (MycoAlert™, Lonza, Walkersville, USA). The experiments were performed on HepG2 cells between cell passage 15 and 25.

2.2. Determining citotoxicity of samples - MTT assay

Cells were seeded onto 96-well microplates (Nunc, Naperville, IL, USA) at a density of 40 000 cells/ml and incubated for 24 hours at 37^{o}C/5% CO_2 to attach. The medium was then replaced by fresh medium containing different concentration of PCL/SeNPs (0.0001, 0.001, 0.01, 0.1 and 1 v/v %) and incubated for 24 h. Experiments were conducted in five parallels and also in each experiment a negative control (non-treated cells; C), solvent control (1% ethanol; 0) and positive control (etoposide 125 µg/mL; PC). After the incubation with PCL/SeNPs, MTT (final concentration 0.5 mg/ml) was added and incubated for additional 3 hours. Finally, medium with MTT was removed and formed formazan crystals were dissolved in DMSO. The optical density (OD) was measured at 570 nm (reference filter 690 nm) using a microplate reading spectrofluorimeter (Synergy MX, BioTek, Winooski, VT, USA). Viability was determined by comparing the OD of the wells containing the treated cells with those of the solvent control. Statistical significance between treated groups and solvent control was determined by One-way analysis of variance and Dunnett's Multiple Comparison Test, using GraphPad Prism 5 (GraphPad Software, San Diego, CA, USA) and $P<0.05$ was considered as statistically significant.

2.3. Determination of intracellular reactive oxygen species formation – DCFH-DA assay

Dichloro-dihydro-fluorescein diacetate (DCFH-DA)DCFH-DA is a fluorescent probe which readily diffuses through the cell membrane where is hydrolyzed by intracellular esterases to non-fluorescent 2′,7′-dichlorofluorescin. In the presence of intracellular ROS, it is then rapidly oxidized to highly fluorescent 2′,7′-dichlorofluorescein. The DCF fluorescence intensity is proportional to the amount of reactive oxygen species formed intracellularly. The cells were seeded at a density of 75 000 cells/ml into 96-well, black, tissue culture treated microtiter plates (Nunc, Naperville IL, USA) in five replicates and wereincubated for 24 h at 37°C/5 % CO_2 to attach. Afterwards, the cells were loaded with DCFH-DA (20 μM) and after 30 min DCFH-DA was removed. Subsequently the cells were treated with different concentration of PCL/SeNPs (0.0001, 0.001, 0.01, 0.1 and 1 v/v %). Negative (non-treated cells-C), solvent (1% ethanol) and positive (tert-butyl hydroperoxid -TBHP at a concentration of 500 μM) controls were included in each experiment. For the kinetic analyses of the intracellular ROS formation, the plates were maintained at 37°C and the fluorescence intensity was determined (at excitation wavelength 485 nm and an emission wavelength of 530 nm) every 30 min during the 5 h incubation using a microplate reading spectrofluorimeter (Synergy MX, BioTek, Winooski, VT, USA). Statistical significance between treated groups and controls was determined by One-way analysis of variance (ANOVA) and Dunnett's Multiple Comparison Test, using GraphPad Prism 5 (GraphPadSoftware; San Diego, CA, USA); $P<0.05$ was considered as statistically significant. Three independent experiments with five replicates were performed and the results of one representative repetition are presented.

2.4. DNA damage (comet assay)

HepG2 cells were seeded at a density of ≈80 000 cells/ml into 12-well microtiter plates (CorningCostar Corporation, Corning, NY, USA) and incubated for 24 h at 37°C and 5 % CO_2 to attach. The growth medium was then replaced with fresh medium containing different concentration of PCL/SeNPs (0.001, 0.01, 0.1 and 1 v/v %) and incubated for 24 h. In each experiment negative (non-treated cells-C), solvent (1% ethanol) and positive (benzo[a]pyrene - BaP at a concentration of 30 μM) controls were included. Three independent experiments were performed for each of the treatment conditions. One-way analysis of variance (ANOVA, Kruskal–Wallis) was used to analyze the differences between the treatments within each experiment. Dunnet's tests were used for comparing median values of percentage tail DNA; $p<0.05$ was considered as statistically significant.

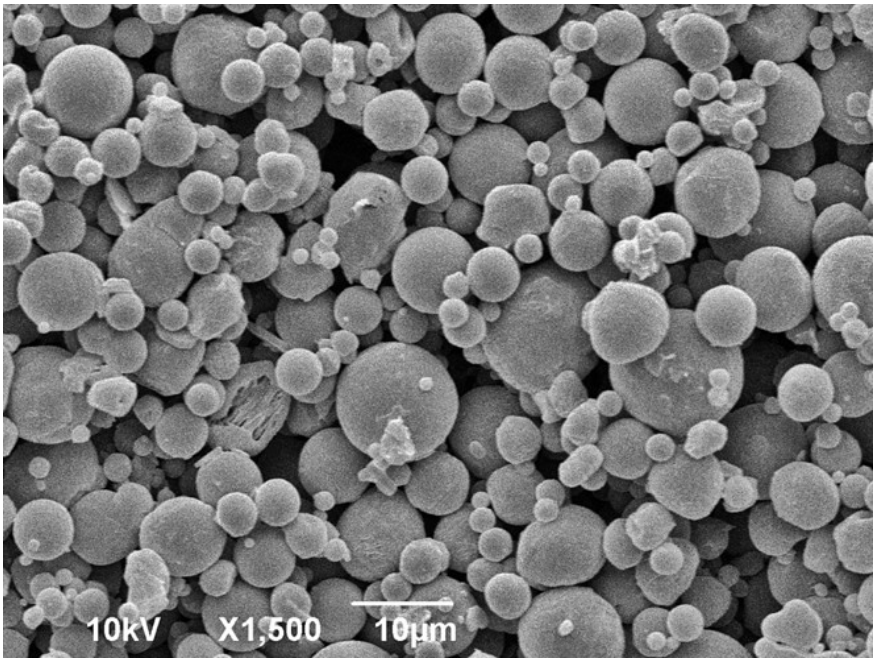

Figure 1. SEM image of blank PCL microspheres.

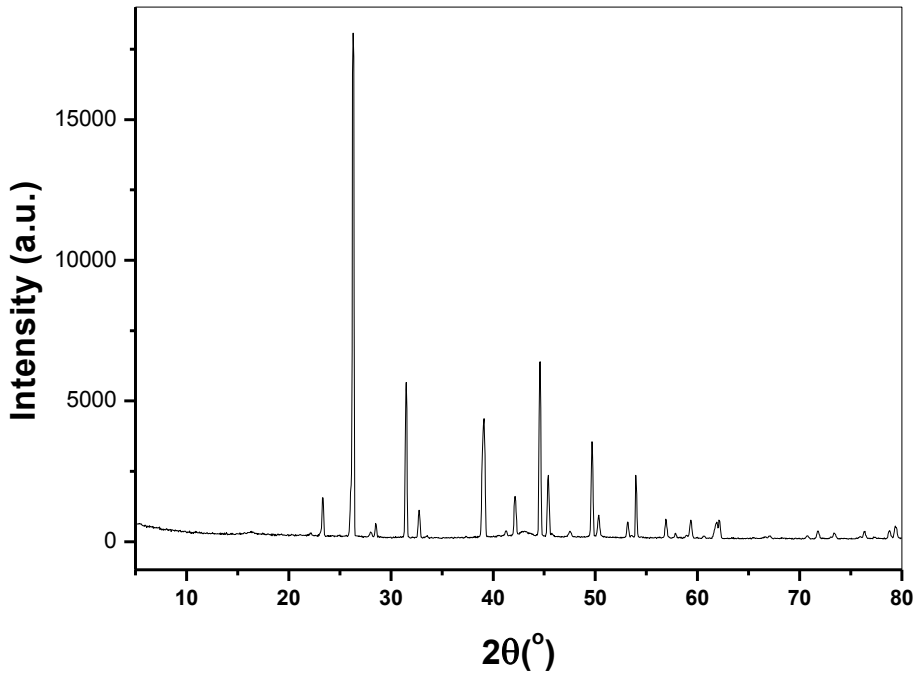

Figure 2. XRD pattern of commercial PGA powder used in the preparation of PCL/SeNPs. Dominant reflection is detected at 26.3 ° of 2θ angle.

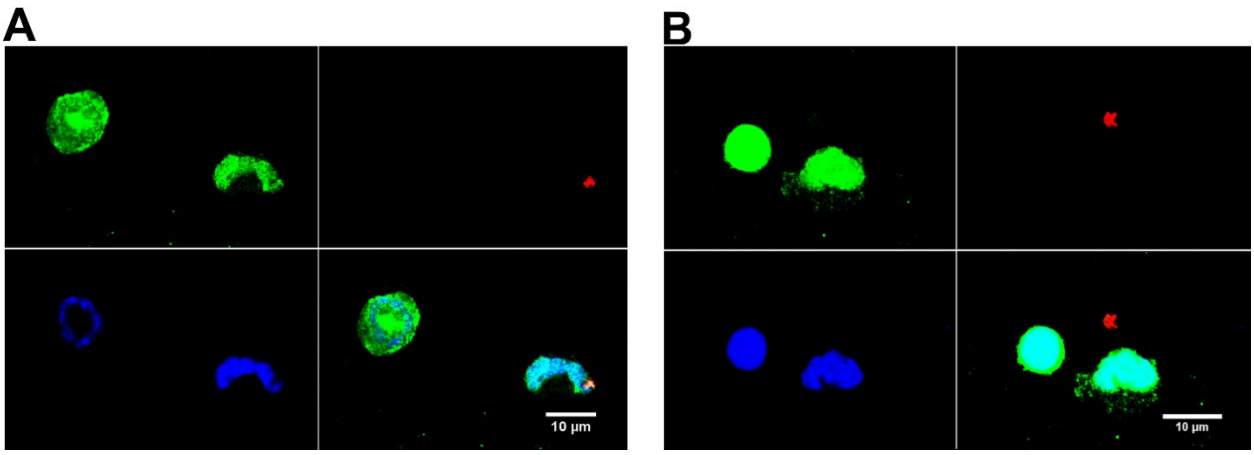

Figure 3. Interaction with PCL/SeNPs *in vivo* by infiltrating cells. PCL/SeNPs (4mg/animal) were injected into sterile polyvinyl sponges implanted subcutaneously. The infiltrating cells were collected from the sponges after 3h and stained to anti-CD45/IgG Alexa 488 (Green) and Syto59 nuclear stain. PCL/SeNPs were detected as brightly scattering particles sized about 1-4 µm after 546nm laser excitation either intracellularly within granulocytes (A) or extracellularly (B). Note that some cells expressed strongly CD45 on the membrane and the cytoplasm, whereas others displayed a weak membrane expression and a strong expression in the granular ER at the nucleus level.

Table 1. Melting temperatures T_m and corresponding enthalpies (heat) of fusion ΔH_f of PCL/SeNPs samples taken at predetermined times from different degradation mediums.

Time intervals (days)	**PBS** T_m(°C)/ΔH_f(J/g)	**PBS+lipase** T_m(°C)/ΔH_f(J/g)	**HCl** T_m(°C)/ΔH_f(J/g)
7	65.0 / 73.52	65.7 / 82.25	65.5 / 80.16
14	65.3 / 76.48	66.0 / 84.76	65.6 / 85.15
21	65.7 / 82.40	66.1 / 89.14	65.6 / 83.38
36	65.5 / 86.34	66.2 / 89.66	65.3 / 86.52
50	66.0 / 86.35	66.2 / 88.73	65.9 / 87.40
108	66.1 / 88.97	66.6 / 94.84	65.8 / 87.70
660	67.1 / 95.55	67.2 / 95.99	67.0 / 95.81

Table 2. Melting temperatures and corresponding enthalpies of PCL/SeNPs samples taken after different degradation periods from P. aeruginosa CFE medium.

Time intervals (days)	T_m (°C)	ΔH_f (J/g)
1	63.0	74.3
7	64.6	80.1
14	65.0	82.2
21	65.6	94.9