IgG-Fe3O4@TiO2 MAGNETIC NANOPARTICLES AS THE PHOTO-KILLING AGENTS FOR PATHOGENIC BACTERIA

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Introduction

IgG-bound magnetic nanoparticles have been used to recognize several pathogenic bacterial strains including *Staphylococcus aureu*s, *S. saprophyticus*, and *Streptococcus. pyogenes* owing to the pseudo-immune interactions between the Fc sites of IgG molecules and the binding proteins on the surfaces of these bacteria [1]. We herein extended the application of this type of affinity magnetic probes as photo-killing agents by immobilizing a shell of photocatalytic material, i.e., TiO_2, onto the surface of the magnetic nanoparticles prior to binding with IgG molecules. Because titania materials have the capacity of adsorbing electron-donating bidentate enediol compounds such as dopamine onto their surfaces [2], IgG can be readily bound onto the surface of the nanoparticles via amide bonding using carbodiimide as the coupling reagent. The IgG-bound magnetic nanoparticles then possess multi-features including magnetic property, the capacity of targeting several pathogenic bacteria, and antimicrobial activity under UV light irradiation [3].

Methods

Iron oxide magnetic nanoparticles were prepared via co-precipitation by dissolving $FeCl_2$ and $FeCl_3$ in aqueous hydrochloric acid followed by addition of aqueous ammonia under stirring. Tetraethyl orthosilicate was immobilized onto the surface of the iron oxide nanoparticles followed by immobilized with a layer of titanium. The $Fe_3O_4@TiO_2$ magnetic nanoparticles obtained above were vortex-mixed with dopamine prepared in deionized water for 1 h. The dopamine-bound $Fe_3O_4@TiO_2$ magnetic nanoparticles were reacted with succinic anhydride prepared in DMF for 6 h under nitrogen protection. Then the nanoparticles were reacted with N-(3-dimethylamino-propyl)-N'-ethylcarbodiimide hydrochloride (EDC) for 10 min followed by rinsing with 2-(N-morpholino)-ethanesulfonic acid (MES) buffer (pH 6.3). The nanoparticles were then reacted with IgG prepared in MES buffer (pH 6.3) for 24 h. Scheme 1 shows the fabrication steps of IgG-$Fe_3O_4@TiO_2$ magnetic nanoparticles.

The IgG-bound $Fe_3O_4@TiO_2$ magnetic nanoparticles were then vortex-mixed with bacterial samples with a cell concentration of 10^9~10^{10} cfu/mL for 30 min. The nanoparticle-bacteria conjugates were isolated by magnetic separation followed by rinse with TSBY (5× 1 mL), which was prepared by dissolving tryptic soy broth (TSB, 24 g) and yeast (4 g) in deionized water (800 mL), and re-suspension in TSBY. Then 0.2 mL of the nanoparticle-bacteria conjugate suspension was diluted with TSBY (0.8 mL). The diluted suspension was irradiated with a UV b lamp (λ_{max} = 306 nm) for a given time (2~20 min). After irradiation, the suspension was $5 \times 10^4 \sim 5 \times 10^5$-fold diluted prior to culture on TSBY agar plates. The bacterial cells were then counted after incubation for overnight.

Results

Figures 1a-e show the plots of the survival ratio (%) of *S. pyogenes* M9141204, *S. aureus*, *S. pyogenes* M9022434, *S. saprophyticus*, and *S. pyogenes* JRS4, respectively, as a function of illumination time of UV light. *S. pyogenes* M9141204 and *S. pyogenes* M9022434 are multi-antibiotic resistant bacteria. The plots show the results obtained from control experiments by

incubating bacteria in the absence of the NPs, and the other experimental plots were obtained by incubating the bacterial samples with the IgG-Fe_3O_4@TiO_2 NPs under UV light illumination for 2~20 min. The cell concentration of these bacterial samples (1 mL) was 10^9~10^{10} cfu/mL. The results indicate that IgG-Fe_3O_4@TiO_2 NPs can be used as effective photo-killing agents under illumination of UV light within 20 min. This approach is also effective for antibiotic-resistant bacteria.

Conclusions

We have demonstrated that IgG-Fe_3O_4@TiO_2 nanoparticles are effective for inhibiting the cell growth of several pathogenic bacteria under UV light illumination. The nano-sized probes contributed to highly efficient energy transfer from UV light to the bacteria targeted by the probes, which result in the effective inhibition of the cell growth of the bacteria under illumination of a low power of a UV lamp within 20 min. This approach is potentially suitable for the treatment of cutaneous bacterial infections.

References:

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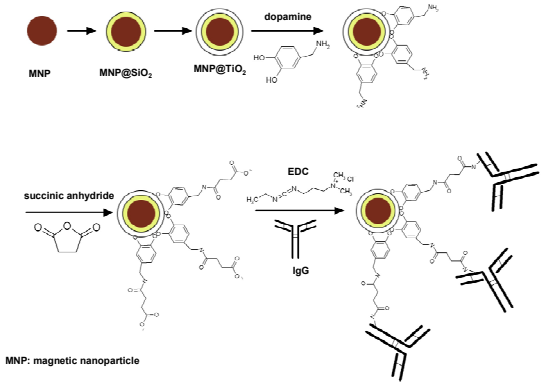
Scheme 1. Preparation steps for fabrication of IgG-Fe_3O_4@TiO_2 magnetic nanoparticles.



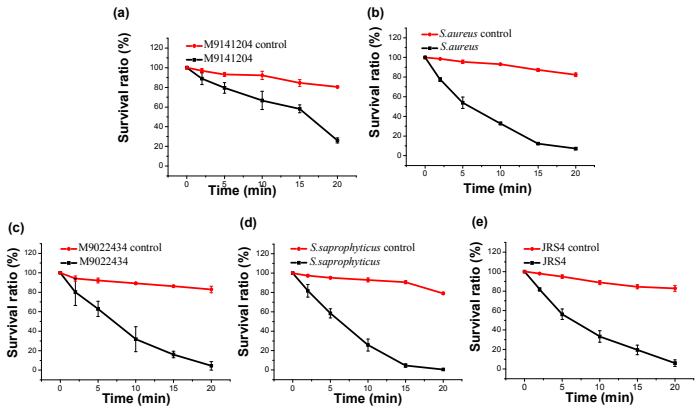


Figure 1. Plots of the survival ratio (%) of (a) *S. pyogenes* M9141204, (b) *S. aureus*, (c) *S. pyogenes* M9022434, (d) *S. saprophyticus*, and (e) *S. pyogenes* JRS4, as a function of the illumination time of UV light. The red plots were the control results obtained by incubating the bacteria under UV light irradiation for 2~20 min in the absence of the nanoparticles, respectively. The black plots were obtained by irradiating the bacteria with UV light for 2~20 min in the presence of the IgG-Fe_3O_4@TiO_2 nanoparticles. The cell concentration of these bacterial samples (1 mL) was 10^9~10^{10} cfu/mL.