



Title	Design of Quantum Dot-Based High Performance Nanoparticle Platform for Nanoparticle-Based Drug Delivery (NDD) system [an abstract of dissertation and a summary of dissertation review]
Author(s)	TAN, ROGER SALVACION
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学 位 論 文 審 査 の 要 旨

Doctoral Dissertation Evaluation Review

博士の専攻分野の名称 博士（生命科学） 氏 名 ROGER SALVACION TAN
Degree requested: Doctor of Life Science Name: ROGER SALVACION TAN

審査担当者	主査 /Chief examiner	教 授	<Professor Shin-Ichiro Nishimura >
Examiners	副査 /Associate examiner	教 授	<Professor Kenji Monde >
	副査 /Associate examiner	教 授	<Professor Masataka Kinjo >
	副査 /Associate examiner	准教授	<Associate Professor Hiroshi Hinou>

学 位 論 文 題 名

Title of Doctoral Dissertation

Design of Quantum Dot-Based High Performance Nanoparticle Platform for Nanoparticle-Based Drug Delivery (NDD) system

(ナノ微粒子による薬物送達システム
ー量子ドット型高性能ナノ微粒子プラットフォームの設計)

博士学位論文審査等の結果について（報告）

Results of Evaluation of the Doctoral Dissertation (Report)

A lot of studies have reported crucial problems that the nanoparticle-based drug delivery (NDD) system has been facing and that is endolysosomal entrapment of the drug/therapeutic agent nanocarrier after endocytosis. These endolysosomal entrapments of NDD carrier can cause degradation of these drugs/therapeutic agents and hamper their delivery to the desired target due to the presence of hydrolytic enzymes inside the lysosome. In order to successfully deliver the therapeutic agents into the desired intracellular compartment, the nanocarriers have to escape successfully from this entrapment. To alleviate and solve this problem, scientists have been using cell-penetrating peptides (CPPs). CPPs are a group of short amphipathic, hydrophobic or cationic peptide that has the ability to penetrate cell membrane and permeabilize lysosomal membrane to allow nanoparticles to escape. However, these CPPs that they have been using have long peptide sequences, which could interfere in the delivery of small molecules such as small peptides, glycopeptides and glycans. These CPPs have also high concentration-dependent cytotoxicities that render them not suitable for long-time intracellular delivery and live cell imaging. Also, the endolysosomal escaping ability of the commonly used CPP, to date, is very slow; it usually takes 3-4 days, as reportedly observed. During this long period of encapsulation, the therapeutic agents carried by the nanocarrier could have been degraded. Therefore, the need of an efficient, simple, robust, and non-cytotoxic CPP sequence is crucial.

In this study, the author designed a high performance nanoparticle platform for NDD based on quantum dots (QDs) utilizing his newly designed hexahistidine CPP for efficient endolysosomal escape of the nanoparticle. The author modified the QD surface through ligand exchange of TOPO-QDs with 11,11'-

dithiobis[undec-11-yl 12-(aminooxyacetyl)amino hexa(ethyleneglycol)] (AOSH) linker and 11-mercaptoundecylphosphorylcholine (PCSH) ligand, which were previously reported from previous researches conducted in the laboratory where he currently belongs to. The modification brought the cell-surface mimicry of the nanoparticle due to the cell-surface mimetic ligand on its surface. The presence of AOSH allowed for an easy attachment and controlled spatial orientation of ketone-functionalized peptides and glycopeptides onto the QD surface through an oxime bond formation. The author has proven that this design worked by delivering a serglycin-like peptides and glycopeptides intracellularly on different normal and cancer human cell lines.

The author's results also showed that QDs displaying only serglycin-like glycopeptide on its surface greatly suffered from endolysosomal entrapment in all cell lines used. However, once the hexahistidine CPP was co-displayed onto the QD's surface together with the serglycin-like glycopeptide, the QD conjugate managed to escape the endolysosomal entrapment after 2 hours of co-incubation with different cell lines. This escaping mechanism is way faster than the previously reported escape of QD conjugates using the commonly used CPPs. Moreover, during this co-incubation period, QD conjugates were found to enter the Golgi apparatus after their escape from endolysosomal entrapment. This result was observed after the author repeated the experiment and stained the Golgi apparatus with the golgi marker. What is also exciting is that the intracellular itinerary of the QD conjugates was also observed. During live cell imaging experiment, QD conjugates were clearly seen entering the cell, being channeled and sorted inside. The organelle is responsible for this channeling we believe to be the sorting endosome, as this is the known organelle that initially sorts intracellular cargos. As also seen in the real-time video presented by the author, the QD conjugate was clearly delivered into the bigger organelle after being sorted. This bigger organelle was believed to be the Golgi apparatus after the author's additional results showed that the frequent Golgi localization was observed after 2 hours of co-incubation of the QD conjugates with the different human cell lines. Exocytosis of QD conjugate was also observed in different parts of the cell during the co-incubation period, for the first time, indicating an exit mechanism after Golgi localization. This exocytic mechanism could prevent the build-up of nanoparticles in organs that would lead to systemic cytotoxicity.

In conclusion, the author's designed hexahistidine CPP surpasses the amount of time of escape from endolysosomal entrapment than those reported common CPPs, which could greatly help attain the desired escaping mechanism for an ideal NDD system. The author's QD-based nanoparticle platform design is very efficient and it cannot only display ketone-functionalized peptides and glycopeptides but also display any ketone-functionalized molecule through a stable oxime bond formation. The characteristics of the author's new nanoparticle platform such as versatility and reproducibility, efficient endolysosomal escaping ability, controlled spatial orientation and density of ligands to be displayed, and its biocompatibility for live cell imaging belong to the required criteria for an ideal NDD system. We recognized the novelty of the author's presented work in attaining an ideal NDD system. Therefore, we hereby acknowledge that the author is qualified to be granted the Doctorate of (Life Science) from Hokkaido University.