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"Nano- and microparticles from polylactides, poli(ethylene oxide) and their derivatives as carriers of bioactive molecules„

Polymeric nano- and microparticles are considered as the future of drug delivery. Amongst numerous synthetic polymers, polyethers and polyestrers can be distinguished for their biocompatibility. Proteins and nucleic acids are those bioactive substances which especially requires a suitable carrier which would enable to use them as dugs.

The first aim if this work was to determine the possibility of encapsulation of insulin inside polyether-polyester block copolymer micelles. The polyether part was built from polyglycidol, which, similarly to PEG, is a biocompatible, water-soluble polymer. However, its hydroxyl side groups facilities micelle's corona further modification. For a hydrophobic block of micelles polylactide was chosen. Within this project I was focused on the determination of the influence of the length of hydrophilic block of copolymer on physicochemical properties of micelles and micellar formulations with insulin, including encapsulation process itself and drug release. The second aim of this work was to evaluate whether linear polyethers bearing amine groups could be used as efficient plasmid DNA carriers. The influence of molecular weight and the degree of substitution of nitrogen atoms on cytotoxicity, DNA complexation and transfection efficiency was established.

It was revealed that insulin can be efficiently encapsulated into micelles formed by block copolymers of glycidol and lactide with constant average molecular weight of polylactide block ca. 5000 and the average molecular weight of polyglycidol varying from ca. 3800 to 12800. Simultaneously, it was demonstrated that the molecular weight of polyglycidol block has a profound impact on formulation's properties. The best result were obtained in case of PGL_PLLA1 copolymer (DP_n (glycidol) = 52). Micelles formed by this copolymer were characterized by the lowest average diameter. Moreover, it showed the highest encapsulation efficiency (ca. 75%) and the best insulin release profile with low initial burst effect and slow drug release for about 24 hours. CMC of examined copolymers, which in case of PLG_PLLA1 was 0,4o mg/ml, is also considered to have substantial impact on formulation properties.

In order to determine the possibility of usage of polyamineethers of different molecular weight and nitrogen atoms' substitution degree as gene carriers, a series of poly(glycidyl amines) (including its block copolymers with ethylene oxide), poly(2,3-epoxypropyldiethylamine) (PEPDEA) and qurartenized poly(2,3-epoxypropyldiethylamine) was synthesized (PEPDEA_Q4) was synthesized. Poly(glycidyl amines) was obtained in three-step reaction (i.e. a one-pot sequence of Appel reaction, chloride substitution reaction with azide group and finally Staudinger reaction) leading to the conversion of hydroxyl groups of polyglycidol to primary amines groups. Poly(2,3-epoxypropyldiethylamine) was obtained by anionic polymerization of 2,3-epoxypropyldiethylamine) initiated with potassium *tert*-butoxide. Quarternized derivatives of poly(2,3-epoxypropyldiethylamine) were synthesized by reacting Poly(2,3-epoxypropyldiethylamine) with bromoethane (average quarternization degree = 70%). The cytotoxicity of synthesized polymers was examined using HeLa, K562 and HUVEC cells. Poly(glycidyl amines) which average polymerization degree varied form 59 to 210 are highly cytotoxic for all used cell lines. Homopolymers of 2,3-epoxypropyldiethylamine which average polymerization degree is below 90 are nonotoxic for

HUVEC cells. In case of HeLa cell, PEPDEA which average polymerization degree is equal 30 (PEPDEA1) is nontoxic. However, LC_{50} of homopolymer PEPDEA2 (DP_n = 90) was equal 10 μg/ml. In case of K562 cells LC_{50} were equal 70 and 30 μg/ml for PEPDEA1 and PEPDEA2 respectively. Quarternized derivatives of PEPDEA (PEPDEA1_Q4 and PEPDEA2_Q4) were found to be less toxic than their precursors. It was revealed that they are nontoxic for HeLa and HUVEC cells. In case of K562 cell, PEPDEA1_Q4 is nontoxic while LC_{50} of PEPDEA2_Q4 was estimated to be 100 μg/ml. Generally, obtained results shows that the cytoxicity of examined polymers rises with their molecular weight. On the other hand, it is lower for polymers with higher substitution degree of nitrogen atoms.

The ability of PEPDEA and its quartenized derivatives was examined using gel electrophoresis, ethidium bromide assay, and photon correlation spectroscopy. Gel electrophoresis revealed that PEPDEA and its quartenized derivatives fully complex plasmid DNA at weigh excess equal 10 and 20 respectively. Ethidium bromide assay revealed that polymers with higher average polymerization degree more efficiently complex plasmid DNA. Nonetheless, none of examined polymers was able to efficiently transfect cells. The reason of their poor efficiency is the size of formed polyplexes, which exceeds the optimal size for efficient endocytosis (below 200 nm). Pictures of cell in the medium with polyplexed formed with BOBO-3 marked plasmid shows that complexes gathers around cells but cannot be found inside cells.