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Biodegradable dextran-based nanoparticles as potential anticancer drug carrier

Introduction

Nanotechnology is nowadays a big hope for an effective solution in anticancer therapies. Due to diameter of about 100 nm nanoparticles manage to penetrate leaky structure of newly formed blood vessels and spontaneously accumulate in tumor area. What is more, such small size allows them to avoid opsonisation process and fast clearance from the bloodstream, resulting in longer circulation time and greater chance of reaching the tumor. Nanoparticles biocompability and biodegrability is an important issue when designing an effective drug carrier. That is why described nanoparticles are composed of dextran – natural polymer, already approved by FDA (*Food and Drug Administration*, USA).

The aim of the studies is to obtain nontoxic nanocarrier and adjust reaction conditions to form nanoparticles ranging in size from 50 to 150 nm. Nanoparticles are obtained by self-assembly method, preceded by chemical modification of dextran chain with coiling agent. Thanks to self organizing strategy, nanoparticles are easy to prepare without addition of toxic components.

Materials and methods

Nanoparticles are composed of dextran (MW: 70 kDa) and polycaprolactone which is the coiling agent attached to the polysaccharide frame. Reaction is carried out in anhydrous conditions in dimethyl sulfoxide (DMSO) dried on molecular sieves with addition of dibutylzinc dilaurate as a catalyst. The spontaneous formation of nanoparticles occurs during purification by dialysis against pure water. A catalyst precipitates during purification process and is separated by filtration. Nanoparticles are dried using spray drying and rehydrated in cell culture medium to investigate cytotoxicity of the possible drug carrier.

Nanoparticles preparation

Nanoparticles synthesis is based on ring opening polymerization (ROP) [*Oktay et al., 2013*] of ε-caprolactone (*Sigma Aldrich*) on hydroxide groups of dextran *(Nobilus-Ent*, Poland). As a catalyst of the reaction dibutylzinc dilaurate (*Sigma Aldrich*) was used. Divalent zinc ion activates the carbonyl group of the monomer and starts polymerization. Reaction mechanism requires organic environment, therefore DMSO (*Carlo Erba*) was used as a solvent and reaction medium. [*Shi and Burt, 2004; Gref et al., 2002*] To investigate the process and influence of catalyst amount on the yield and nanoparticles diameter number of experiments was performed changing the percentage of dibutylzinc dilaurate added. Fig. 1 presents reaction of spontaneous polymerization of ε-caprolactone.

Fig. 1. Conditions and mechanism of ring opening polymerization of ε-caprolactone

Polymerization was carried out in DMSO dried on molecular sieves (*Sigma Aldrich*; Ø = 3Å) and run for 24 h in 50°C. Dextran was dissolved in DMSO in concentration of 2.5%. Amount of ε-caprolactone added was theoretically estimated to substitute every tenth glucose unit in polysaccharide chain. Five concentrations of catalyst added to the reaction medium were compared to adjust its amount to obtain possibly the highest yield and size distribution in appropriate range. Taken amount of catalyst was described as the percentage of molar mass of all reagents taken to reaction including solvent and was 0, 0.25, 0.5, 0.75 and 1%.

After reaction the product was purified by step dialysis in dialysis bag of MWCO 14 kDa (*Carl Roth*) against pure water. According to the literature dialysis of DMSO should be carried out slowly in stages to avoid maring of the solvent and provide total dialysis. Dialysis was carried in the first step placing the sample in a volume of water equal twice the volume of sample, and every 20 minutes the water volume was doubled. After an hour water was replaced with fresh one and the process continued for another 24 h, replacing the water from time to time. Simultaneously, modified dextran chain assembles into nanoparticles through hydrophilic-hydrophobic interactions and catalyst (insoluble in water solution) precipitates.

Diameter of nanoparticles was determined using NTA (Nanoparticle Tracking Analysis) (*NanoSight, Malvern Inst.*). Each sample before measurement was filtered on syringe filter (PTFE 0.45µm) to make sure that any microscopic pollutions will not interfere with the outcome. Yield of reaction was defined as concentration of nanoparticles measured after reaction, also estimated by *NanoSight*. Product was dried by spray drying (*Mini Spray Dryer B-290, Büchi*) [*Faure et al., 2010*], inlet temperature about 150°C and outlet temperature of 80°C.

Cytotoxicity assay

Safety of acquired nanocarrier was confirmed by cytotoxicity assay MTT (*Sigma Aldrich*) examining enzymatic activity of living cells. MTT is the yellow fluorescent tetrazolium (3-(4, 5-dimethylthiazolyl-2) -2,5-diphenyltetrazolium bromide) and is reduced by living cells by active dehydrogenase enzymes and as a result purple formazan crystals are forms. Crystals are dissolved in DMSO with addition of *Sorensen* buffer and quantified by spectroscopic measurement, readout of absorbance at 570nm [*ATCC MTT, 2014*]. Test was performed on mice fibroblast cells (L929) adherent cell line cultured on DMEM without phenol red (*Invitrogen*) supplemented with 10% FBS, 1% penicillin, 1% streptomycin and 1% L-glutamine. Cells were cultured in 37°C with 5% CO_2. Cells were grown on 96-well plate, sterile solutions of nanoparticles were added and plate was incubated for 24h.

Results and discussion

Nanoparticles' diameter and concentration measurements are presented in Tab. 1. To obtain reliable results samples were estimated using the same values of key parameters.

Tab. 1. Influence of amount of catalyst added to reaction on diameter of nanoparticles and concentration.

	Diameter [nm]		Concentration [part./ml]
% Sn	mean	mode	
0	145	134	$2.59 \cdot 10^8$
0.25	168	138	$10.71 \cdot 10^8$
0.5	113	99	$13.31 \cdot 10^9$
0.75	110	79	$3.13 \cdot 10^8$
1	125	110	$12.01 \cdot 10^8$

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Mode value of NPs diameter gives the size of the majority of nanoparticles present in suspension and mean value provides an overview on dispersity of the sample. The closer the mean value to the mode value the lower dispersity. Comparing the results, all variants are acceptable when it comes to the size demand, however two proportions: 0.5% and 0.75% are preferable due to diameter below 100 nm. Thus it can be assumed that after drug loading these nanoparticles will retain appropriate size. Further discussion then narrowed to these two proportions. Concentration turned to be the highest when 0.5% of dibutylzinc dilaurate was added to reaction. It was 100 times higher than for 0.75% catalyst addition, therefore that proportion was chosen for further experiments.

Nanoparticles were dried by spray drying method and measured again after rehydratation. The diameter 104 nm (mean: 142) shows that majority of NPs returned to their original size distribution.

Particles were easily soluble in cell culture medium. The cytoxicity was inspected both by MTT assay and by observation of cell morphology. The range of NPs concentrations was 1 mg/ml to 10^{-7} mg/ml with a change in an order of magnitude. Results were corresponded to the control raw – cells growing on culture medium without addition of nanoparticles. Results of MTT assay are presented in Fig. 2.

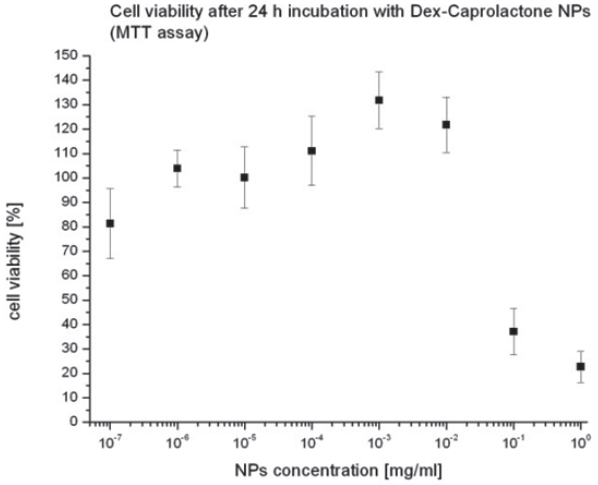

Fig. 2. Call viability after 24 h incubation with Dex-caprolactone nanoparticles

The plot shows that only very high concentrations of nanoparticles were toxic for cell viability. Solutions of concentration 10^{-2} mg/ml and smaller did not show any cytotoxic influence on cells.

Viability value is referred to the viability of control raw, therefore the result of over 100% is related to various amount of cells present in the wells and should be considered as systematic deviation. Toxicity of two the highest concentrations tested could correspond with too many additives in the medium and as cells being exposed directly to the NPs solution are forced to incorporate them, these amounts effectively disrupts cell's metabolism. Moreover the concentration is far too high when compared with doses of drugs administrated in clinic.

In Fig. 3 microscopic photographs of cells after 24 h incubation were presented. It can be clearly seen that cells treated with the highest concentration have apoptotic structure. In this case shrunken non-adherent cells were observed whereas the two remaining photographs showed no significant difference when compared to the control. Pictures confirmed the result of the cytotoxicity assay.

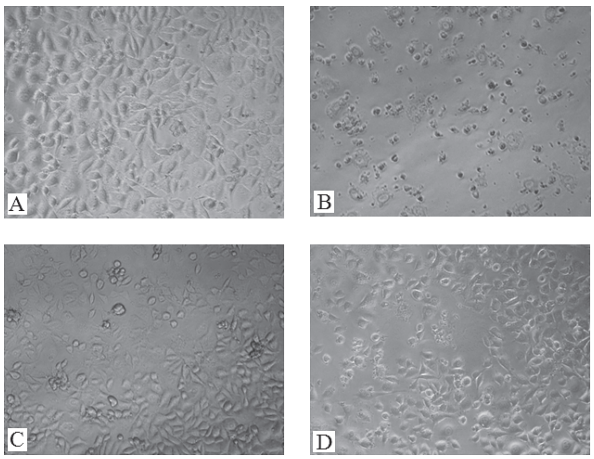

Fig. 3. Photographs of L929 cells exposed to different concentrations of nanoparticles (optical microscope OPTA TECH, magnification 10x10): A) control – cells grown in supplemented DMEM; B) cells exposed to NPs solution of concentration 10^{0} mg/ml; C) cells exposed to NPs solution of concentration 10^{-3} mg/ml; D) cells exposed to NPs solution of concentration 10^{-7} mg/ml

Conclusions

Nanoparticles were prepared in simple one-step chemical modification and spontaneous assembly by physical interactions and meet the basic requirements posed to the efficient drug carrier.

Obtained nanoparticles due to the size distribution and low cytotoxicity are promising drug carrier. Both dextran and caprolactone are fully biodegradable and what was experimentally confirmed biocompatible with living cells, when administrated in clinically defined doses.

Nanoparticles are stable in solution and were successfully dried by spray drying method. Moreover, particles can be stored in this form for longer time and when rehydratated, returned to their original structure and size.

Having character of the surfactant nanoparticles are soluble in polar organics and water, which creates opportunities for future drug entrapment. NPs due to their long polycaprolactone chains most likely have hydrophobic zones, which would a preferable place for majority of commonly used anticancer drugs or precipitated drug conjugates.

LITERATURE

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