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# Nanoantibiotic Particles for Shape and Size Recognition of Pathogens

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## ABSTRACT

We have developed a novel class of colloidal particles capable of shape and size recognition as well as specific binding to the target cells. These colloid particles were fabricated using a nanoimprinting technology which yields inorganic imprints of the chosen target microorganisms. The products of the templating process are partially fragmented inorganic shells which can selectively bind to their biological counterparts, therefore impairing microbial cell growth, replication and infection. We have named this class of particles, which are capable of selectively recognizing bacterial shape and size, "nanoantibiotics", which can be further functionalized to kill the target cells. The selective binding is driven by the increased area of contact upon recognition of the cell shape and size between the cells and their matching inorganic shell fragments. Here, we demonstrate the cell recognition and binding action of such particles using two different microbial test organisms.

## INTRODUCTION

The emergence of bacterial strains resistant event to vancomycin questions our ability to create a universal and lasting defense against novel strains of antibiotic-resistant bacteria[1]. New strategies are needed to confront these growing threats which involve unconventional approaches for containing such microorganisms that use different principle of antibiotic action[2]. One new approach involves nanoparticle formulations with engineered biocidal effect designed to target specific bacteria[3-6]. In another approach Dickert and Hayden[7] used shape recognition of yeast cells by patterned solid surfaces imprinting the surfaces of three different type of yeast, which allowed selective cell binding and distinguishing between them. Similar approached for shape selective binding of microbes and spores on imprinted surfaces and hydrogel beads was employed by Cohen et al.[8] and Harvey et al. [9].

In this paper we have fabricated a new class of the nanoantibiotic colloid particles using a common process of producing silica shells on target cells via the Ströber process of base catalyzed hydrolysis of tetraethoxysilane[10]. Yeast cells were used as model target cells for this templating process. The silica shell deposition was followed by the shell fragmentation using ultrasonic agitation and subsequent bleaching of the yeast cells. After suitable surface treatment, the obtained shell fragments were used to test their binding selectivity in a dispersion of the yeast cells and in a mixture of the same yeast cells and rod-shaped bacterial cells (*B. subtilis*). This methodology is the first step of the fabrication of cell shape-recognizing colloid particles which can be further loaded with highly concentrated biocide that can be delivered directly onto the

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target cell surface. This combination would make these "nanoantibiotic" particles highly efficient selective biocides. In the present work we demonstrate only the selective binding and cell recognition by such nanoparticles. Figure 1 illustrates the fabrication process for these cell shape recognizing nanoantibiotic particles.

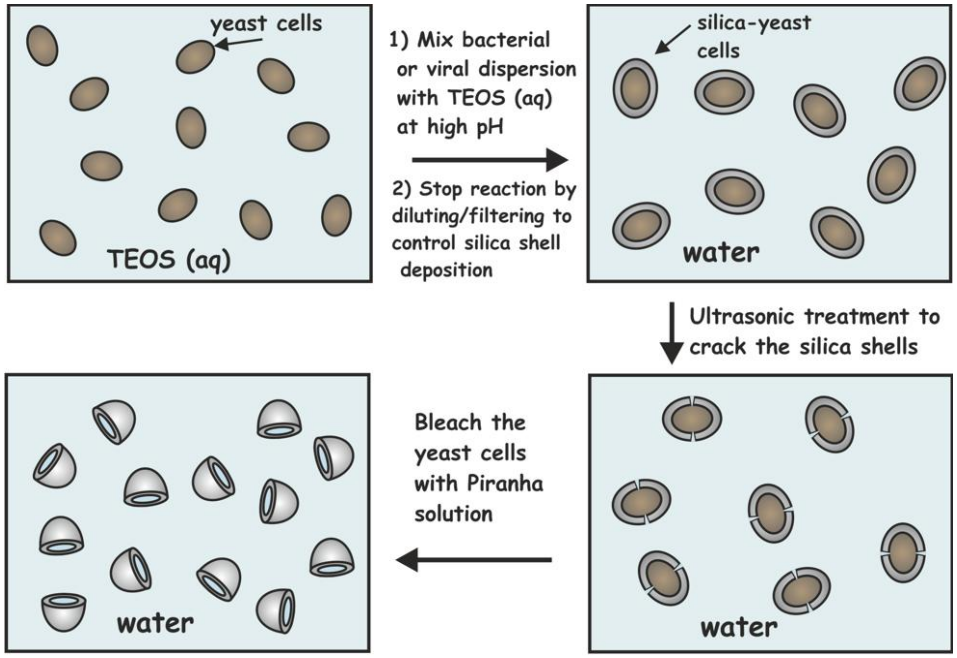


**Figure 1.** Fabrication scheme of the novel nanoantibiotic particles. The nanoantibiotic particles were fabricated via silica deposition onto yeast cells templates which was followed by the silica shell fragmentation and cell removal via a bleaching process. These inorganic templates of the yeast cells surface were then used to test the shape and size recognition in a dispersion of yeast cells as wells as a mixture of yeast and other bacterial cells.

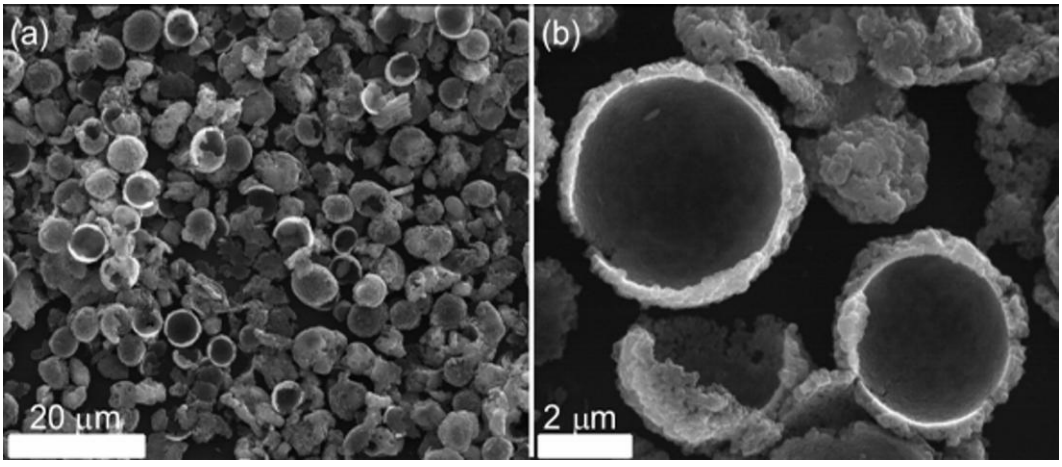


**Figure 2.** SEM images of the silica shell fragments obtained by templating yeast cells.

## RESULTS AND DISCUSSION

The silica shell fragments were produced on yeast cell templates with very good quality. The SEM micrographs of the core/shell particles revealed that some of the produced shell species were already disrupted at the end of the silica deposition procedure. The fragmentation of the silica shells was done using sonication in conjunction with bleaching process, which led to the formation of good quality silica shell fragments which are negative replicas of a part of the yeast cells surface (see Figure 2). These fragmented shells were then utilized in the recognition experiments (Figure 3). The average shell thickness was estimated from the SEM images to be around ~ 220 nm. This thickness, however, depends on the amount of deposited silica and the time of treatment of the target cells.

### a) Yeast cells recognition by matching nanoantibiotic particles

The capability of the nanoantibiotic particles to *"*remember*"* the size and shape of the target microorganisms was first tested by mixing the shell fragments with their matching yeast cell targets. In these experiments, we also probed the role of the surface chemistry of the nanoantibiotic particles and their matching target cells by coating them with polyelectrolytes. Yeast cells and the silica shell fragments were then incubated together in an aqueous suspension and the cell recognition events were then observed using optical and fluorescence microscopy (Figure 3).

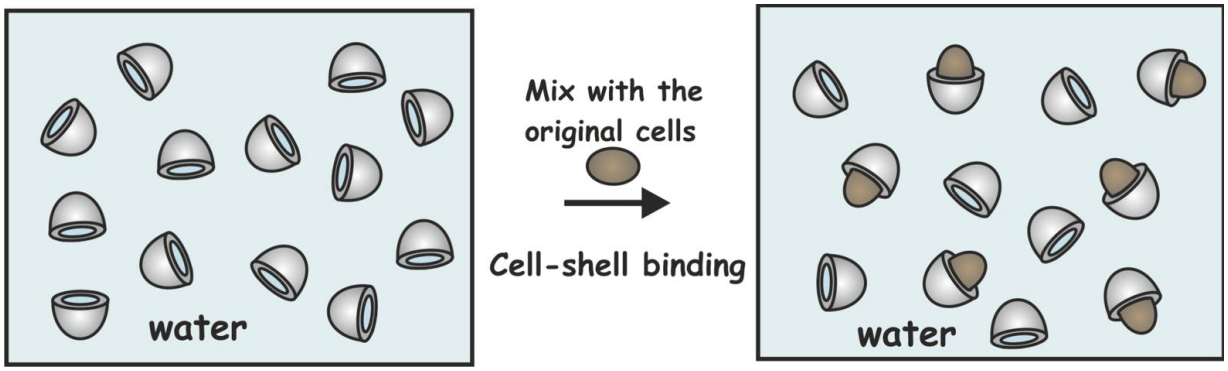


**Figure 3.** Experimental set up for studying the interaction between yeast cells and their matching silica shell fragments after surface treatment with polyelectrolytes to control their interaction.

The silica shell fragments and the cells were coated with monolayers and bilayers of cationic and anionic polyelectrolytes in order to induce attractive and repulsive electrostatic forces between the matching species. We expected that this would enhance or deter the combination of the negative replicas and their targets. The positive surface charge was induced via the coating of the shell fragments and/or the cells with polyelectrolytes using the layer-by-layer method, where the last coat was done with polyallylamine hydrochloride (PAH), while the negative surface charge was induced using polystyrene sulfonate (PSS) as a last coat. The yeast cells used: (a) were untreated with polyelectrolytes; (b) treated with a monolayer of PAH; or (c) treated with a double layer of PAH and PSS. The silica fragments were (d) left untreated; (e) treated with a monolayer of either PAH or PSS; or (f) treated with a double layer composed of each of those two polyelectrolytes. We tagged the silica shell fragments with Rhodamnine isothiocyanate

(RBITC) while their yeast cell targets were stained with perylene. This was undertaken in order to visualize the recognition incidents as it was realized that the silica shell fragments were often found on the retrograde side of the cell surface with respect to the microscope objective. As expected, we found that the species coated with oppositely charged polyelectrolytes attracted each other and lead to cell recognition by the oppositely charged shell fragments. We also found that the fluorescently tagged silica shell fragments recognized the natively negatively charged yeast cells. Figure 4 contains a graphical summary of the results and a set of sample micrographs.

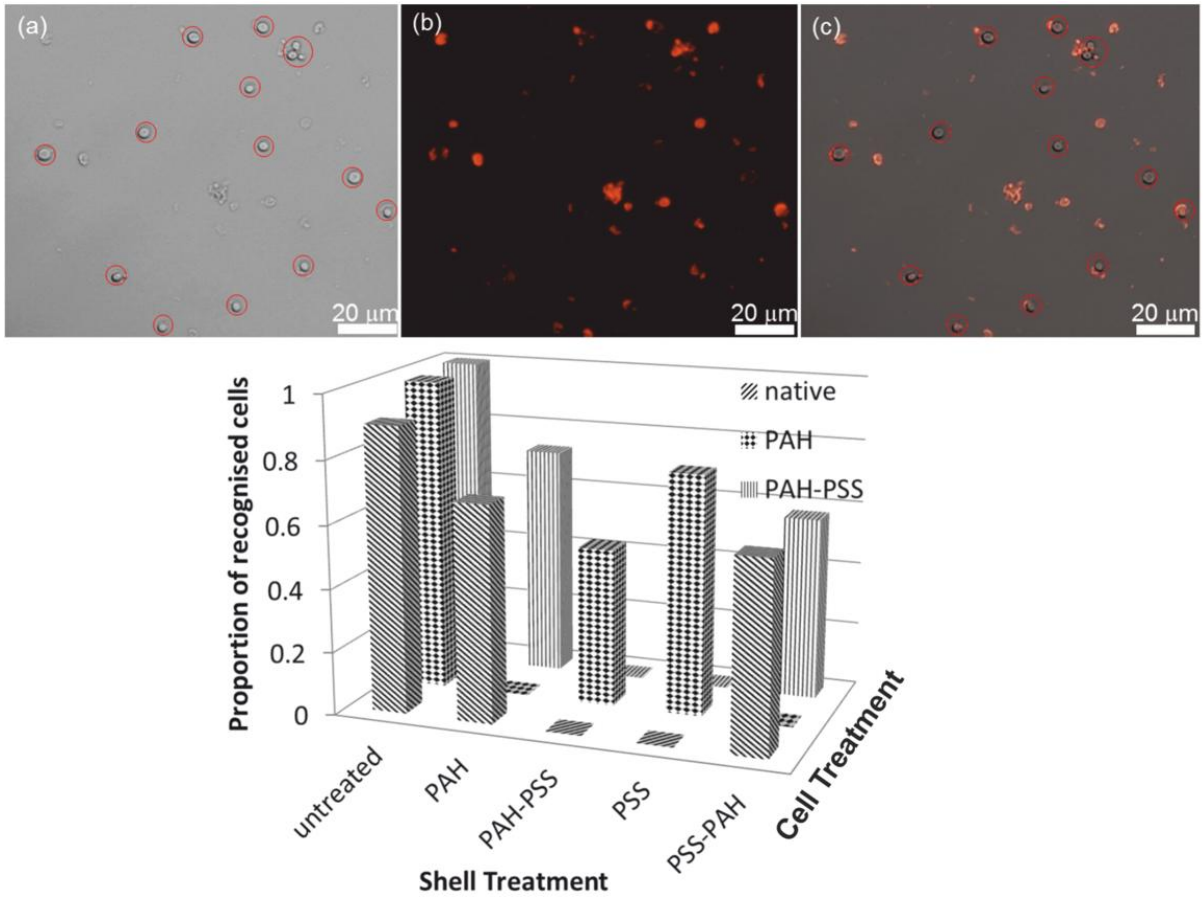


**Figure 4.** A cell recognition experiment which involved untreated yeast cells and untreated silica shell fragments leading to a match of the silica shell fragments and their cell targets. The bright field image (a) and the fluorescence image (b) were overlaid to produce the image (c). The diagram summarizes the findings from the nanoantibiotic-yeast cell recognition experiments.

In the graphical representation of the results in Figure 4 one sees that the highest rate of recognition interactions between the silica shell fragments and the yeast cell targets was observed for the untreated nanoshells, regardless of the surface coating of the cells. The thickness of the polyelectrolyte coating may also play a certain role in the recognition events resulting in less favorable interactions and a lower recognition rate. It is also expected that other interactions, like van der Waals forces, between the matching silica shell fragments and the target cells, can be strong enough to bind them to their matching cell targets together if their orientation is favorable. We expect that this contribution plays a major effect for untreated cells

and non-treated shell fragments. In the case of successful recognition, the shell fragments are attached to their cell counterparts via the concave side which corresponds to the largest achievable area of surface contact with the cell and maximal shell-cell adhesion. Our observations by high resolution optical microscopy also revealed that in the experiments where recognition occurred most of the cells did have attached silica shell counterparts.

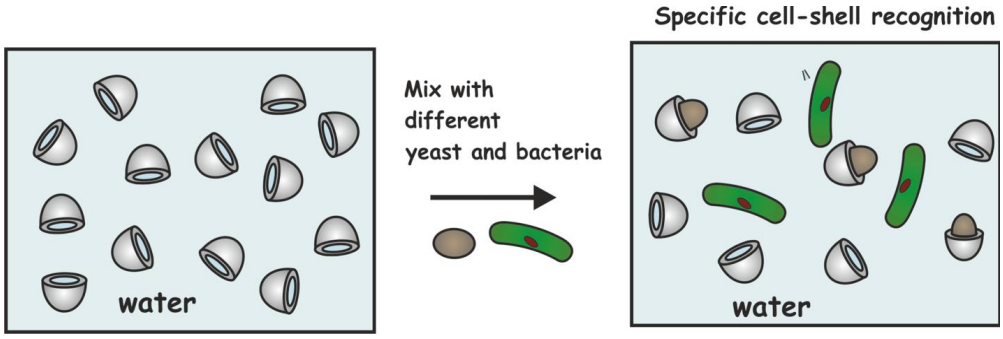


**Figure 5.** Experimental set up for probing the selective action of nanoantibiotics in a mixture of two types of test microbial organisms of different shape and size.

### b) Yeast cells recognition by nanoantibiotic particles in a mixture of bacterial cells

Following the successful demonstration of the specific recognition of individual yeast cells by matching nanoantibiotics, we investigated the nanoantibiotic selectivity in aqueous suspension containing a mixture of two microbial organisms. The nanoantibiotic particles were matching only the shape of the yeast cells whilst the other microbial cells were rod-shaped bacteria (*B. Subtilis*) which are much smaller. Figure 5 illustrates the experimental setup in this case.

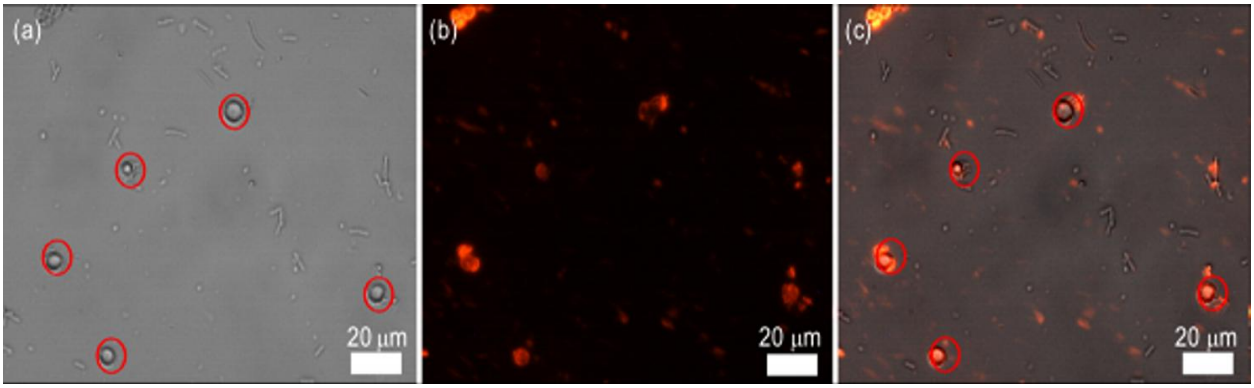


**Figure 6.** Sample results from the experiments involving the combination of bacterial and yeast cells together with the silica nanoshell fragments designed to match yeast cells. (a) The bright field optical microscopy image and (b) fluorescence microscopy image ( b) of the cell mixture incubated with yeast matching nanoantibiotic particles; (c) is the overlay of (a) and (b) which helped us to localize the position of the different cells and the nanoantibiotic particles.

The yeast-templated silica shells fragments were fluorescently tagged with RBITC in the same way as in the experiments involving silica shell fragment-yeast cell interaction (see Figure 6). The recognition was quantified in terms of the percentage of recognized cells and the

mismatches. An average 85 ± 11% recognition of yeast cells with no mismatches was observed. Apart from very small silica fragments with no specific shape, there was no evidence of yeast-templated shell fragments binding to *B. subtilis*.

## CONCLUSIONS

We have developed a novel concept for cell shape recognizing colloid particles based on a combination of nanoimprining of cells and shape-specific colloid interactions. These colloid particles are the first step in fabrication of more complex particles which we called "nanoantibiotics". Such particles were fabricated via several preparation steps which involve producing "negative" inorganic replica of the targeted cells in the form of shells fragments that match closely the cell shape and size. We present experimental results illustrating the shape-specific binding of matching silica shell fragments to model target cells like yeast and *B. subtilis* and analyze the effect of the shell fragments surface coating on the cell-shell binding efficiency and the cell recognition. It is anticipated that this novel class of nanoantibiotic particles could be designed to bind shape-specifically and potentially deliver a high dose of biocides directly onto the surface of target bacterial cells. This will allow a single nanoantibiotic particle to deactivate highly antibiotic resistant bacteria where most conventional antibiotics are ineffective. Nanoantibiotic particles can also find applications as non-toxic antibacterial agents, for example to prevent harmful bacteria from growing on home and personal care formulations.

## ACKNOWLEDGMENTS

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