

# Photothermal Colloid Antibodies for Shape-Selective Recognition and Killing of Microorganisms

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## Abstract

We have developed a class of selective antimicrobial agents based on the recognition of the shape and size of the bacterial cells. These agents are anisotropic colloid particles fabricated as negative replicas of the target cells which involve templating of the cells with shells of inert material followed by their fragmentation. The cell shape recognition by such shell fragments is due to the increased area of surface contact between the cells and their matching shell fragments which resembles antibody–antigen interaction. We produced such “colloid antibodies” with photothermal mechanism for shape-selective killing of matching cells. This was achieved by the subsequent deposition of (i) gold nanoparticles (AuNPs) and (ii) silica shell over yeast cells, which were chosen as model pathogens. We demonstrated that fragments of these composite AuNP/silica shells act as “colloid antibodies” and can bind to yeast cells of the same shape and size and deliver AuNPs directly onto their surface. We showed that after laser irradiation, the localized heating around the AuNPs kills the microbial cells of matching shape. We confirmed the cell shape-specific killing by photothermal colloid antibodies in a mixture of two bacterial cultures of different cell shape and size. This approach opens a number of avenues for building powerful selective biocides based on combinations of colloid antibodies and cell-killing strategies which can be applied in new antibacterial therapies.

The discovery of antibiotics has enabled the successful treatment of many infectious diseases. However, due to extensive use of antibiotics in recent decades, many pathogenic bacteria have developed multiple resistances to a number of antibiotics.(1-4) Development of novel strategies for combating multiple antibiotic resistant pathogens is recognized as a major challenge of modern medicine.

Here, we are introducing a new class of antibiotic agents based on colloid particles, which are capable of recognition and binding to bacterial cells based on their shape and size, which was used for their selective killing by photothermal therapy. Surface recognition of bacteria based on the cell morphology has already been demonstrated in biosensors based on microorganism-imprinted surfaces.(5-8) Key-lock interactions have also been used in non-biological colloidal systems for programmable self-assembly of colloid particles into composite clusters.(9, 10) Recently, several teams have looked into the encapsulation of cells in inorganic shells to create novel microstructures.(11-18)

Figure 1.

In this study we created the colloid analogue of antibodies which recognize the shape of target cells by fabricating silica shell fragments templating the cell surface. We produced such shell fragments by depositing silica by a sol-gel process onto the surface of target cells, forming core-shell particles. This was followed by their fragmentation by mild ultrasonic treatment and further removal of the cells' cores by a bleaching process.

Figure 2.

Figure 1A shows schematically the fabrication steps of such shell fragments after pre-coating of the target cells with gold nanoparticles. The surfaces of these shell fragments can be treated further in order to increase the specific affinity of the shell fragment to the matching bacterial species. We demonstrated that even without biospecific interactions, the shell fragments recognize the target cells and bind preferentially to them due to the increased area of surface contact, which resembles the antibody-antigen interaction. However, in our case the colloid particle recognizes the cell of matching shape, hence the term "colloid antibody". This strategy allows for selective killing of bacterial cells with matching colloid antibodies by combining the cell recognition with the delivery of biocidal agent directly to the target cell surface. The latter can be achieved, for example, by loading the colloid antibody with antibacterial payload. Here we demonstrate selective killing of target microbial cells by delivery of gold nanoparticles (AuNPs) onto their surfaces by photothermal treatment. When AuNPs are irradiated with light at a frequency which is resonant with their surface plasmon resonance, the absorbed light energy is converted into heat. This effect has been utilized in the so-called photothermal therapy to heat and consequently kill cancer cells;(19-27) it has also been used to kill bacteria(28-33) and damage viruses.(25-27) We demonstrate the action of these "photothermal" colloid antibodies powered by the localized heating of the AuNPs delivered directly onto the surface of the target microorganisms. The cells recognition combined with the photothermal treatment leads to their selective killing in a mixture with other non-matching microorganisms. We envisage that this strategy could also work for selective deactivation of viruses.

Figure 3.

Figure 1B illustrates the principle of action of such photothermal colloid antibodies (PCAs) in a mixture of bacteria of different morphologies. We fabricated PCAs matching the shape of yeast cells, *Saccharomyces cerevisiae*, which we used as model target microorganisms. The silica shells were templated over yeast cells, pre-coated with 10 nm AuNPs. Silica was deposited on the cells by a Stöber process, which led to integration of the AuNPs on the inner silica shell surface. The silica shells were partially fragmented by ultrasonic bath treatment and cleaned from the yeast cell cores by oxidation with Piranha solution. Here we describe the fabrication methodology of PCAs and the tests with them in more details (see also Supporting Information).

The AuNPs were synthesized as follows.<sup>(34)</sup> All reagents were purchased from Sigma and used as supplied. A mixture of 79 mL of Milli-Q water and 100  $\mu$ L of 10 wt% chloroauric acid (98%) was heated to 60 °C and rapidly mixed with vigorous stirring with a solution prepared from 4 mL of 1 wt% aqueous trisodium citrate, 100  $\mu$ L of 1 wt% tannic acid, 0.5 mL of 0.025 M potassium carbonate, and 20 mL of Milli-Q water. The mixture was heated until boiling, which produces a AuNPs dispersion, and subsequently cooled at room temperature. The PCAs for yeast cells were fabricated as follows. First, 25 mL of the AuNP dispersion was combined with 25 mL of 10 mg/mL of aqueous poly(allylamine hydrochloride) (PAH, 15 kDa, from Sigma) and agitated in an ultrasonic bath for 20 min. Next, 1.5 g (wet weight) of hydrated baker's yeast cells (from a local supermarket) was added to the AuNPs/PAH solution and incubated with shaking for 10 min. This was followed by triple centrifugation and washing with Milli-Q water, leaving pink pellets of the AuNP-coated yeast cells which were then redispersed in 3 mL of 1:1 methanol/water mixture. Finally, 0.25 mL of 25% ammonia as catalyst and 2.5 mL of tetraethylorthosilicate, both from Sigma, were added to the cell suspension and then agitated for 3 h at 25 °C to form silica shells.<sup>(18)</sup> The product was recovered by centrifugation and washed three times with methanol following two washing steps with deionized water. Afterward, the resulting pellet was dried overnight at 105 °C. The yeast core/silica shell particles were washed with Milli-Q water, and the sediment was exposed to 15 mL of Piranha solution (3:1 concentrated sulfuric acid and hydrogen peroxide) in order to remove the organic templates. The resulting shells were washed three times with water. Microscopy observation revealed that the Piranha solution treatment led to partial fragmentation of the silica shells which was further aided by their incubation in an ultrasonic bath for 10 min. The silica shell fragments were fluorescently tagged with Rhodamine B isothiocyanate (RBITC) in order to facilitate the identification of the recognition instances with the target microorganism. This was performed via a 1 h incubation in a solution of 10% 3-aminopropyltriethoxysilane in toluene. A triple washing of the silica fragments with methanol was then followed by incubation in 1 mM solution of RBITC in methanol for 3 h, multiple washings with methanol, and redispersing in water. Figure 2 shows typical SEM and TEM images of the AuNP-covered yeast cells and their matching shell fragments at different stages of fabrication of the PCAs. Fresh yeast and *Bacillus subtilis* cells were each washed with water and gently centrifuged to produce a pellet. About 0.02 g wet weight of each of the microorganism pellets were then dispersed in 5 mL water, to which the sediment of 10 mL of PCA dispersion was added and agitated for 10 min. We designed a test experiment for selective killing of yeast cells by PCAs in a mixture with *B. subtilis*, which is a rod-like bacterium (see Figure 1B). The model system was placed in a custom-made cylindrical glass cuvette with inner diameter 2 mm and length 20 mm, and

the sample was irradiated across its length with a Nd:YVO<sub>4</sub> laser (model AOT) at 532 nm. The laser pulse duration was 2 ns full width at half-maximum (fwhm) with energy ~10 μJ and a pulse repetition frequency of 20 kHz.

Figure 4.

The viability of the microorganisms was tested using fluorescein diacetate (FDA). A 10 mL aliquot of the FDA in acetone (10 mg/mL) was added to the irradiated sample, and the suspension was agitated for 20 min and examined using fluorescence microscopy, where the viable cells emitted green fluorescence due to internal hydrolysis of the FDA to fluorescein by the cell esterases. We also conducted three control experiments. These included testing the viability of cells which were not incubated with PCAs and/or treated by a laser. We examined the viability of the microorganisms which were incubated with PCAs but were not irradiated, and finally we also studied the effect of the exposure to PCAs and laser irradiation in a binary microbial mixture. Figure 3 shows a typical set of micrographs, which show over 85% specific recognition and killing in a mixture of two microorganisms by PCA. A summary of the experiments can be found in Figure 4. The photothermal effect of the AuNPs on PCAs by laser irradiation led to disruption of the cell walls of matching yeast cells and their death while the *B. subtilis* cells retained their viability.

In summary, we have fabricated and demonstrated the selective bactericidal action based on the recognition of microbial shape by colloid antibodies. Selective killing based on cell shape recognition was achieved in a mixture of microorganisms combined with a photothermal effect. We anticipate that similar colloid antibodies can potentially become a powerful weapon in the fight against antibiotic-resistant bacteria as well as some viruses against which current medicine is powerless. They can also find applications as non-toxic antibacterial agents, preventing growth of harmful bacteria in various formulations.

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