Synthesis and Characterization of Doxorubicin-bearing Cetuximab-PAMAM Dendrimer Bioconjugates

Gunjan Saxena[1], Hu Yang[1,2]
[1]Department of Biomedical Engineering, [2]Massey Cancer Center, Virginia Commonwealth University, Richmond, VA 23284

Statement of Purpose:
Brain cancer remains a challenging medical problem. Brain cancer therapy would benefit from enhanced selective delivery. In this work, we designed and synthesized a tumor targeted drug delivery system based on polyamidoamine (PAMAM) dendrimer and used it to deliver anticancer drug doxorubicin (DOX) oriented toward brain cancer treatment. To ensure timely release of DOX from the delivery system, DOX was covalently conjugated to the dendrimer via an acid-labile hydrazone linkage. The synthesis and characterization of the novel bioconjugates are reported herein.

Methods:
Synthesis. The synthesis of Doxorubicin-bearing Cetuximab-PAMAM dendrimer bioconjugates followed three steps: **(1)** NH_2-PEG-maleimide (Mn=3500 Da) was coupled to PAMAM dendrimer G4.5 (Dendritech) using EDC/NHS chemistry; **(2)** DOX was covalently attached to the dendrimer surface via acid sensitive hydrazone linkage (Bae et al. Angew. Chem. Int. Ed. (2003), 42(38): 4640-4643); and **(3)** Cetuximab (CTX) was thiolated and then coupled to the dendrimer via maleimide-thiol reaction (Quan et al. ACS Chem. Neurosci. (2011), 2(11), 676-683).
Characterization. The characterization methods included ^{1}H NMR spectroscopy, fluorescence anisotropy, ultraviolet-visible spectroscopy, size and zeta measurements using Malvern Zetasizer Nano ZS90.
Drug release studies. DOX release at different pHs (7.4, 5.5, and 4.5) was studied.
Cytotoxicity assay. Toxicity effects of bioconjugates with various equivalent DOX concentrations on HN12 cells was assessed using Trypan blue assay following incubation for 24, 48 and 72 h. Drug uptake was imaged under a Zeiss Axiovert 200M inverted microscope.

Results:
Polyamidoamine (PAMAM) dendrimer G4.5 was chosen as the underlying carrier. It consists of 128 surface carboxylate groups and has higher cytocompatibility and low non-specific cellular uptake. The delivery system was synthesized using a layer-by-layer assembly strategy to attach three functional entities—anticancer drug doxorubicin, monoclonal antibody Cetuximab against EGF receptor, and polyethylene glycol (PEG)—to the dendrimer surface. In particular, DOX was attached via an acid-sensitive hydrazon linkage to the dendrimer. As shown in Fig. 1, more DOX was released at pH 4.5 indicating the acid sensitivity of the hydrazone linkage between the drug and dendrimer. Cytotoxicity studies indicated that combination of DOX and CTX exerted a significant toxic effect over a period of 72 hours (Fig.2). The cellular uptake of the delivery system was higher than that of free doxorubicin. Free DOX localized mainly in the nucleus, whereas DOX-G4.5-PEG-CTX conjugates localized within both cytoplasm and nucleus after 6 h-incubation (Fig.3).

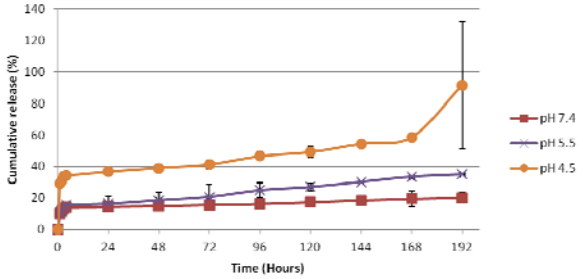

Fig. 1. *Cumulative release of DOX from the bioconjugates at different pHs.*

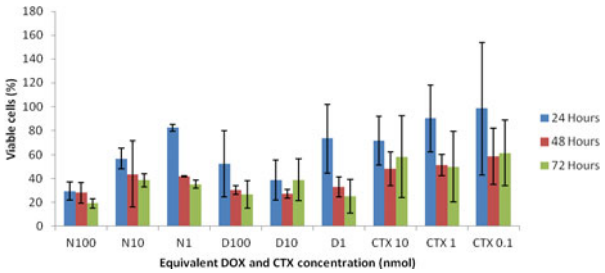

Fig. 2. *Cytotoxicity studies of DOX-G4.5-PEG-CTX (N), free DOX (D) and free CTX at the indicated equivalent concentrations (100, 10, 1 or 0.1 nmol)*

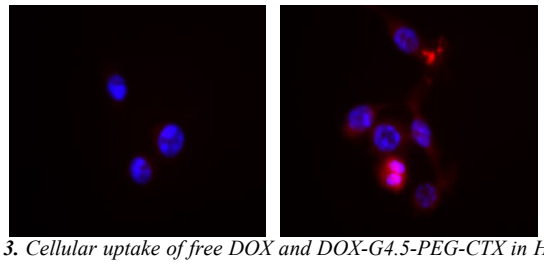

Fig. 3. *Cellular uptake of free DOX and DOX-G4.5-PEG-CTX in HN12 cells.*

Conclusions:
We designed a brain tumor-targeted drug delivery system on the basis of overexpression of EGFR in brain tumor. Dendrimers have proven to be a suitable carrier to construct targeted delivery systems because of their well-defined structures and numerous terminal groups. We demonstrated the success in synthesis. In vitro results showed that cellular uptake of dendrimer nanomedicine can be enhanced by the use of the targeting ligand. Release of CTX became more pronounced at low pH due to the acid sensitivity of the linkage. Future studies include transport of the synthesized bioconjugates across the blood-brain barrier and in vivo efficacy measurements.