Next Generation Cancer Therapeutics: Active Targeting of Drug Encapsulated Nanogel for Her2 Positive Breast Cancer

Peidong Wu[1], Uyen Huynh[1], Jingyi Qiu[2], Theo Prachyathipsakul[1], Khushboo Singh[1], Jingjing Gao[1,3], Sankaran Thayumanavan[1,2,4*]

[1]Department of Chemistry, University of Massachusetts, Amherst, Massachusetts 01003, USA. [2]Department of Biomedical Engineering, University of Massachusetts, Amherst, Massachusetts 01003, USA. [3]Current address: Harvard Medical School, Boston, MA 02115, USA. [4]Center for Bioactive Delivery, The Institute for Applied Life Sciences, University of Massachusetts Amherst, Amherst, Massachusetts, USA

Statement of purpose: Targeted delivery of therapeutics using supramolecular nanoassemblies is an attractive prospect in tackling life-threatening diseases such as cancer. Antibody-drug conjugates (ADC) have become the mainstay for cancer chemotherapy in the clinic in recent times. Due to the limited number of receptors present on the targeted tumor cell, people have been working on either increasing the drug to antibody ratio (DAR) or conjugation of highly potent drugs for enhanced anti-tumor efficacy. However, ADC with a high DAR number (>8) is not as effective compared to DAR at around 4, at which the targeting capability of antibody was not influenced significantly by the additional hydrophobicity from the drug molecules. Also, the exposure of linker between drug and antibody might cause a pre-mature release of the highly potent drug which leads to significant side-effect. To overcome the limitations of conventional ADCs, we have developed a drug delivery platform Antibody Nanogel Conjugate (ANC).

Methods: Here we have designed block copolymer based nanoassemblies where polymers were prepared by RAFT polymerization. Azide or tetrazine functional group was modified on the poly(ethylene glycol) terminal for the later conjugation of DBCO or TCO modified antibody. Hydrophobic drug molecules were non-covalently or covalently encapsulated and further stabilized in the interior of nanoassemblies by disulfide crosslinkers. The size and surface charge of ANCs was evaluated by DLS and TEM. The encapsulation stability and release kinetics were studied using UV or HPLC based methods. Antibody conjugation efficiency was evaluated by gel electrophoresis. Drugs with varying potencies and physical properties were selected to test the feasibility of this platform. Trastuzumab was selected as the model antibody to test this platform in Her2+ cancers. Drug to antibody ratio was determined by HPLC and BCA assay. To test the selectivity of ANC towards Her2+ cell lines, we utilized dye encapsulated ANC compared with nanogel without antibody decoration in cell lines with different extents of Her2 expression. Then, we have evaluated ANCs, based on four different drugs, in vitro towards Her2+ and Her2- cell lines. Finally, we evaluated the efficacy of ANC and ADC (homemade Trastuzumab DM1 and Kadcyla) in Her2+ and Her2- cell lines in vitro and in vivo (in progress).

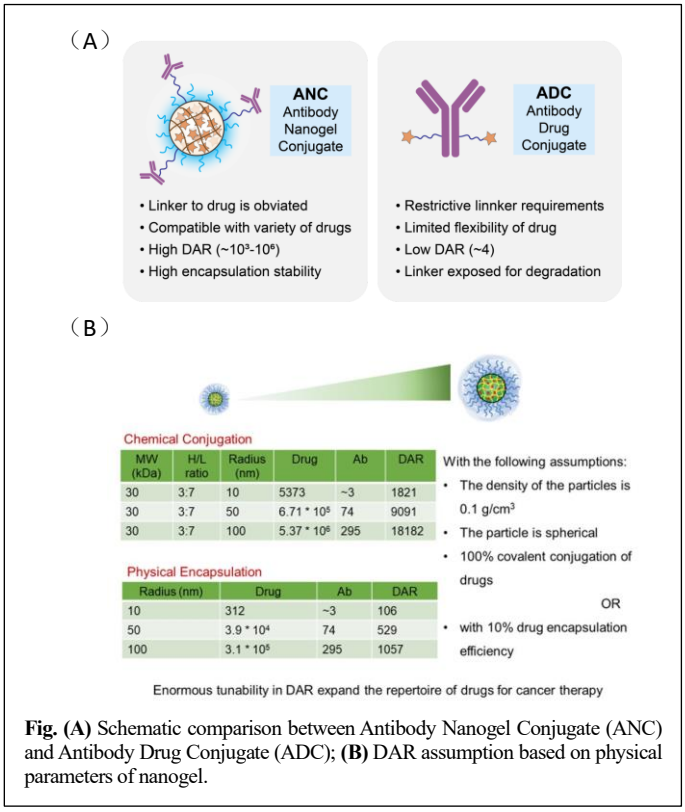

Fig. (A) Schematic comparison between Antibody Nanogel Conjugate (ANC) and Antibody Drug Conjugate (ADC); **(B)** DAR assumption based on physical parameters of nanogel.

Results: We firstly found that all drugs have been successfully encapsulated into the nanogel. The size of the drug encapsulated nanogels increases accordingly, along with a change in surface charge, upon antibody conjugation. The efficiency of antibody nanogel conjugation is better using TCO-tetrazine-based click chemistry compared to DBCO-azide. Compared to nanogel without antibody decoration, ANC shows significant uptake in Her2+ cell lines which indicated a high selectivity after antibody conjugation. DAR of ANCs could be fine-tuned from 100 – 5000. The cytotoxicity of ANC is higher compared to nanogel without antibody decoration in Her2+ cell lines. However, in Her2- cell lines the toxicity of nanogel is higher or similar to ANC which indicated that the decoration of antibody might potentially minimize the non-specific uptake of nanogels. Then, we compared the efficacy of ANC and ADC in Her2+ and Her2- cell lines. Both ANC and ADC show selectivity towards Her2+ cells, where higher efficacy was observed for ANCs. While in Her2- cells, at the same drug concentration range, both ANC and ADC did not show any toxicity. In vivo evaluation of ANC benchmarking with Kadcyla is in progress.