Doxorubicin-loaded and Antibody-Conjugated Liposome-QD Hybrid Vesicles for Targeted Cancer Therapy and Imaging

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ABSTRACT

Quantum dots (QD) have been extensively explored for *in vitro* and *in vivo* imaging due to their superior fluorescence properties compared to organic fluorophores. The hydrophobic nature of QD hinders their biomedical applications in biological milieu, therefore many efforts have been made to construct water-soluble QD by substituting the organic surface ligands with hydrophilic moieties. However such surface modifications adversely affected the QD optical properties and colloidal stability. Previously, our group offered an alternative approach to improve QD hydrophilicity by incorporating hydrophobic QD into liposome lipid bilayer (L-QD) which efficiently labelled cancer cells *in vitro* and *in vivo*.In this study, we report the engineering of multimodal liposome-QD hybrids (L-QD) for cancer imaging and therapy. L-QD hybrids were loaded with Dox using the osmotic gradient technique, achieving high encapsulation efficiency comparable to liposome alone. Structural elucidation using cryogenic electron microscopy (cryo-EM) clearly showed that QD were incorporated in the lipid bilayer and Dox crystals were encapsulated into the liposome aqueous core. Furthermore, the surface of Dox-loaded hybrids were functionalized with anti-MUC-1 antibody for active targeting, using the post-insertion technique. The specific binding of antibody-targeted hybrids was studied against the MUC1 epitope by surface plasmon resonance (BIACORE) showing higher binding affinity than the antibody alone due to a multivalent effect. In addition, cellular uptake studies of the antibody-targeted hybrids were conducted using confocal laser scanning microscopy (CLSM). The antibody-targeted hybrids showed high binding and uptake by human breast cancer cells (MCF-7) that overexpress MUC-1 receptors in contrast to human pulmonary adenocarcinoma cells (Calu-6) exhibiting low level of MUC-1 expression. Finally, cytotoxicity assays indicated higher toxicity of antibody-targeted hybrids in MCF-7 compared to Calu-6 cells. In conclusion, MUC-1 antibody-targeted L-QD hybrids encapsulating doxorubicin are thought to constitute a potential multimodal system for the simultaneous delivery of therapeutic and diagnostic agents to cancer cells *in vitro* and *in vivo*.

Keywords: QD, doxorubicin, cancer targeting, imaging, MUC1

In recent years, quantum dots (QD) have been widely used for fluorescence imaging applications *in vitro* and *in vivo* due to their superior fluorescence properties compared to organic fluorophores, such as high photostability and bright fluorescence[1]. However, the hydrophobic nature of QD hinders their direct biomedical applications in biological milieu, therefore many efforts have been made to construct water-soluble QD by substituting the organic surface ligands with hydrophilic moieties. However such surface modifications adversely affected the QD optical properties and colloidal stability.

Liposomes are one of the most-developed nanometer-scale drug delivery systems.[2] Liposomes encapsulating cytotoxic drugs have been widely used for the treatment of various tumors.[2-4] Liposomes (smaller than 200 nm in mean diameter) can preferentially accumulate in tumors *in vivo* due to enhanced permeation and retention effect (EPR).[2-5] Furthermore, the attachment of targeting ligands to the liposome surface facilitate their cellular uptake [6], resulting in a significant improvement in cancer therapy.

Previously, our group offered an alternative approach to improve QD hydrophilicity by incorporating hydrophobic QD into liposome lipid bilayer (L-QD), which were capable of efficiently labeling both human epithetial lung cells (A549) *in vitro* and human cervical carcinoma (C33a) xenografts *in vivo*[7]. Surface modification with polyethylene glycol (PEG) dramatically prolong the blooding circulation half-life of zwitterionic L-QD after intravenous administration[8]. The engineering of L-QD hybrid vesicles offers simplicity and effectiveness in creating water-compatible QDs, most important of all, the construction of L-QD hybrid vesicles that set up a bridge between liposomes, the most-established nanometer-scale delivery

carriers, and QDs, one of the most processing fluorescent imaging agents. This provides enormous opportunities for L-QD based fluorescence imaging of various tumors. In addition to imaging, L-QD hybrid vesicles offer the potential to encapsulate various anticancer drugs in their aqueous compartment for cancer therapy. It was recently reported that QD-aptamer covalently linked with Dox could be used for cancer imaging and therapy in vitro[9]; however, one QD could only be attached to one doxorubicin molecule, which comprised the therapeutic effect.

In this study, we attempted to further develop L-QD hybrids with therapeutic effect (*Fig. 1*) for cancer imaging and therapy. This was achieved by loading anti-cancer drug Dox into the aqueous core of L-QD using the classical osmotic gradient technique[10], with high encapsulation efficiency comparable to liposome alone. Structural elucidation using cryo-EM clearly showed that QD were incorporated in the lipid bilayer and Dox formed crystals-like structures into the liposome aqueous core. Furthermore, the surface of Dox-loaded hybrids was functionalized with anti-MUC-1 antibody for targeted delivery into cancer cells, using the post-insertion technique. The specific binding of antibody-targeted L-QD hybrids was studied against the MUC1 epitope by surface plasmon resonance (BIACORE) showing higher binding affinity than the antibody alone due to a multivalent effect. In addition, cellular uptake studies of the antibody-targeted L-QD hybrids were conducted using CLSM. The antibody-targeted hybrids showed high binding and uptake by human breast cancer cells (MCF-7) that overexpress MUC-1 receptors in contrast to human pulmonary adenocarcinoma cells (Calu-6) exhibiting low level of MUC-1 expression. Finally, cytotoxicity assays using MTT assay indicated higher toxicity of antibody-targeted hybrids in MUC-1 positive cell line (MCF-7) compared to MUC-1 negative Calu-6 cells. In conclusion, MUC-1 antibody-targeted L-QD hybrids encapsulating doxorubicin are thought to constitute a targeted theranostic system for the simultaneous delivery of therapeutic and diagnostic agents to cancer cells *in vitro* and *in vivo*.

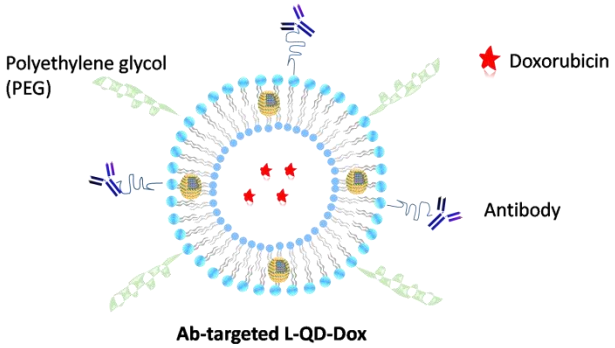

Figure 1: Schematic representation of Dox-loaded and antibody-targeted L-QD.

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