Tumor-targeting, pH-responsive and stable unimolecular micelles as drug nanocarriers for targeted cancer therapy

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Statement of Purpose: Unimolecular micelles have attracted significant attention in various biomedical fields due to their desirable properties such as high drug loading capacity, well-controlled nanoparticle sizes, and especially *in vivo* stability (Criscione J M et al., Biomaterials 2009; 30: 3946-55). Traditional multimolecular micelles are thermodynamically stable only above the critical micelle concentration (CMC) of the amphiphilic copolymers, while unimolecular micelles do not disassemble upon dilution and remain stable during environmental changes due to their covalent nature. In addition, the highly branched structure of unimolecular micelles can provide many end groups for further functionalization (Kreutzer G et al., Macromolecules 2006; 39: 4507-16).

To enhance the efficacy of targeted cancer therapy, drug nanocarriers need to be internalized by the tumor cells effectively; once internalized, they need to release a sufficient amount of drug to effectively kill the cells. In this work, we developed a new type of multifunctional unimolecular micelle based on Boltron® H40 as a drug nanocarrier for targeted cancer therapy, which exhibited a pH-triggered drug release profile. These unimolecular micelles were formed by amphiphilic hyperbranched copolymer, H40-((poly(β-malic acid)-hydrazone-doxorubicin)-*co*-poly(ε-caprolactone))-methoxy poly(ethylene glycol)/poly(ethylene glycol)-folate in aqueous solution. Folate was used as the active tumor-targeting agent and selectively conjugated onto the distal end of PEG arms on the surface of the unimolecular micelle. The anticancer drug, doxorubicin (DOX), was conjugated onto the hydrophobic segments through an acid-sensitive hydrazone bond. This acid-cleavable linkage reduces the unwanted DOX release during blood circulation yet initially releases DOX quickly once inside tumor cells. This tumor-targeting, unimolecular micelle nanosystem with a pH-triggered drug release profile and potentially excellent *in vivo* stability will provide many exciting opportunities for targeted cancer therapy.

Methods: The polymer H40-(poly(benzyl malic acid)-*co*-poly(ε-caprolactone))-methoxy poly(ethylene glycol)/poly(ethylene glycol)-folate (H40-(PMABz-*co*-PCL)-MPEG/PEG-FA was prepared by the ring-opening polymerization of benzyl malolactonate and ε-caprolactone using H40 as a macroinitiator and $Sn(Oct)_2$ as a catalyst, followed by conjugating with the mixture of $HOOC\text{-}PEG\text{-}OCH_3$ and HOOC-PEG-FA. The benzyl groups of H40-(PMABz-*co*-PCL)-MPEG/PEG-FA were substituted with hydrazide groups for DOX binding by an ester-amide exchange aminolysis reaction. The unimolecular micelles based on H40-((PMA-Hyd-DOX)-*co*-PCL)-MPEG/PEG-FA were prepared using the dialysis method.

Results: H40-((PMA-Hyd-DOX)-*co*-PCL)-MPEG/PEG-FA formed unimolecular micelles that were characterized by both TEM and DLS (Figure 1). The umimolecular micelles were spherical and most had a relatively uniform size, which was about 20 nm. Moreover, the unimolecular micelles were stable over several weeks and displayed no changes in size and size distribution within experimental accuracy during this period. The unimolecular micelles showed a pH-sensitive DOX release behavior. A negligible amount of drug release was observed at pH 7.4, while the DOX released rate increased significantly within the range of pH 4.5 to 6.5. This PH-dependent releasing behavior is of particular interest in achieving the tumor-targeted DOX delivery with micelles.

The flow cytometry and CLSM studies showed that the cellular uptake of the FA-conjugated unimolecular micelles was much higher than that of the non-targeted unimolecular micelles, which was attributed to the FA-receptor mediated endocytosis. This is consistent with the higher cytotoxicity exhibited by the FA-conjugated unimolecular micelles.

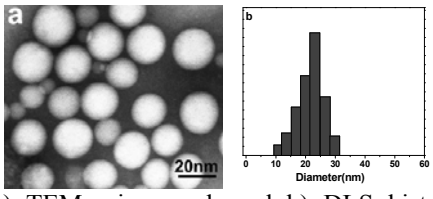

Fig. 1 a) TEM micrograph and b) DLS histogram of unimolecular micelles based on H40-((PMA-Hyd-DOX)-*co*-PCL)-MPEG/PEG-FA copolymers.

Conclusions: Tumor-targeting, multifunctional unimolecular micelles that exhibit a pH-triggered drug release profile and potentially excellent *in vivo* stability were developed for targeted cancer chemotherapy. The unimolecular micelles were formed by hyperbranched amphiphilic H40-((PMA-Hyd-DOX)-*co*-PCL)-MPEG/PEG-FA copolymers. The unimolecular micelles exhibited a pH-sensitive drug release profile. The flow cytometry and CLSM studies showed the cellular uptake of the H40-((PMA-Hyd-DOX)-*co*-PCL)-MPEG/PEG-FA was greatly enhanced due to folate-receptor-mediated endocytosis. These novel tumor-targeting, pH-responsive, and potentially *in vivo* stable unimolecular micelles may provide a very promising approach for targeted cancer therapy and greatly improve the quality care for cancer patients.