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An aptamer cocktail-functionalized photocatalyst with enhanced antibacterial efficiency towards target bacteria

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HIGHLIGHTS

- Aptamer-conjugated TiO_2 was developed for target-specific bacterial inactivation.
- TiO_2-aptamer cocktail can enhance inactivation of target bacteria faster than TiO_2.
- TiO_2-aptamer cocktail can enhance inactivation of target bacteria in mixed culture.
- Efficient ROS transfer to the bacteria is caused by close contact of TiO_2-aptamer.

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ABSTRACT

We developed TiO_2 particles conjugated with an *Escherichia coli* surface-specific ssDNA aptamer cocktail (composed of three different aptamers isolated from *E. coli*) for targeted and enhanced disinfection of *E. coli*. We examined the target-specific and enhanced inactivation of this composite (TiO_2-Apc), which were compared to those of TiO_2 conjugated with a single aptamer (one of the three different aptamers, TiO_2-Aps) and non-modified TiO_2. We found that TiO_2-Apc enhanced the inactivation of targeted *E. coli* under UV irradiation compared to both the non-modified TiO_2 and TiO_2-Aps. A higher number of TiO_2-Apc than TiO_2-Aps particles was observed on the surface of *E. coli*. The amount of TiO_2-Apc required to inactivate ~99.9% of *E. coli* (10^6 CFU/ml) was 10 times lower than that of non-modified TiO_2. The close proximity of functionalized particles with *E. coli* resulting from the interaction between the target surface and the aptamer induced the efficient and fast transfer of reactive oxygen species to the cells. In a mixed culture of different bacteria (*E. coli* and *Staphylococcus epidermidis*), TiO_2-Apc enhanced the inactivation of only *E. coli*. Taken together, these results support the use of aptamer cocktail-conjugated TiO_2 for improvement of the target-specific inactivation of bacteria.

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1. Introduction

Safe water that is free from pathogens and hazardous substances is a basic need for all humankind. The presence of microbial pathogens in an aqueous environment and the diseases associated with such pathogens have become a considerable risk to public health. Therefore, securing safe water for the general public is a critical concern worldwide [1–3]. Chemical disinfection of waterborne pathogens is a practice that is widely followed to prevent disease, with the most common chemical disinfectant being chlorine. However, chlorination often results in the formation of harmful by-products [4–6]. Ozone is used mostly for the removal of organic micropollutants; however, like chlorine, it also forms by-products [7,8]. Filtration is another commonly used disinfection method; however, waterborne pathogens are neither inactivated nor killed by this technique, and membrane modules are not as cost-efficient as chemical disinfectants [9]. UV irradiation has been used worldwide for disinfection for many years to help reduce the amount of chlorine input in the environment and to consequently minimize the potential formation of by-products [10,11], and has been reported to effectively inactivate *Cryptosporidium* and other pathogenic protozoa [12–14].

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Recent advances in nanotechnology have provided several possible solutions for water disinfection using nanocatalysts and bioactive nanoparticles. In particular, TiO_2 nanoparticles have emerged as promising antibacterial agents for prevention of waterborne disease in combination with UV irradiation. TiO_2 is excited under UV irradiation ($\lesssim$380 nm) to generate electrons for holes on its surface [15,16]. These electrons interact with water to produce reactive oxygen species (ROS) such as hydroxyl radicals ($^{\bullet}OH$) and superoxide anions ($O_2^{\bullet -}$), which decompose most organic materials via oxidation [17–19]. The properties of ROS generated from TiO_2 under UV irradiation have also garnered extensive research attention for application in bacterial inactivation [20–22]. Numerous reports have suggested that these ROS cause oxidative damage to bacteria by disrupting the cell wall or damaging DNA/RNA and proteins [22–24]. It is believed that TiO_2 particles must be proximate to bacteria for effective disinfection, because reactive radicals have a very short life span and diffusion length in water [25–30]. Recently, enhanced antibacterial activity was also demonstrated by using TiO_2 particles doped with metals (such as Mn, Co, or Cu), fluorine, or carbon nanotubes under visible light [31–34]. However, these TiO_2 particles generally show non-selective inactivation in suspended conditions. A few studies have been conducted on the conjugation or absorption of TiO_2 with biomolecules such as DNA or antibodies; however, these studies did not focus on the selective inactivation of bacterial targets [35–38]. To overcome this limitation, we used bacterial target-specific aptamers that can enhance the specificity of TiO_2 particles to the target bacteria resulting in the efficient transfer of ROS to the cellular surface.

Aptamers are bio-receptors that are widely used for molecular recognition and can bind to a target with high selectivity and specificity owing to their ability to adopt a three-dimensional structure to target DNA or RNA [39–41]. Aptamers present numerous advantages for specific molecular recognition, such as ease of storage, chemical synthesis and modification, and handling [39,42]. Considering the high target affinity and specificity of aptamers, we hypothesized that TiO_2 particles conjugated with bacteria-specific aptamers would show selective and enhanced inactivation of target bacteria. We previously isolated several aptamers, which had high affinity and specificity to *Escherichia coli*, and investigated the advantage of combining these aptamers (aptamer cocktails) to enhance sensing compared to the use of a single aptamer for detecting bacteria [43]. To our knowledge, this is the first report about aptamer-TiO_2 conjugation for the enhancement of target-specific bacterial inactivation.

In this study, we synthesized a target-specific photocatalyst comprising TiO_2 and bacterial-specific aptamers to support our investigation of enhanced inactivation of target bacteria that can occur following proximate contact of TiO_2-aptamer conjugates to the bacterial surface in water. Specifically, we used *E. coli* aptamers as a model and compared the bacterial inactivation ability of a single aptamer-conjugated TiO_2 with that of an aptamer cocktail-conjugated TiO_2. The cocktail comprised three different aptamers that were all *E. coli*-specific, but possessed different affinities and target motifs. Furthermore, the specific enhancement of inactivation of aptamer-conjugated TiO_2 was verified by measuring its antibacterial effect on the bacterial co-existence of *E. coli* (target) and *Staphylococcus epidermidis* (non-target).

2. Materials and methods

2.1. Materials

TiO_2 particles (P-25, 75% anatase, 25% rutile; average diameter 25 nm) used in this study were purchased from the Degussa Company (Germany). Polyacrylic acid (PAA) was purchased from Wako Pure Chemical Industries, Ltd. (Japan). *N,N*-dimethylformamide (DMF), acetone, ethanol, ethanolamine, and 1-ethyl-3-[3-dimethylaminopropyl] were purchased from Sigma-Aldrich (St. Louis, MO, USA). Carbodiimide hydrochloride (EDC), *N*-hydroxysulfosuccinimide (Sulfo-NHS), 2-(*N*-morpholine) ethanesulfonic acid (MES), and sodium phosphate (PBS) were purchased from ThermoFisher Scientific Inc. (Rockford, IL, USA). Coumarin-3-carboxylic acid (3-CCA) was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). *E. coli* (KCTC 2571, KCTC 2617, and KCTC 2618) and *S. epidermidis* (KCTC 1917) were purchased from the Korean Collection for Type Culture (KCTC). Nutrient broth and nutrient agar were purchased from Becton, Dickinson and Company (Franklin Lakes, NJ, USA).

2.2. ssDNA aptamers

Three ssDNA aptamers (E1, E2, and E10), all of which exhibited specificity for *E. coli*, were used in this study [44]. Aptamers were synthesized by Genotech Inc. (Daejeon, Korea), and the sequence of each aptamer was as follows: E1 (5′-GCA ATG GTA CGG TAC TTC CAC TTA GGT CGA GGT TAG TTT GTC TTG CTG GCG CAT CCA CTG AGC GCA AAA GTG CAC GCT ACT TTG CTA A-3′), E2 (5′-GCA ATG GTA CGG TAC TTC CCC ATG AGT GTT GTG AAA TGT TGG GAC ACT AGG TGG CAT AGA GCC GCA AAA GTG CAC GCT ACT TTG CTA A-3′), and E10 (5′-GCA ATG GTA CGG TAC TTC CGT TGC ACT GTG CGG CCG AGC TGC CCC CTG GTT TGT GAA TAC CCT GGG CAA AAG TGC ACG CTA CTT TGC TAA-3′). Before use, each aptamer was modified with a 3′-amino group.

2.3. Preparation of aptamer-conjugated TiO_2 particles and measurement of ROS

PAA-TiO_2 particles activated by EDC and sulfo-NHS in 1 ml of MES buffer were synthesized following a previously described method [45]. These re-suspended PAA-TiO_2 particles were mixed with 50 μl of single aptamers (E1, E2, or E10) or aptamer cocktails (E1:E2:E10 = 1:1:1 molar concentration) and incubated overnight at 4 °C. The molar concentration (10 μM) of each single aptamer and aptamer cocktail was adjusted to be the same to give a similar number of aptamers on the surface of TiO_2 particles. Subsequently, the activated carboxyl groups were blocked with 0.5 ml of 0.1 M ethanolamine solution, and the mixture was incubated for 30 min at 4 °C. The single aptamer-conjugated TiO_2 particles (TiO_2-Ap_{E1}, TiO_2-Ap_{E2}, and TiO_2-Ap_{E10}) or aptamer cocktail-conjugated TiO_2 particles (TiO_2-Apc) were recovered by centrifugation (4000 rpm) for 10 min at room temperature and then washed twice using 1 ml of PBS (pH 7.0) buffer. Washed TiO_2-Ap_{E1}, TiO_2-Ap_{E2}, TiO_2-Ap_{E10}, and TiO_2-Apc were re-suspended in 2 ml of PBS (pH 7.0) buffer up to 0.05 g/ml and stored at 4 °C. The surface of the non-modified TiO_2, TiO_2-PAA, and TiO_2-Apc particles was analyzed using an Infinity Gold Fourier transform infrared (FT-IR) spectrometer (Thermo Mattson). Further, the elemental analysis of TiO_2 and TiO_2-Apc particles was performed using X-ray fluorescence (XRF, ZSX Primus II, RIGAKU, Tokyo, Japan) analysis. Production of ROS was measured from the raw TiO_2 and TiO_2-Apc particles. H_2O_2 production was measured using the Amplex red/horseradish peroxidase (HRP) reaction (Molecular Probes, Eugene, OR, USA) following the manufacturer instructions, and $^{\bullet}OH$ production was measured using a coumarin-3-carboxylic acid (3-CCA, 2 mM in PBS, pH 7.0) solution [46] with 0.1 mg/ml particles during 0, 30, and 60 min of UV irradiation. The fluorescence intensity of the reactant of 3-CCA was measured at an emission maximum of 450 nm.

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