Supplementary Materials

Antibiotic loaded nanocapsules functionalized with aptamer gates for targeted destruction of pathogens

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EXPERIMENTAL

Materials and reagents

MCM-41 type (hexagonal) mesoporous silica particles were purchased from Sigma-Aldrich (Sigma-Aldrich). The properties of the particles were given by the supplier as follows; the unit cell size: 4.6-4.8 nm; pore volume: 0.98 cm^3/g; pore size: 2.3-2.7 nm; specific surface area: ~1000 m^2/g (BET); bp: 2230 °C; mp: >1600 °C and bulk density: 0.34 g/mL. (3-Mercaptopropyl) triethoxysilane (CAS number: 14814-09-6), vancomycin (CAS number: 1404-93-9) and all other chemicals were purchased from Sigma-Aldrich. Sulfo-GMBS (Sulfo-N-succinimidyl 4-maleimidobutyrate sodium salt, CAS number: 185332-92-7) was from Fisher Scientific. *S. aureus* (ATCC 29213) and *S. epidermidis* (ATCC 2222) were obtained from the American Type Culture Collection (ATCC). The DNA oligonucleotides in Table I were synthesized by IDTDNA (USA).

Table S1. DNA aptamer oligonucleotides with modifications

SA20-hp	AF488-GCGCCCTCTCACGTGGCACTCAGAGTGCCGGAAGTTCTGCGTTAT-(PEG)-AGGGCGC-BH1
SA23-hp	AF488-GGGCTGGCCAGATCAGACCCCGGATGATCATCCTTGTGAGAACCA-(PEG)-CCAGCCC-BH1
SA34-hp	AF488-CACAGTCACTCAGACGGCCGCTATTGTTGCCAGATTGCTTTGGC-(PEG)-GACTGTG-BH1
SA20-amino	NH_2- GCGCCCTCTCACGTGGCACTCAGAGTGCCGGAAGTTCTGCGTTATAGGGCGC
Scrambled Sequence	NH_2- GGAGTTGCCGGTTACTGCGTACGTCCAGCTAGGCCATCTATCCGC*CAACTCC*

NH_2: *Amino-C6-modification,* **AF488:** Alexa Flour 488, **BH1:** Blackhole I quencher, **PEG:** 6 polyethylenglycol linker, **Red colored nucleotides:** added nucleotides complementary to 5´end for hairpin formation. The aptamer sequences was as reported by Cao *et al* [1].

Synthesis of aptamer-capped nanoparticles

The silica particles with aptamer gates were synthesized according to procedures as previously reported [2-4]. The details of procedures were briefly summarized below.

Silanization: 50 mg of MCM-41 powder in 20 ml of 95% ethanol containing 1 mM acetic acid and 3% (w/w) of 3-Mercaptopropyl) triethoxysilane were stirred for 30 min at room temperature, followed by washing with ethanol by centrifugation (13000xg, 3 min).

Loading and Capping: The sulfhydryl-modified nanoparticles (1 mg) were loaded with vancomycin by incubation in 1 mM vancomycin solution in PBS buffer (0.01 M phosphate buffered saline; NaCl-0.138 M; KCl-0.0027 M; pH 7.4) overnight and used for coupling with amino-modified oligonucleotides. 2 mg/ml sulfo-GMBS (Sulfo-N-succinimidyl 4-maleimidobutyrate) was mixed with the sulfhydryl-modified nanoparticles in PBS5M buffer and mixed for 30 min, washed 3 times with buffer and followed by 1 hour incubation under mixing in 100 μM solution of amino-modified aptamer (50 nmol in 500 μl PBS buffer containing 1 mM vancomycin). The particles were then washed thoroughly 3 times with buffer. Loading was calculated from the absorbance of the particles at 280 nm by comparing with a calibration curve.

Quantification of Immobilized Aptamer: The amount of aptamer immobilized on the surface of silica particles was measured by using complementary DNA labeled with FAM. The unloaded aptamer-particles were prepared as above and 0.01 mg of particles mixed with 5 μM FAM-labeled complementary DNA, followed by an incubation of 30 min. The particles were washed 5 times and re-suspended in 2 ml buffer for fluorescence reading. Bare particles were used to normalize the readings and the amount of immobilized aptamers was calculated from a calibration. The samples were analyzed by a spectrofluorometer (Horiba, Fluoromax 4) with appropriate filters for Fluorescein (Ex. 480 / Emm. 520).

Particle Size Distribution and Transmission Electron Microscopy (TEM)

The silica particles were characterized by electron microscopy. TEM samples were prepared by ultra-sonication of powders in ethanol for 5 min and drying of a droplet of suspension on a standard holey carbon TEM grid. TEM analysis was performed on Titan 60–300 electron microscope (FEI, Netherlands) operating at 300 kV in TEM mode. The average diameter of particles was determined by dynamic light scattering (DLS) with a Zetasizer Nano-S (Malvern Instruments, Worcestershire, UK). About 1 mg MCM-41 particles were suspended in PBS buffer and analyzed with Dynamic Light Scattering (DLS). Large aggregates of particles were first removed by low speed centrifugation (500 rpm for 30 seconds) and the supernatant was used in DLS experiments.

Bacterial cultures

Staphylococcus aureus and *Staphylococcus epidermidis* cells were grown overnight from frozen stocks at 37 ºC in tryptone soy broth (TSB). For colony counting experiments, the samples were surface plated on tryptone soy agar (TSA). The plates were incubated for 18 h and the single colony forming units were recorded. The turbidity of bacterial cultures, were obtained by measuring absorbance values at 600 nm in a UV/VIS spectrophotometer (Agilent, 8000).

Vancomycin release experiments by UV absorbance

Release of vancomycin from pores was monitored in UV/VIS spectrophotometer (Agilent, 8000) at 280 nm. The aptamer-capped mesoporous silica particles were placed in a cell holder, such that the particles were not exposed to incidence light. The particles were kept from mixing by trapping them in a compartment created by a dialysis membrane (cellulose membrane with molecular cut of 12,000 Da) at the top of spectroscopy cuvette as described in previous report [5]. Silica particles were large enough to be completely retained by dialysis membrane. A similar design of a two-compartment cuvette by using dialysis membrane was previously used to follow release of fluorescein from silica nanoparticles [5]. The solution was stirred continuously to create a homogeneous solution during readings. The stimuli (*S. aureus* cells) were added directly to the solution to trigger the controlled release. The maximum loading of the particles with vancomycin was determined by preparing a mixture of 1 mg aptamer capped silica particle mixture in PBS buffer and measuring the 280 nm absorbance in a well-mixed solution. The half-life of the triggered release was determined as the time at which 50 % of loaded vancomycin had diffused into buffer solution.

Vancomycin release by MALDI-TOF analysis

All mass spectra were recorded using a Voyager-DE Pro MALDI-ToF mass spectrometer (Applied Biosystems, USA) in positive ion mode. Desorption and ionization of samples were performed using a 337 nm nitrogen laser by averaging 200 laser pulses at ca. 10^{-7} Torr. For ion extraction, an acceleration potential of 25 kV was used in the source region. A 10 mg/mL of 2,5-dihydroxybenzoic acid (DHB) matrix solution (20 mg/mL) was prepared in water:acetonitrile:trifluoroacetic acid mixture (1:1:0.001 v/v/v) and sample solutions were mixed with the matrix solution at 1:10 (v/v) ratio. Finally, 1 µl of each final solution was directly spotted onto the MALDI plate and analysis was carried out after air-drying of each spot.

Microbial inhibition concentrations

The bacterial susceptibility was determined by minimal inhibitory concentrations (MICs) in 96-well microtiter plates using the microdilution method according to the Clinical and Laboratory Standards Institute [6]. However, rather than Mueller-Hinton broth, tryptone soy broth (TSB) was used for susceptibility testing as it is the common medium used for *Staphylococcal* cultures.

RESULTS

Aptamers gates

Three potential aptamer sequences as reported by Cao *et al.*,[1] were tested for their ability to design an aptamer-gate. The specific aptamer sequence of this study was experimentally determined from the response of switch probe designs. The probe is an intramolecular signal-transduction aptamer that consists of the aptamer sequence and a short-stem DNA sequence complementary to the other end of the aptamer sequence and separated by a polyethylene glycol (PEG) linker. A fluorophore (Alexa Fluor 488) and quencher (Black Hole 1) were covalently attached at the two ends of the sequence. The interaction of *S. aureus* surface antigens with the aptamer sequence disrupts the hairpin structure and leads to rearrangement of the structure, separating quencher and fluorophore from each other. This leads to an increase in fluorescence that depends on the presence of the target (Fig. S1). Among the three probes, SA20hp responded with highest fluorescence peak in the presence of 10^4 *S. aureus* cells (Fig. S1, black line). Although SA23hp and SA34hp resulted in fluorescence increases, the responses were about 25% and 40% lower than SA20hp respectively. Based on these results, we have

selected SA20hp as the molecular gate aptamer in the rest of the study. The response of SA20hp probe was also challenged with *S. epidermidis* and only a slight response was obtained at about 82 % lower than that of *S. aureus* cells.

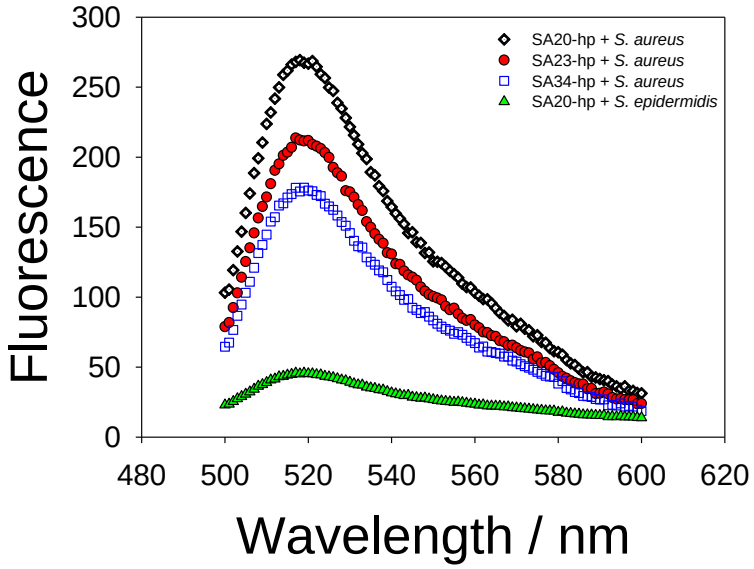

Fig. S1 Fluorescence changes of the aptamer switch probes upon addition of 104 S. aureus or S. epidermidis cells. Emission spectra was recorded for λex. at Green _ 480 nm, The y-axis is fluorescence at arbitrary units.

Synthesis of aptamer-gated silica nanoparticles

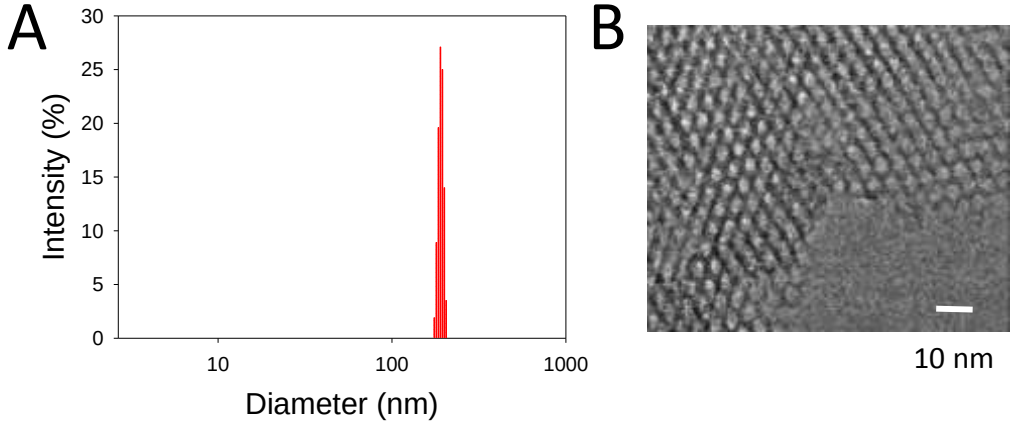

Fig. S2 Size and surface characterization of silica particles. A) Size distribution of MCM-41 nanoparticles as determined by dynamic light scattering. The average diameter of particles was 177.5 ± 2.3 nm. and B) Typical Transmission Electron Microscopy (TEM) image showing the size of nanopores.[7]

Kinetic Evaluation of Drug Release

To study the mechanism of drug release from the beads, the release data (Fig. S3) were fitted to four different kinetic models; the zero-order release equation (Eq.1), Higuchi equation (Eq. 2), and Power-law equation (Eq.3) to find the equation with the best fit.

$$Q = k_0 t \quad (1)$$

$$Q = k_H t^{1/2} \quad (2)$$

$$M_t = M_\infty \; k_p t^n \quad (3)$$

where Q is the mass of drug release at time t and k_0, k_H and k_P are the release rate constant of zero-order, Higuchi, and Power-law, respectively. M_t and M_∞ are the respective mass of drug release at time *t* and infinite (equilibrium) time, respectively. *n* is the diffusion exponent and related to drug release kinetics [8-12].

Fick's first law assumes that the concentration gradient is independent of time. However, during a real diffusion process, the concentration of a diffusing species within the host solid changes with time at a given position. The Fickian mechanics is often applied to diffusion-controlled release behaviour due to simplicity. A situation that does not conform to these conditions is described as either Case II or anomalous behaviour.

The effect of simple geometry on drug release is described by power-law model (Eq. 3). K_p is the kinetic constant and K_p being a constant incorporating structural and geometric characteristics of the device. In fact, the power law approximation described the release behaviour better than the previously mentioned approaches such as the Higuchi model. Therefore, the diffusion coefficient was determined from the ln-ln plots of M_∞ versus time (Equation 4). The diffusion coefficient was calculated from the slope of the curve and Equation (5)

$$\ln (1-M_t /M_\infty) = -k t + \ln B \quad (4)$$

$$k = \pi^2 D /r^2 \quad (5)$$

where *D* is the apparent diffusion coefficient, *r* is the particle radius. The apparent diffusion coefficients, the D of vancomycin from the aptamer-capped nanoparticles were analyzed by eq. 5 and given in Table S1. The D values change between 0.859×10^{-16} – 4.64×10^{-16} $cm^2\ s^{-1}$.

Table S2. Release parameters from aptamer-capped nanoparticles containing vancomycin

	Release kinetic models							
	Power Law				Zero order		Higuchi's model	
	k_p	n	R^2	D $x10^{16}$ ($cm^2\ s^{-1}$)	k_o x 10^2	R^2	k_H x 10^2	R^2
S.aureus	2.6	0.36	0.98	4.6	12.1	0.86	10.4	0.87
S.epidermidis	1.9	0.21	0.98	4.1	4.3	0.90	3.7	0.91
No-bacteria	1.3	0.06	0.95	0.9	1.1	0.97	0.9	0.97

Goodness of fit in different release kinetic models (Power Law, Zero-order and Higuchi's equations) was evaluated in order to understand the mechanism of drug release (Table S1). The high values of the coefficient of linear regression (≥0.97) for Power-law model confirm that it might be valid for nanocapsules. Exponents *n*, related to drug release kinetics, were in all cases lower than 1, indicating that drug release was not time

dependent and controlled by the relaxational process due to the swelling of the polymeric network. Drug release mechanisms were evaluated by using the Korsmeyer-Peppas semi-empirical model.[12] In this model, the value of n identifies the release mechanism of drug and $0.45 \leq n$ corresponds to a Fickian diffusion mechanism, $0.45 < n \leq 0.89$ to non-Fickian transport, $n = 0.89$ to Case II (relaxational) transport, and $n > 0.89$ to super Case II transport. The values of release parameters n and k are inversely related. A higher value of k may suggest a burst drug release from the nanocapsules. Vancomycin release mechanism from aptamer-gated nanocapsules was evaluated and the *n* value was observed to be between 0.060-0.355 (Table S1). The *in vitro* release mechanism in aptamer-capped nanoparticles was therefore identified to be a Fickian diffusion mechanism. The n values were less than 0.5 for all the batches indicating that the release rates exhibit a combined mechanism of diffusion partially through a swollen matrix and partially through water-filled pores [12].

Control Experiments for MIC Analysis

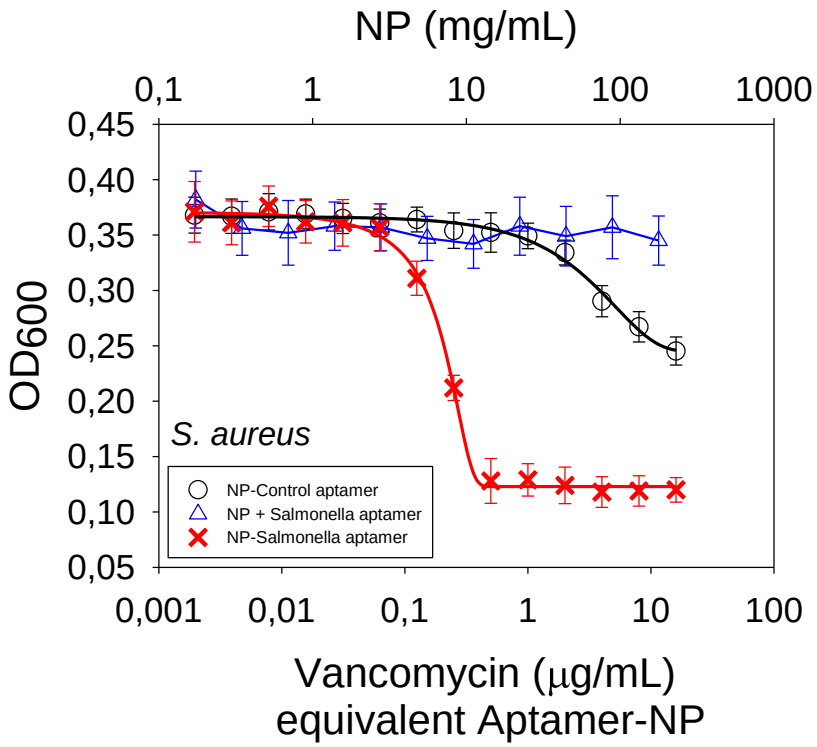

Fig. S3 Control experiments for aptamer-gated antibiotic nanoparticles. Susceptibility of *S. aureus* cultures were challenged against serial two-fold dilutions of vancomycin-nanoparticles (red and black lines) or control samples without vancomycin (blue line). Red line is the experiment with vancomycin loaded nanoparticles capped with Salmonella aptamers (The same experiments as in Fig. 3). Blue line is the silica empty nanoparticles and Salmonella aptamers added separately in the sample solution. Black line represents vancomycin loaded nanoparticles capped with a scrambled sequence. After a 24 h incubation period at 37˚C, the cell viability was determined by measuring optical density (OD) at 600 nm, and the minimum inhibitory concentrations (MICs) were calculated. All the measurements were performed in triplicate; the error bars indicate the standard deviations.

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