In vitro biocompatibility and cell permeability study of biodegradable nanoparticles made of amino acid based poly(ester amide)

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Introduction

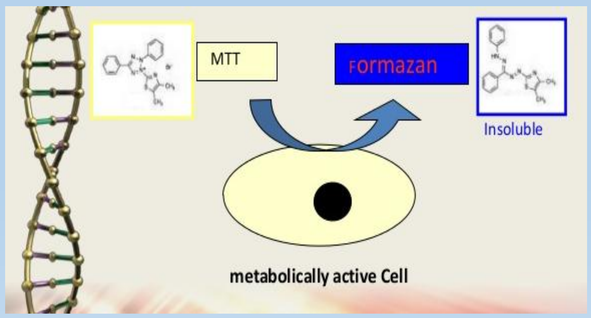
Amino Acid Based Biodegradable (AABB) polymers are promising materials for sophisticated biomedical applications. We have already carried out a systematic study of the AABB polymers NPs' fabrication using a cost-effective method - nanoprecipitation (polymer deposition /solvent displacement) from organic phase into water phase containing a surfactant. This study revealed that in terms of particles size, stability and biocompatibility the best appeared to be: as AABB polymer — poly(ester amide) composed of L-leucine, 1,6-hexanediol and sebacic acid — 8L6, as a solvent (organic phase) — DMSO, and as a surfactant — Tween 20. For *in vitro* biocompatibility assessment of the NPs several established cell lines have been used. In the initial experiments cytotoxicity level of the NPs has been checked only at one time point – after 24 h incubation with the NPs.

Research goal

In vitro study of biocompatibility (cytotoxicity) and cell permeability of biodegradable poly(ester amide) nano-particles using three types of cell lines: A549 – human alveolar epithelial type II cells derived from lung carcinoma, Hepa1-6 – mouse hepatoma derived cells, RAW264.7 – mouse leukemic monocyte macrophage cell line; evaluation of NPs penetration in hepatic spheroids made of Hepa1-6 cells.

Methods

The in vitro biocompatibility study of NPs have been performed using three established cell lines, previously used by us for biocompatibility study at 24 hour time-point: A549 – human alveolar epithelial type II cells derived from lung carcinoma, Hepa1-6 – mouse hepatoma derived cells, RAW264.7 – mouse leukemic monocyte macrophage cell line. The cytotoxicity of NPs was assessed by MTT assay based on the ability of a mitochondrial dehydrogenase enzyme in viable cells to cleave the tetrazolium rings of the pale yellow MTT and form a dark blue formazan crystal (see scheme below). The number of surviving cells is directly proportional to the level of the formazan product created, which can then be quantified by reading absorbance at a wavelength of 570 nm with a multiwell scanning spectrophotometer. In parallel, NPs inside the outer, capsule-like layer of hepatic spheroids have been assessed using TEM.



Results

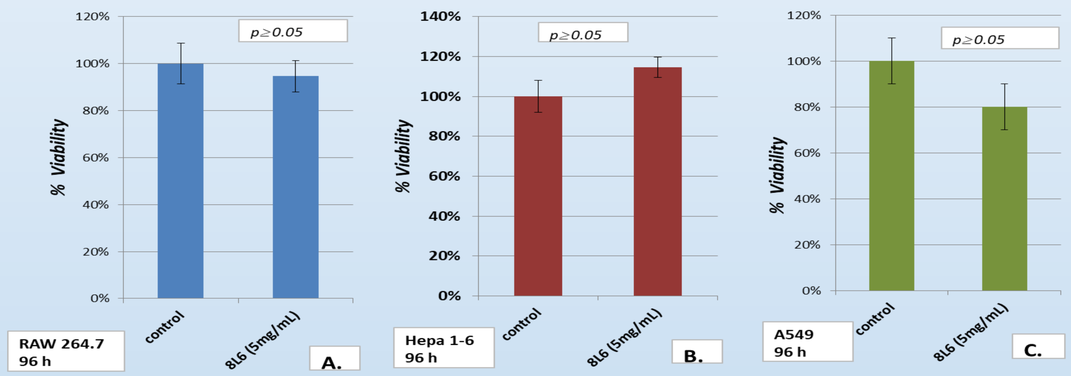


Figure 1 . Percentage of viable cells after 96h incubation with NPs made from 8L6 AABB polymer (5.0 μg/mL) with: (**A**) A549 cell line; (**B**) Hepa 1-6 cell line; (**C**) RAW264.7 cell line. The NPs were fabricated from DMSO solution in the presence of Tween 20. *Data is presented as average ± st.error for four independent experiments, p value calculated according two-tailed Mann-Whitney U test.*

As it can been seen from Fig. 1, according to the obtained results for all three cell lines tested, there was no statistically significant decrease of cell viability (compared to the control) after the 96 hour-long incubation with NPs made from 8L6 AABB polymer, used at concentration 5.0 μg/mL. This data indicates a high biocompatibility of NPs fabricated from 8L6 polymer.

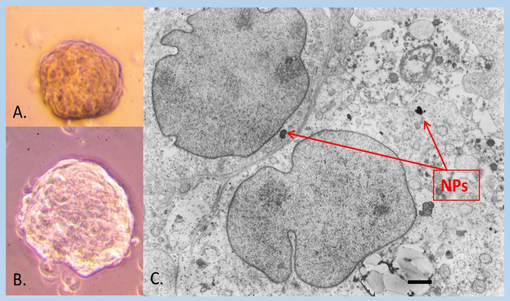


Figure 2. (A, B) 5-day spheroids from Hepa 1-6 cells, Phase-Contrast Microscopy, Nikon TMS: **(A)** X10; (**B)** X40; **(C)** TEM image of ultra-thin section of hepatic spheroid, incubated for 24h with NPs made from 8L6 AABB polymer, arrows show discrete NPs.

Conclusion

In vitro study data indicates a high biocompatibility of biodegradable 8L6 NPs

References

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