Graduate School of Advanced Science and Engineering,
Waseda University

博　士　論　文　概　要
Doctor Thesis Synopsis

Thesis Theme

Cellular Response of Titanium dioxide Nanoparticles via Toll Like Receptor 4

論　文　題　目

チタニアナノ粒子のトル様受容体を介した細胞応答

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Abstract

Nanoparticles (NPs) are minute substances in the size range of 1-100 nanometers. Because of its unique specialized properties in physical, chemical and mechanical aspects, nanomaterials are focused for a wide range of basic studies in industrial and laboratory applications. All people are exposed to nanometer sized foreign particles; we inhale them with breath, and consume them with drink. NPs have the ability to enter, translocate within, and damage living organisms. This ability results primarily from their small size, which allows them to penetrate physiological barriers, and travel within the circulatory systems of a host and induced several deleterious effects. The deleterious effects induced by NPs could be overcome by understanding the interaction between NPs and cells at molecular level. However, very small detailed information is available regarding the molecular mechanism of interaction of NPs to the cells. In order to understand the molecular mechanism of NPs-cell interaction, the first step is to identify the phenomena which occur in the cells after the exposure of NPs, the molecules took part in the interaction of NPs with cells and the mechanism of interaction between that molecules and NPs. It is also necessary to find out the solution to overcome this interaction. Here, the strategies of my Ph.D program are

- To understand the effects of NPs on the cells (Chapter 2)
- To identify the target receptor involved in NPs and cell interaction (Chapter 3)
- To understand the molecular mechanism involved in the interaction of NPs with receptors (Chapter 4)
- How to avoid the interaction of NPs with receptor for the safety usage of NPs (Chapter 3)

In order to understand the molecular mechanism of interaction between NPs and cells, I focused on titanium dioxide (TiO_2) NPs, hence TiO_2 has a long history of use in the commercially available products such as paints, pigments, cosmetics and so on. To understand the molecular mechanism of interaction between TiO_2 NPs and cells, it is essential to know the effects of TiO_2 NPs on cells. TiO_2 NPs decrease cell viability and induce inflammatory responses. In order to reduce their toxicity, TiO_2 NPs is conjugated with polyethylene glycol (PEG). The findings showed that modifying TiO_2 NPs with PEG reduced their cytotoxicity and decreased the induction of inflammatory response. The receptor involved in the interaction of TiO_2 NPs and cells was identified as toll like receptor 4 (TLR 4) and this interaction would be avoided by PEG modification. The bacterial ligand lipopolysaccharide (LPS) is used to compare with TiO_2 NPs. LPS is a well-known inducer of the

innate immune response, and a major natural ligand of TLR 4. Finally, detailed molecular mechanism of interaction between NPs and cells were fined. These are explained in the following chapters.

Chapter 1 described a general introduction on nanomaterials and their toxicity *in vitro* and *in vivo*. This chapter also described about the mode of entry of nanomaterials and importance of innate immunity. Innate immune system is the first physiological system to interact with foreign materials and therefore, key to understand how organisms will affect by exposure to nanomaterials. TLRs play a fundamental role in the activation of innate immunity. TLRs have been studied for their roles in the recognition of microbial pathogens. Each TLR recognizes a specific microbial ligand. TLRs and innate immune system is essential for the interaction between nanomaterials and cells because the cells don't have a specific defense system for nanomaterials. The strategies of this work were shown in this chapter.

Chapter 2 deals about the effects of TiO_2 aggregates and PEGylated TiO_2 NPs on cytotoxicity and gene expressions in human cell lines. To understand the molecular mechanism of interaction between TiO_2 NPs and cells, at first it is essential to know the effects of TiO_2 NPs on cells. Inorder to understand the effects of TiO_2 NPs, different human cell lines were exposed to TiO_2 NPs in a dose and time dependent manner. The cytotoxicity has been analyzed based on the presence of cytoplasmic ATP concentration of the cells. The potential biomarker for TiO_2 NPs has been identified by analyzing the PCR array for human stress and toxicity biomarker induced by TiO_2 NPs. The fold of induction of biomarker has been examined by real time PCR (RT-PCR). The results indicated that TiO_2 NPs aggregates decrease cell viability and induced the expression of stress and toxicity related genes, such as those encoding interleukin-6 (IL-6), suggesting that TiO_2 NPs induce inflammatory responses. In order to reduce their toxicity, TiO_2 NPs is conjugated with PEG. The findings indicate that modifying TiO_2 NPs with PEG reduces their cytotoxicity and reduces the induction of stress-related genes. The results also showed that TiO_2 NP-induced cytotoxicity and gene expression vary depending upon the cell type.

Chapter 3 described the incorporation of TiO_2 NPs to the cell and the role of TLRs in cell- materials interaction. In order to identify the target receptor involved in the interaction of TiO_2 NPs and cells, the cells were transfected with different TLRs expression vectors such as TLR 1, 2, 3, 4 and 7 and the incorporation of TiO_2 NPs has been analyzed. The ratio of incorporation of TiO_2 NPs has been performed by fluorescent activated

cell sorter- side scatter (FACS-SSC) analysis. Among the TLRs expression vector transfected cells, TLR 4 expression vector-transfected cells increased the uptake of TiO_2 NPs and also increased IL-6 mRNA induction by TiO_2 NP exposure. Inorder to confirm the role of TLR 4 in the uptake and inflammatory signal transduction induced by TiO_2 NPs, PEG modified TiO_2 NPs were also used. The results showed that modifying TiO_2 NPs with PEG reduced their uptake and inflammatory-signal transduction via TLR 4. The results indicated that TLR 4 was involved in the interactions between cells and NPs, and that PEGylation of TiO_2 NPs reduced the cellular response via interaction with TLR 4.

Chapter 4 demonstrated the comparison of bacterial ligand and NPs in TLR 4 mediated cellular responses. In the case of bacterial ligand LPS, LPS binds to LPS binding protein (LBP) and cluster of differentiation 14 (CD 14), and then this complex binds to TLR 4. The results indicated that for cellular uptake of TiO_2 NPs, TLR 4 did not need to form complex with LBP and CD 14. In TiO_2 NP-mediated inflammatory response, TLR 4 acted as the signaling receptor without the protein complex of LBP and CD 14. The results also showed that the character of TiO_2 NPs might be similar to the complex of LPS, LBP and CD 14.

Chapter 5 deals about the general conclusion and future prospects. In order to know the effects of TiO_2 NPs on cells, the cytotoxicity and biomarker expressions for TiO_2 NPs exposure have been analyzed. The cytotoxicity and inflammatory related gene expression induced by TiO_2 NPs have been reduced by PEG modification. In order to identify the target receptor involved in the interaction of TiO_2 NPs and cells, the cells were transfected with different TLRs expression vectors. The results indicated that TLR 4 is involved in the uptake and inflammatory signal transduction induced by TiO_2 NPs. Modifying TiO_2 NPs with PEG reduced their uptake and inflammatory-signal transduction via TLR 4. The molecular mechanism of TLR 4 mediated cellular uptake and inflammatory response induced by TiO_2 NPs are compared with LPS. This is the first time showed that co-receptors such as LBP and CD 14 are not involved in the activation of TLR 4. This is speculated to ascribe to that TiO_2 NPs has a similar character with LPS, LBP and CD 14 complex. This suggested that TiO_2 NPs could bind to TLR 4, similar to complexes of LPS, LBP, and CD 14.

In this work, detailed molecular mechanism of interaction between NPs and cells were studied via TLR 4. Based on these findings, the results would be very useful information for the safety usage of nanomaterials and safer nanomaterials development.

早稲田大学　博士（理学）　学位申請　研究業績書

(List of research achievements for application of doctorate (Dr. of Science), Waseda University)

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	6. Effects of TiO_2 nanoparticles on cytotoxicity and gene expressions in human cell lines. Tsukuba Biomedical Engineering Collaboration Forum 2011, NIMS, Tsukuba, Japan (January 18, 2012). **Sharmy Saimon Mano** and Akiyoshi Taniguchi 7. Effects of TiO_2 nanoparticles on cytotoxicity and gene expressions in human cell lines. 3rd NIMS- Waseda International Symposium, Waseda University, Tokyo, Japan (November 1, 2011). **Sharmy Saimon Mano** and Akiyoshi Taniguchi