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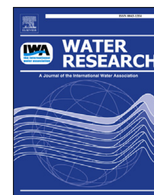

Interaction between bacterial cell membranes and nano-TiO_2 revealed by two-dimensional FTIR correlation spectroscopy using bacterial ghost as a model cell envelope

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A B S T R A C T

The interaction between microorganisms and nanoparticles is a crucial step towards understanding the subsequent biological effect. In this study, the interaction between TiO_2 nanoparticles and bacterial cell membrane was investigated by Two-dimensional Correlation Fourier Transformation Infrared spectroscopy (2D-FTIR-COS) using bacterial ghosts (BGs), which are non-living bacterial cell envelopes devoid of cytoplasm. The synchronous map of 2D-FTIR-COS results indicated that the functionalities in proteins of BGs preferentially interacted with TiO_2 nanoparticles; whereas the interaction of TiO_2 nanoparticles with characteristic functionality in polysaccharides (C—OH) and phospholipids (P=O) were very weak or insensitive. This conclusion was further corroborated by settling of TiO_2 nanoparticles in the presence of pure protein, polysaccharide and phospholipid represented by bovine serum albumin (BSA), alginate and phosphatidylethanolamine (PE). Additionally, the asynchronous map of 2D-FTIR-COS indicated a sequential order of functionalities bonded to TiO_2 nanoparticles with the order of: COO^- > aromatic C=C stretching > N—H, amide II > C=O, ketone. These findings contribute to deeper understanding of the interaction between TiO_2 nanoparticles and bacterial cell membrane in aquatic systems.

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1. Introduction

Titanium dioxides (TiO_2) nanoparticles is one of the most widely used nanomaterials, with applications as cosmetics (Auffan et al., 2010), sunscreens (Nohynek et al., 2007), food additives (Weir et al., 2012) and photocatalysts (Hoffmann et al., 1995). The annual production of TiO_2 nanoparticles is rapidly increasing and estimated to reach 2.5 million metric tons by 2025 (Menard et al., 2011). Due to the increased production and application of synthetic TiO_2 nanoparticles, their release into the environment is inevitable. However, information regarding the TiO_2 nanoparticles toxicity, transport and fate in both natural and engineered systems is still scarce. Based on the existing studies, it is proposed that the interaction between nanoparticles and the membranes of microorganisms can be a critical initial process that precedes the toxicity pathways as well as influences the environmental fate of nanoparticles (Chen and Bothun, 2014).

To date, most related investigation on the interaction between cells and nanoparticles were mainly focused on how water chemistry, such as pH and ionic strength, affect the interaction between cells and nanoparticles (French et al., 2009; Ma et al., 2015). For example, solution pH determines the surface charges (i.e. zeta potentials) of both cells and nanoparticles, and thus influences the electrostatic interaction profile between the two objects. It was observed in many studies that low pH enhanced this interaction due to the nanoparticles being more positively charged while cells remaining negatively charged (Khan et al., 2011; Schwegmann et al., 2013). Salt ions can compress the electro-double layer of nanoparticles and cells, thereby reducing or eliminating the

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electro-double layer interaction. As a consequence, the commonly attractive van der Waals force becomes dominated and results in enhancement of cell surface and nanoparticle interaction under high ionic strength in water (Mukherjee and Weaver, 2010; Li et al., 2011; Shih et al., 2012). These results could be well described by the Derjaguin-Landau-Verwey-Overbeek (DLVO) theory due to the fact that the sizes of both microorganism cells and nanoparticles aggregates are within the scale of colloids. More recently, more comprehensive studies on the interaction between cell surface and nanoparticle in the presence of natural organic matters (NOM) have been carried out to mimic natural water environment (Lin et al., 2012). It was pointed out that both the bulk and nanoparticle-bound NOM can inhibit the interaction between cells and nanoparticles due to the delivery of negative charge to the surface of TiO_2 nanoparticles by NOM.

Approaches to probe the nanoparticle-membrane interaction are quite diverse, including by atomic force microscopy (AFM) (Leroueil et al., 2007; Roiter et al., 2008), optical tweezers (Rusciano et al., 2009), and quartz crystal microbalance with dissipation monitoring (QCM-D) (Keller and Kasemo, 1998; Zhang and Yang, 2011). QCM-D is the most extensively used tool for nanoparticle-membrane interaction due to its ability to in situ detection of nanoparticles adsorption on model cell membranes at a sensitivity level as low as tens of nanograms (Chen and Bothun, 2014). However, these techniques cannot distinguish which constituents or functionality of cell membrane correspond to the interaction when a membrane with multiple constituents is applied. Therefore, the binding affinities of cell surface constituents or functional groups to nanoparticles are unexplored.

Fourier transform-infrared (FTIR) spectroscopy is a versatile technique that offers a comprehensive insight into the molecular structure of principle constituents in bacterial cell membranes, such as protein, polysaccharide and lipid (Mecozzi et al., 2009). Two-dimensional correlation spectra (2D-COS), developed by Noda (1993), can be applied to resolve the overlapped peaks by distributing the spectral intensity trends along a second dimension with the data set collected as a function of a perturbation (e.g. time, temperature, concentration, etc.) (Noda, 1993; Dluhy et al., 2006). More importantly, it can also provide the information about the relative direction and sequential orders of structural variations in response to the perturbation. Thus, 2D-COS has been successfully applied to explore the interaction processes of NOM and TiO_2 nanoparticles (Chen et al., 2014). To the best of our knowledge, there is no literature reporting the application of 2D-FTIR-COS in the investigation on the interaction between nanoparticles and biological relevant components.

So far, the molecular mechanisms of the interaction between the NPs and bacterial cell membrane remain unclear, particularly, information on adsorption affinities of individual molecular constituents and functional groups is lacking. One of the major challenges is that cell membrane is dynamic and heterogeneous comprising multiple components such as phospholipid, protein, and polysaccharide (Chen and Bothun, 2014) that can lead to a more elaborate analysis of the mechanisms involved. Another challenge is that live cells undergoing metabolic process would secrete soluble microbial product (SMP) into the reaction solution and undoubtedly affect the interaction profile between cell surface and nanoparticles (Ni et al., 2011). A strategy to carry out nanoparticle-membrane interaction studies is to employ model membrane systems based on the phospholipid bilayer backbone of the cell membrane such as lipid vesicles (Hou et al., 2012; Lesniak et al., 2013; Chen and Bothun, 2014). Such systems can be further elaborated on by introducing other relevant components (i.e. protein and polysaccharides) that will make them more resemble the structure of cell membrane. Nevertheless, the synthesis of multi-components membrane architectures requires complex procedures and studies employing model membranes with embedded constituents such as protein and polysaccharide is still lacking to completely explore the interaction mechanism.

Bacterial ghosts (BGs) have recently emerged as novel vaccine candidates owing to their properties of being non-living bacterial cell membrane structure (cell envelopes) devoid of cytoplasmic constituents, and maintaining the full cellular morphology and surface constituents of their living counterparts (Jalava et al., 2002; Kudela et al., 2010). Moreover, the BGs can be easily produced by genetic methods or chemical methods (Mayr et al., 2005; Amara et al., 2013b). Therefore, it will be advantageous to employ BGs to study the interaction mechanisms of nanoparticles and cell membrane.

The purpose of this study, therefore, is to investigate the interaction between TiO_2 nanoparticles and cell membrane, by 2D-FTIR-COS technique, with BG as a model system. The settling experiments of standard protein, polysaccharide and phospholipid with TiO_2 were carried out to further verify and support the results.

2. Material and methods

2.1. Cell cultures and TiO_2 nanoparticles

Escherichia coli (*E. coli*) K-12 was used as model bacterium in this study. The bacterial cells were cultured in 50 mL Nutrient Broth 'E' (Lancashire, UK) with agitation at 200 rpm for 16 h. The cultures were then washed twice with sterile saline solution (0.9% NaCl) and resuspended in 50 mL sterilized saline solution with a cell density of ~2×10^9 colony forming unit per milliliter (cfu/mL). Degussa TiO_2 (P25, German) was used as a model TiO_2 nanoparticles in this study. The crystalline structures of the TiO_2 nanoparticles were identified through X-ray diffraction (XRD) analysis (Fig. S1). Its crystal structure consists of 80% anatase and 20% rutile, with an average primary size of 20–30 nm as revealed by transmission electron microscopic (TEM) analysis (Fig. S2), which were consistent with the description of manufacturer and published literature (Chowdhury et al., 2011; Tong et al., 2013a). A stock solution containing 10 g/L P25 solutions was used to prepare different concentrations of TiO_2 solutions.

2.2. BGs preparation and characterizations

The BGs were prepared according to a chemical method named "sponge-like" protocol (Amara et al., 2013a, 2013b). This method based on using active chemical reagents in concentration between Minimum Inhibition Concentration (MIC) and Maximum Growth Concentration (MGC) for bacteria. The MIC and MGC of were determined according to a previous report (Andrews, 2001), and shown in Table S1. Four chemical reagents were used in this protocol and their applied concentrations are determined as 4 mg/mL for SDS, 0.02 M for NaOH, 1.05 μg/mL for $CaCO_3$ and 64 mM for H_2O_2. In brief, 50 mL of washed cells were incubated with SDS, $CaCO_3$ and NaOH for 1 h to produce micropores on the surface of bacteria cells. Then the mixtures were centrifuged at 4000 rpm (Hermle Z323, Germany) for 10 min to evacuate the cytoplasmic constituent. The cell pellets were then washed with sterilized saline solution and resuspended in H_2O_2 solution for 30 min to guarantee the degradation of the residual DNA. Finally, the cells were collected by centrifugation at 4000 rpm and resuspended in 60% ethanol to remove any soluble organic residual. Then BGs were harvested by centrifugation at 4000 rpm and resuspended in 50 mL ultrapure water.

Light microscopy, scanning electronic microscope (SEM) and atomic force microscope (AFM) were used to observe the BGs as

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