pH and Reduction Dual-Sensitive Copolymeric Micelles for Intracellular Doxorubicin (DOX) Delivery

Liu, S[1,2,3]; Chen, J[1,2]; Zhong, W[3] and Xing, M[1,2+]
[1]Mechanical Engineering, [2]Manitoba Institute of Child Health, [3]Textile Sciences
University of Manitoba, Winnipeg, MB R3T 2N2, Canada

Introduction: Polymeric micelles formed from amphiphilic copolymers in aqueous solutions have been extensively explored as nanoscale carriers for hydrophobic anticancer drugs[1]. These nanocarriers exhibit prolonged circulation time by avoiding rapid clearance by the renal and reticuloendothelial systems (RES). Stimuli-responsive nanocarriers have been developed to release drugs with efficacy into tumor tissues upon changes in physical and chemical environments, such as redox potential, pH, temperature and ultrasound. pH change and redox potential are major chemical stimuli to trigger drug release from cargos. In this study, we hypothesized that DOX releases faster from micelles in a weakly acidic environment (pH 6.5) than at pH 7.4 or in the presence of a higher concentration (5 mM) of reducing agent (DTT). The release is even more effective in a scenario of both stimuli (pH 6.5 and 5 mM DTT). The objective of this study is to prepare a series of novel pH and reduction dual-responsive micelles and study the intracellular release of DOX from these micelles. To this end, we designed and synthesized a novel copolymer based on poly(ethylene glycol) (PEG) and reducible poly(β-amino ester)s (RPAE), containing disulfide bonds in the backbone of poly(β-amino ester)s. This copolymer can self-assemble into stable micelles in a physiological environment, with the RPAE constituting the core and the PEG as the shell.

Materials and Methods:
2,2′-dithiodiethanol diacrylate (DSDA) was synthesized. RPAE-PEG copolymer was synthesized based on DSDA and trimethylene dipiperidine and mPEG- NH_2.

Statistical significance was tested by using a one way analysis of variance (ANOVA) with 95 % confidence interval. When $P<0.05$, differences were considered to be statistically significant.

Results:
A series of RPAE-g-PEG amphiphilic graft copolymers via a simple one-step polymerization were successfully synthesized and characterized (Figure 1and 2A). Micelles self-assembled from the copolymer have pH and reduction dual sensitivities, which have been verified via fluorescence spectroscopy and dynamic light scattering (Figure 2B and 3). When entrapped in the RPAE-PEG micelles, the anticancer drug, DOX, was found to become rapidly released in an acidic PBS (pH 6.5) or in a higher concentrated DTT solution (5 mM). The most important advantage of this design is that the highest rate of DOX release from the copolymeric micelles was observed in an environment at a low pH value and with a high level of reductive agent, which is analogous to pH and reduction conditions in the endosomes (Figure 4). CLSM images indicated a fast internalization of the DOX-loaded micelles and efficient intracellular drug delivery (Figure 5). All these results suggested that this copolymeric micelle is

promising in the development of a potential intracellular anticancer drug delivery system.

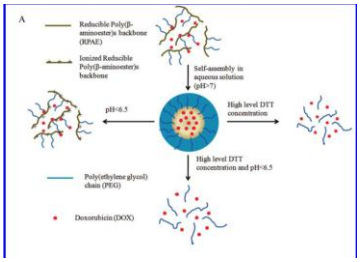

Figure 2 (A) Schematic illustration of DOX-loaded RPAE-PEG copolymeric micelles dissociation and release of DOX upon changes of pH value, reducing agent concentration or dual factors.

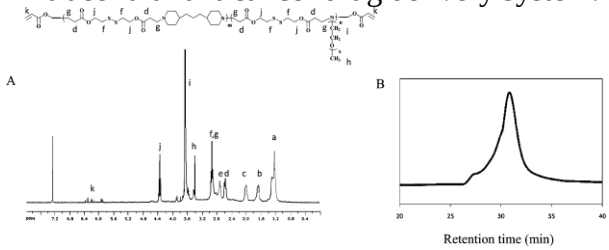

Figure 1 (A) 1H NMR spectrum of RPAE-PEG5000 (B) GPC trace of RPAE-PEG5000 copolymer which was determined by N,N-dimethylformamide based gel permeation chromatography.

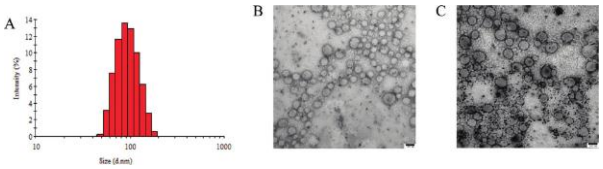

Figure 3 (A) Diameter and size distribution of RPAE-PEG-5000 micelles by DLS. (B) TEM micrograph of blank and (C) of DOX-loaded RPAE-PEG5000 micelles. The bar is 100 nm.

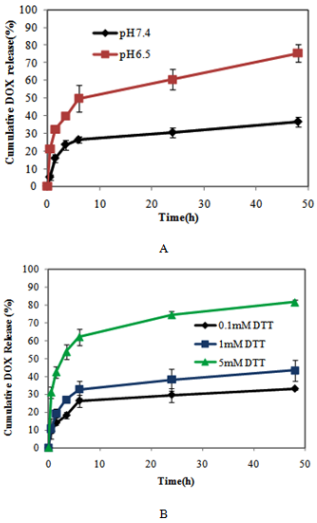

Figure 4(A) pH and (B) DTT dependent DOX release curves

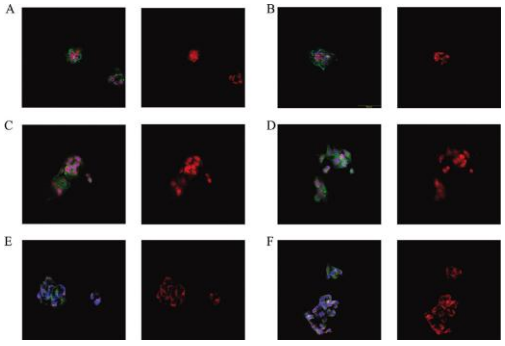

Figure 5 Confocal microscopy images of human liver carcinoma cell line (HepG2) incubated with free DOX and DOX-loaded RPAE-PEG5000 micelles for 15 min, 3 h, and 24 h. The nuclear was stained by Topro-3(blue) and F-actin staining by BODIPY FL phallacidin (green).

Reference

1. Lu, C., M. Xing, and W. Zhong, *Shell cross-linked and hepatocyte-targeting nanoparticles containing doxorubicin via acid-cleavable linkage.* Nanomedicine-Nanotechnology Biology and Medicine, 2011. **7**(1): p. 80-87.