Oxidation-sensitive polymeric micelles for cancer cell-selective drug release

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Statement of Purpose: Reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), superoxide anion radical, and the hydroxyl radical, are ubiquitously produced in the body as a consequence of aerobic metabolism. While ROS production in healthy tissues is maintained at basal levels, cancer cells typically generate these species at elevated levels, making the tumor microenvironment more oxidative. This characteristic oxidative microenvironment is the hallmark of cancer and provides a unique opportunity for cancer-specific drug delivery. Thus far, significant efforts have been made to develop oxidation-sensitive drug delivery systems (DDS) that release drugs in response to ROS. Those include polymeric micelles and polymersomes composed of amphiphilic polymers containing an oxidation-sensitive building block that alters its water solubility upon oxidation by ROS. Although these drug carriers show enhanced drug release in cancer cells, the drug release in normal cells is not negligible since those cells also generate ROS at basal levels. Therefore, careful fine-tuning of the ROS sensitivity of drug carriers is required to achieve cancer-specific drug delivery.
Here, we report oxidation-sensitive polymeric micelles containing different thioether groups with varying oxidation sensitivities. We evaluated the effect of different thioether groups on micelle destabilization in the presence of H_2O_2 as well as in human liver cancer cells (HepG2) and human umbilical vein endothelial cells (HUVECs). Furthermore, cytotoxicity of the micelles loaded with doxorubicin (Dox) was evaluated to demonstrate the potential applications in cancer chemotherapy.

Methods: Amphiphilic diblock copolymers consisting of a hydrophilic poly(*N*-acryloyl morpholine) (PAM) block and a hydrophobic block having different thioether groups were synthesized by reversible addition−fragmentation chain-transfer (RAFT) polymerization. The polymers were dissolved in *N*-methyl pyrrolidone (NMP), dispersed in water and dialyzed against water to prepare micelles by self-assembly. The destabilization of the micelles was evaluated by dynamic light scattering (DLS). The micelles containing self-quenched fluorescein isothiocyanate (FITC) fluorophores were used to confirm micelle destabilization in HepG2 cells and HUVECs. The cells were cultured in the presence of the micelles for 1 d and the fluorescence due to the destabilized micelles was observed by confocal laser scanning fluorescence microscopy (CLSFM). The images were analyzed by Image J to compare fluorescence intensity within cells. To prepare the Dox-loaded micelles, polymers were mixed with Dox in NMP, dispersed in water and dialyzed against water. Cells were treated with the Dox-loaded micelles for 3 d and the metabolic activity of the cells were measured by MTT assay.

Results: We prepared micelles containing three different thioether groups within the core (TP, TM and TPh micelles, **Figure 1**). These thioether groups have different oxidation sensitivities: TP group is most sensitive towards oxidation by H_2O_2 followed by TM and TPh groups as confirmed by the experiments using thioether amide model compounds.

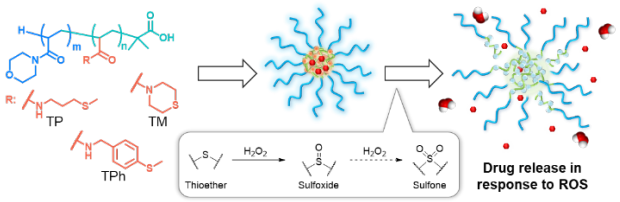

Figure 1. Oxidation-sensitive polymeric micelles containing different thioether groups within a micelle core.

The micelles were incubated with H_2O_2 at different concentrations and destabilization of the micelle structure was studied by DLS. TP micelles dissociated in the presence of H_2O_2 at concentrations as low as 0.0003 wt%. As for TM micelles, while no clear change was observed at 0.0003 wt% of H_2O_2, the scattered light intensity significantly decreased at > 0.03 wt% of H_2O_2 showing dissociation of the micelles. TPh micelles were stable and the effect of H_2O_2 was only observed at 0.3 wt% or higher. Micelle destabilization was also tested in human liver cancer (HepG2) cells and human umbilical vein endothelial cells (HUVECs). In this experiment, we used micelles containing self-quenched FITC in the micelle core. The intact micelles are non-fluorescent due to self-quenching of FITC, whereas dissociation of the micelles will cause a recovery of fluorescence. Strong fluorescence due to the destabilization of the TP micelles was observed in both HepG2 cells and HUVECs. The TM micelles were destabilized in HepG2 cells but were stable in HUVECs. The TPh micelles, on the other hand, were stable in both cell lines. These results show that the TM micelles, which have moderate oxidation sensitivity, are selectively destabilized in cancer cells. Furthermore, the micelles loaded with Dox were used to treat HepG2 cells and HUVECs to evaluate their potential in drug delivery applications. Among the micelles, the TM micelles loaded with Dox enhanced the relative toxicity in HepG2 cells over HUVECs while the TP and TPh micelles did not show obvious beneficial effects compared to Dox alone. Therefore, our approach to fine-tune the oxidation sensitivity of the micelles has potential for expanding the therapeutic window of Dox in cancer treatment.

References:

A.J. van der Vlies *et al.*, Biomacromolecules, accepted.