Abstract

The first work in my doctoral thesis described a novel rapid and eco-friendly reversible addition-fragmentation chain transfer (RAFT) polymerization reaction of the *N*-(2-hydroxypropyl) methacrylamide (HPMA) monomer under microwave irradiation (MWI). Optimal conditions for the polymerization such as reaction time, solvents, monomer stoichiometry and RAFT agents was determined. The polymerization kinetics demonstrated the linear increase in the number-average molecular weight (*Mn*) with monomer conversion. Good agreement between the theoretical and experimental *Mn* values was verified with pseudo-first-order kinetic plots, with low dispersities ($Đ \leq 1.04$). Furthermore, this publication demonstrated the ability of MWI to facilitate copolymer formation by the preparation of relevant copolymers, such as poly(HPMA-*b*-bocAPMA), poly(HPMA-*b*-MABH) and poly(HPMA-*b*-PDPA) which were used as a base for the following work in the thesis.

The second and third study are devoted to delivery of therapeutic molecules by using cargo-delivery self-assemblies in the form of polymersomes (PS). Such drug delivery systems (DDS) potentially minimize the premature degradation of drug, fast clearance from bloodstream and dosing frequency which leads to lower toxicity. The main advantage of DDS is the controlled manner of drug release at a specific site via active or passive targeting strategies. Passive targeting is a preferential accumulation of nanocarriers (NCs) in tumor tissue based on pathophysiological features (*i.e.*, leaky tumor vasculature and poor lymphatic drainage) of the growing solid tumor. For passive targeting, suitable particles size (from 10-100nm), surface charge and surface modification can promote effective extravasation as well as reduced liver capture and renal clearance. Active targeting refers to ligands' affiliation to receptors or with physicochemical engineering of structure for recognition by specific antigen/receptor of targeting cell. More novel stimuli-responsive programmed specific targeting in combination with previously mentioned strategies could achieve higher accumulation in tumor and enhance cellular internalization.

Stimuli-responsive drug-loaded polymersomes can respond to the inherent features of tumor microenvironments (TME), such as extracellular acidosis or higher levels of reactive oxygen species (ROS) in tumor sites. In this thesis novel TME-responsive amphiphilic block copolymers (BCs) based on HPMA were synthesized by a reversible addition-fragmentation chain transfer (RAFT) polymerization and characterized by standard techniques (^{1}H NMR and SEC). Hydrodynamic flow-focusing nanoprecipitation microfluidics (MF) was used in the preparation of well-defined ROS or pH-responsive PSs. The obtained PSs with desired size (hydrodynamic diameter - D_H ~ 100 nm) were evidenced by dynamic light scattering (DLS), static light scattering (SLS), transmission electron microscopy (TEM), and cryogenic electron microscopy (cryo-TEM). PSs loaded with doxorubicin (DOX) were evaluated by the cellular uptake and cytotoxicity in EL4 lymphoma cancer cells. The *in vivo* biodistribution studies were performed in nude mice using covalently attached fluorescent dye (DBCO-Cyanine7) to the polymersomes by copper-free, strain promoted alkyne azide cycloaddition "click chemistry". The obtained results demonstrated PSs circulation for a longer time (~ 144h). and accumulation to a greater extent compared to the free fluorescent probe. *In vivo* antitumor efficacy was analyzed in mice bearing EL4 lymphoma tumor. The results evidenced enhanced suppression of tumor cell growth and extended survival rate compared to the administration of free DOX. Side-effects

characteristic of therapeutic treatments based on DOX, such as hair loss and cardiotoxicity, were remarkably reduced.

The final part of the thesis reports about giant stimuli-responsive PS. Polydimethylsiloxane (PDMS) microfluidic device casted with sol-gel process with a coating rendered hydrophilic on selected junction channels was used for preparation of giant non-responsive and stimuli-responsive PS by w/o/w double emulsion method. The pH-responsive behavior was studied in detail by confocal microscopy and the results demonstrated the spatial and temporal pH-controlled PS disruption under simulated relevant physiological conditions. Cytotoxicity studies demonstrated excellent biocompatibility of produced PS. The giant PS could find application in pH-responsive drug and gene delivery, microreactors and as a model of artificial cell studies.

Keywords: polymersomes, self-assembly, ROS, drug delivery, stimuli-responsive, microfluidics, DOX.