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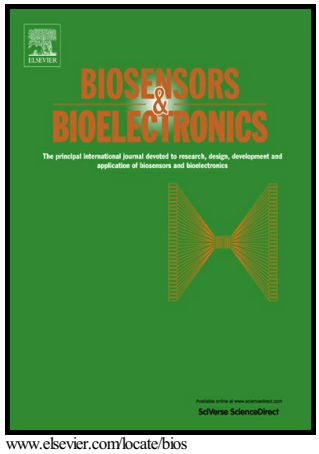

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Staphylococcus aureus detection in blood samples by silica nanoparticle-oligonucleotides conjugates

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Abstract

A fast, specific and sensitive homogeneous assay for Staphylococcus aureus detection was developed by measuring the activity of secreted nuclease from the bacteria via a modified DNA oligonucleotide. As biosensor format, an effective system, Nanokeepers as previously reported, were used for triggered release of confined fluorophores, and hence specific detection of S. aureus on nuclease activity was obtained. The interference from blood components for fluorescent quantification was eliminated by a pre-purification by aptamer-functionalized silica magnetic nanoparticles.The reported assay system was exclusively formed by nucleic acid oligos and magnetic or mesoporous silica nanoparticles, that can be used on blood samples in a stepwise manner. The assay was successfully used as a sensing platform for the specific detection of S. aureus cells as low as 682 CFU in whole blood.

Keywords: Micrococcal nuclease, Staphylococcus aureus, Aptamers, Nanoparticles, Magnetic separation

1. Introduction

Traditional culturing methods have been the gold standard in pathogen diagnosis. However, they are extremely time-consuming and labour intensive. Hence, culture-based techniques are far from satisfying the urgent needs of health care systems, which demand the instant determination of infections at point-of-care locations. To satisfy the need for quick pathogen detection, we developed a homogenous solution assay based on activity measurement of Staphylococcus aureus specific exogenous nuclease for quick detection of the bacteria directly from blood samples.

S. aureus is one of the major Human pathogens that are responsible from diverse disease symptoms with their infections. Micrococcal nuclease (MNase) of S. aureus is a naturally-secreted nucleic acid (RNA or DNA) degrading enzyme with important role in the spread of the bacterial cells in the infected host (Sibbald et al. 2006). MNase (also denoted as Themonuclease) is one of the most prominent biomarker of S. aureus for developing fast sensing platform in food and clinical samples. As an exo- and endo-5'-phosphodiesterase (EC 3.1.31.1) effectively degrading single stranded forms of both DNA and RNA (Alexander et al. 1961; Trémillon et al. 2010), MNase, also called thermonuclease, has been utilized in many molecular biology applications such as RNA sequencing (Krupp and Gross 1979), or as nucleic acid removal method for reduction of the viscosity of a cell lysate, improvement of protein purification, and development of in vitro translation systems (Cooke et al. 2003; Craig et al. 1992). MNase has been recognized as biomarker for the presence of the specific pathogen. Due to its potential as a quick indicator of S. aureus presence in the sample solutions, MNase has been employed in biosensor development for a long time. For example, heat treated blood samples were assayed for toluidine blue-DNA spot test for MNase activity assay (Xiong et al. 2014). Similar to other rapid detection tests such as coagulase, biochemical and immunological, MNase-based assays were shown to have variable sensitivity and reliability when applied to direct blood samples (Cao et al. 2009). PCR-based methods have been developed for S. aureus detection in food and clinical samples (Rodriguez et al. 2016; Wang 2016). Although PCR-based sensing assays significantly decrease time to results, they are still labour intensive and require sophisticated instrumentation with trained personnel. Similarly, Mass Spectroscopy-based methods suffer from the same kinds of limitations (Fleurbaaij et al. 2015; Jordana-Lluch et al. 2015). Some approaches based on MNase activity have been reported for quick detection of S. aureus, such as monoclonal antibody based quantum dot platform (Chandan et al. 2016), or graphene oxide.

Aptamer-nanoparticles conjugates have been used to develop biosensors in many sensor formats(Ozalp et al. 2015c; Pavlov et al. 2004). We developed previously a magnetic pre-concentration of microorganisms for Salmonella detection from milk samples by PCR, real-time PCR (rtPCR) and quartz Crystal Microbalance (QCM) (Ozalp et al. 2013; Ozalp et al. 2015b; Ozalp et al. 2014). Later, similar strategies were adapted by other research groups (Eslami and Alizadeh 2015; Xu et al. 2015). Bloodstream infections are life-threatening condition, that can lead to septic shocks. The antibiotic therapy is recommended within the first hour as soon as infection was determined (Dellinger et al. 2013). Since blood sample analysis remains the best approach to detect any infection and to monitor the effectiveness of subsequent antibiotic therapy (Opota et al. 2015), the assay system to be developed in this report utilized a pre-concentration approach in blood samples.

We also reported fluorophore loaded mesoporous silica nanoparticles designed to widely block the porous orifice and in this way could retain the loaded drug inside the nanocapsules, called Nanokeepers (Hernandez et al. 2014a). In this report, such particles were exploited to obtain an efficient and specific S. aureus detection when combined with magnetic pre-concentration procedure from blood samples. The utility of NanoKeepers as a biosensor approach depends on the ability to identify specific bacterial nucleases and thus, certain types of bacteria. This molecular approach for the specific detection of S. aureus use secreted nuclease, MNase, as a trigger mechanism for activatable-fluorescent probes. This oligonucleotide probe sequence has two central thymidines, flanked by 2'-O-Methyl nucleotides modified at the ends with a fluorophore and a quencher (named as TT probe) (Hernandez et al. 2014b). TT probe confers specificity to MNase (most likely by the TT sequence) while resistance to endogenous nucleases in mouse and human serum is provided by the 2'-O-Methly nucleotides. Based on this knowledge, we integrated a similar TT probe oligonucleotide sequence with hairpin structure into mesoporous silica nanocapsules as molecular gates (Hernandez et al. 2014a). In this report, we combined magnetic pre-concentration of target bacteria through aptamer specific capture from blood samples to Nanokeepers in order to measure micrococcal nuclease activity, thus detection of S. aureus. The overall strategy as employed in this report was presented in Figure 1.

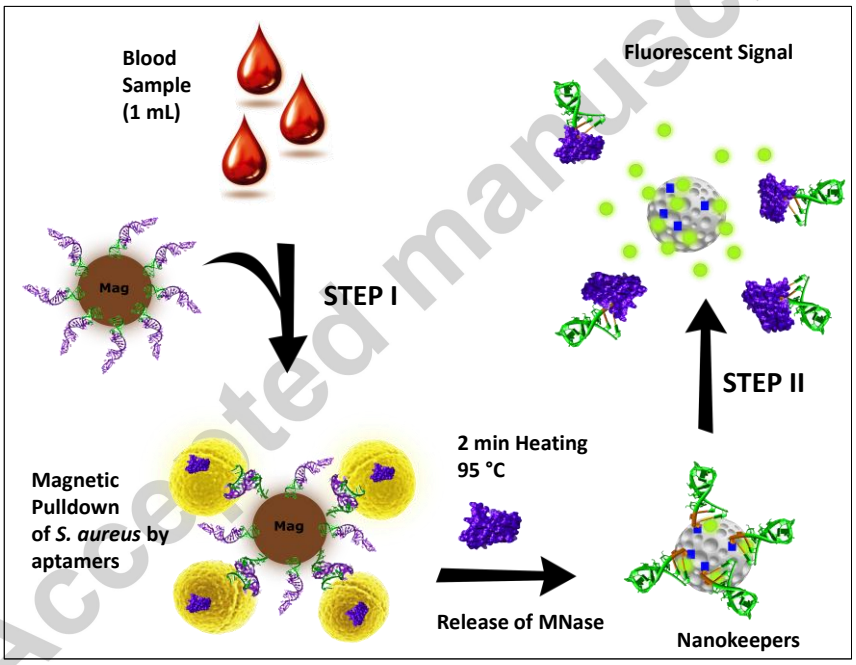

Fig. 1 Schematic representation of stepwise determination of S. aureus by specific opening of the pores of fluorescein loaded Nanokeepers. In Step I, 1 mL of blood sample with S. aureus cells was mixed with specific aptamer functionalized magnetic silica nanoparticles and a magnetic pulldown procedure resulted in isolation of S. aureus cells from blood. Subsequently, a brief heating treatment of the isolated cells released MNase into solution, to which fluorophore loaded and nuclease resistant olignucltides-capped nanokeepers (mesoporous silica nanoparticles) were added in Step II. Finally, the fluorescence signal was monitored when S. aureus MNase was present.

2. Materials and methods

2.1 Materials and reagents

Ferric chloride ($FeCl_3$), ferrous chloride ($FeCl_2$), 3-(triethoxysilyl)-propylamin (APTES), tetraethoxysilane (TEOS), glutaraldehyde, tris-HCl, and thetrahydrofuran (THF) were also obtained from Sigma-Aldrich. All other chemicals were analytical grade products and purchased from Merck AG (Darmstadt, Germany). The oligonucleotides were synthesized by IDTDNA (Europe).

2.2 Synthesis of silica coated magnetic nanoparticles

The magnetite nanoparticles were synthesized by chemical co-precipitation of Fe(III) and Fe(II) ions with a molar ratio of 2 : 1 as reported before (Bayramoglu et al. 2015b; Kim et al. 2007; Oktem et al. 2007; Ozalp et al. 2015a). For thermal co-precipitation reaction, $FeCl_3.6H_2O$ (5.8 g, 0.02 mol) and $FeCl_2 \cdot 4H_2O$ (1.72 g, 0.01 mol) were dissolved in 100 mL water under nitrogen gas, and the solution was mixed in a reactor with aqueous ammonia (50 mL, 25% w/v) by continuous stirring and refluxed under nitrogen at 40 ◦C for 2.0 h, and then at 80 ◦C for 1.0 h. Finally, the black magnetic particles were separated magnetically using a magnet and washed with HCl solution (10 mmol L^{-1}) and distilled water. The resulting magnetic particles were dried under vacuum at 40 ◦C for 6.0 h.

APTES/TEOS was grafted on the magnetic nanoparticles by a co-condensation reaction under alkaline conditions to obtain free amino groups on their surface. APTES (1.0 g) and TEOS (1.5 g) was dissolved in 300 mL ethanol, and mixed with magnetic nano-particles (about 2.0 g) in purified water (100 mL). The magnetic nano-particles in the reaction solutions were dispersed by sonication for 30 min. The co-condensation reaction of APTES and TEOS was initiated by the addition of ammonia (20 mL, 25%, v/v) and allowed to react by refluxing at 40 ◦C reflux for 8.0 h under continuous stirring. The resultant silica coated magnetic hybrid nanoparticles were collected by means of a magnet, washed with methanol and ultrapure water. The magnetic hybrid particles were dried under low pressure at room temperature.

2.3 Preparation of aptamer functionalized hybrid magnetic-silica particles

The free amine groups on the surface of the hybrid magnetic-silica particles were first activated with glutaraldehyde, then covalently coupled with amino modified aptamers (Bayramoglu et al. 2013; Bayramoglu et al. 2015a). About 250 mg of amino functionalized hybrid magnetic-silica particles was transferred in a reactor containing 2.0 % glutaraldehyde in phosphate buffer (0.1 M, pH 7.0). The coupling reaction was carried out at 35 ◦C for 3.0 h and washed sequentially with 0.1 M acetic acid solution, and 0.1 M phosphate buffer. Then, the amino functionalized hybrid silica nanoparticles (about 50 mg) were left in phosphate buffer (50 mM, pH 8.5) for 2 h, and transferred to the phosphate buffer containing S. aureus binding aptamers (100 µL, 200 µM). The aptamer sequence (GCGCCCTCTCACGTGGCACTCAGAGTGCCGGAAGTTCTGCGTTAT) was used in this work as reported by Cao et. al. (Cao et al. 2009). A 5' amino labelled form of the aptamer were immobilized on the magnetic beads at 25 °C for 18 h, while continuously mixing the reaction medium. Finally, non-covalently adsorbed aptamer molecules from the magnetic beads were washed away with PBS buffer. The amount of immobilized

aptamer was measured using UV-absorption method at 265 nm using an UV–Vis spectrophotometer (Agilent 8000, USA).

2.4 Characterization of silica nanoparticles

The free amino group content of the APTES/TEOS coated silica particles was determined by potentiometric titration (Bayramoglu et al. 2015a). The silica coated magnetic particles (about 0.1 g) was put into hydrochloride acid solution (0.1 M, 10 ml) and incubated in a shaking water-bath at 35 ◦C for 6 h. Subsequently, the final HCl concentration in the solution was determined by potentiometric titration with 0.05 M NaOH solution. ATR-FTIR spectra of the magnetic and silica coated magnetic nano-particles were obtained in the one-bounce ATR mode in a Spectrum 100 FTIR spectrometer (Perkine Elmer Inc., Norwalk, CT, USA) equipped with a Universal ATR accessory. Samples were scanned from 4000 to 525 cm^{-1}. The specific surface area of the magnetic and silica coated magnetic particles was determined by BET (Brunauer, Emmett, and Teller) method using a Quantachrome Nova 2200 E, USA. The magnetization of the magnetic and silica coated magnetic particles versus the applied magnetic field was determined with a vibrating-sample magnetometer (VSM; Model 155, Digital Measurement System Inc., Westwood, MA, USA) at room temperature (Ozalp et al. 2014). Size distribution patterns of magnetic-silica nanoparticles and mesoporous silica particles were determined using Particle Size Analyzer from Malvern Instruments Limited (Malvern Zetasizer-Nano ZS90 ZEN 3600) afte they were sonicated for 20 min at room temperature in phosphate buffer (50 mM, pH 7.0).

Nanokeepers were prepared from mesoporous silica nanoparticles according to previously published procedure(Hernandez et al. 2013). Briefly, MCM-41 type particles were incubated in 100 µM Fluorescein solution in PBS buffer overnight and nuclease resistant oligonucleotides were covalently immobilized on the surface of the particles, encapsulating the fluorophore molecules inside the pores. The particles were characterized with FTIR as explained above and TEM analysis showed mesoporous surface of the particles.

2.5 Bacterial cultures

Staphylococcus aureus, Staphylococcus epidermis, Escherichia coli and Salmonella enterica cells were grown overnight from frozen stocks at 37 °C in tryptone soy broth (TSB culture medium). The desired concentrations of cultures were prepared by serially diluting with PBS buffer (phosphate buffer (10 mM, pH 7.4), 0.137 M NaCl). For colony counting experiments, the samples were surface plated on TSB agar plates and were incubated for overnight at 37 °C and the single colony forming units were counted.

2.6 Magnetic pre-concentration by magnetic cell capture

Various numbers of bacterial cells (S. aureus or S. epidermis) were prepared in PBS buffer (50 mM Phosphate buffer, pH=8.0, 150 mM NaCl) or whole blood. The procedure was adapted from a previous report for milk samples (Ozalp et al. 2014). Briefly, the aptamer-conjugated beads of 1 mg were added into 1.0 mL of sample, mixed thoroughly by vortexing and incubated at 25 °C by constant shaking for 15 min. The captured cells were isolated by magnetic pull-down after 2 times washing with a total of 2 mL of PBS buffer. The isolated cells captured on aptamer-magnetic beads were directly used in assays.

2.7 Assay

The samples spiked with the specific bacterial cells were mixed with 1 mg of aptamer-nanoparticles and subjected to fluorescent assay or to culture plating for colony counting. Serial dilutions of eluted cells were plated on TSB agar plates and grown overnight at 37 °C. The viable colonies were enumerated and expressed as CFU/mL. For fluorescent assay, the captured cells from 1 ml blood samples were incubated at 95 °C for 2 min., cooled down to room temperature in 1 min. and then directly mixed with nanokeepers.

3. Results and discussion

3.1 Synthesis of aptamer-gated silica nanoparticles

The APTES/TEOS coated magnetic hybrid silica particles were synthesized as previously reported (Kim et al. 2007) and used for bacterial cell capture experiments. First, the magnetic particles were coated with 3-aminopropyltriethoxysilane and tetraethyl orthosilicate via co-condensation reaction. Then, the amine groups on the surface of the particles were activated by a bifunctional agent, glutaraldehyde. The silica shell and amine functionalization on magnetite particle core were confirmed by ATR-FTIR in the one-bounce ATR mode. Figure 2 shows the ATR-FTIR spectra of the magnetic nanoparticles (A), APTES/TEOS coated magnetic silica particles (B) and aptamer functionalized magnetic-silica particles (C). The characteristic absorption of Fe-O vibration is observed at 586 cm^{-1} (Figure 2A). The Sharp and revealing peak at around 580-631 cm^{-1} can be observed in (A), (B) and (C) spectra is attributed to the absorption peak Fe-O-Fe bond of Fe_3O_4 nanoparticles. In order to confirm the modification of the magnetic nanoparticle surface through the grafting with APTES/TEOS, an FTIR spectrum of the APTES/TEOS coated magnetic silica was obtained (Figure 2B). In the case of APTES/TEOS grafted particles the sharp band at 1119 cm^{-1} corresponds to Si-O-Si antisymmetric stretching vibrations, being indicative of the existence of SiO_2 in the particles. Therefore, the adsorption of silane polymer onto the surface of magnetite particles was confirmed by bands at 1119, 1046 and 968 cm^{-1} assigned to the SiO–H and Si–O–Si groups. The absorption band at 968 cm^{-1} revealed the presence of Si–O–H stretching and OH vibrations on the surface of magnetic nanoparticle. The characteristic absorption band for N-H bond was observed at 1631 cm^{-1}. Hydrogen-bonded silanols also absorb at around 3414 cm^{-1}. The presence of the propyl group of APTES was confirmed by C–H stretching vibrations that appeared at 2924 and 2853 cm^{-1} (Figure 2B). ATR-FITR spectrum is presented in Figure 2C for aptamer immobilized magnetic particles. As seen in this figure, the characteristic adsorptions at 1653 and 1539 cm^{-1} are attributed to -CONH- (amide I) and amide II vibrations, respectively (Figure 2C). Immobilized aptamer also displays these bands, indicating that aptamer has been successfully immobilized on the APTES/TEOS coated magnetic silica nanoparticles.

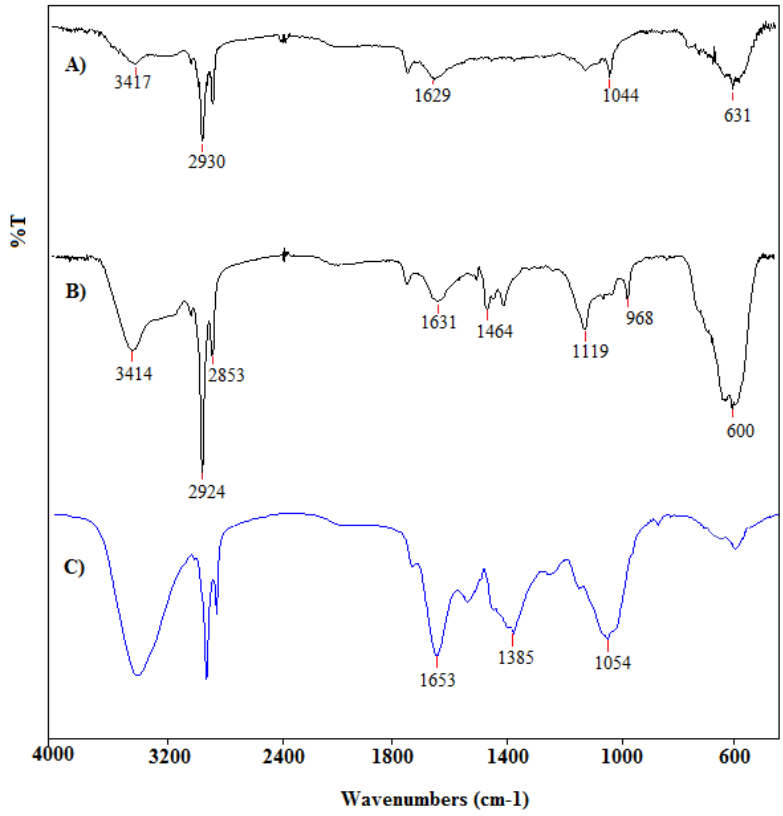

Figure 2. The FTIR spectra of the magnetic nanoparticles (A), APTES/TEOS coated magnetic silica particles (B) and aptamer functionalized magnetic-silica particles (C).

The peak value and average particles size distribution were found to be 145 and 283 nm, respectively (Supplementary data, Figure S1B). The presented separated from the reaction medium without needing high magnetic intensity. In addition, the coating efficiency of the silica grafted magnetic particles was found to be 81.2% as calculated by weighing the magnetic nanoparticles before and after coating with APTES/TEOS silica layers. The free amine group of the APTES/TEOS coated magnetic silica particles was determined to be about 2.87 mmol. The free amine groups of the coated silica particles were used for activation of glutaraldehyde for covalent coupling of aptamer ligands the APTES/TEOS grafted magnetic particles. Aptamer molecules with $-NH_2$ ending can be immobilized on the glutaraldehyde activated magnetic silica particles under very mild experimental conditions.

The Brunauer–Emmett–Teller (BET) surface area of the APTES/TEOS coated magnetic particles was found to be 27.6 $m^2\ g^{-1}$. The surface area measurement indicated that the silica coated magnetic particles had a large pore volume and a porous surface structure, which would favour high immobilization capacity for the aptamer ligands.

Results of magnetization measurements as a function of applied magnetic field are presented in Figure 3. Magnetic properties were determined at room temperature using a vibrating sample magnetometer (VSM). It is observed that saturation magnetization of silica coated magnetic particles was found to be 71.5 emu/g while the saturation magnetization of the bare Fe_2O_4 particles was 85.9 emu g^{-1}. The decrease in the saturation magnetization of silica coated magnetic particles can be explained by the decrease in the amount of the magnetic moments per unit weight due to the diamagnetic contribution of grafted silica

layers on the magnetic particles. The presented silica coated magnetic particles can be easily dispersed or separated from the medium without needing high magnetic intensity.

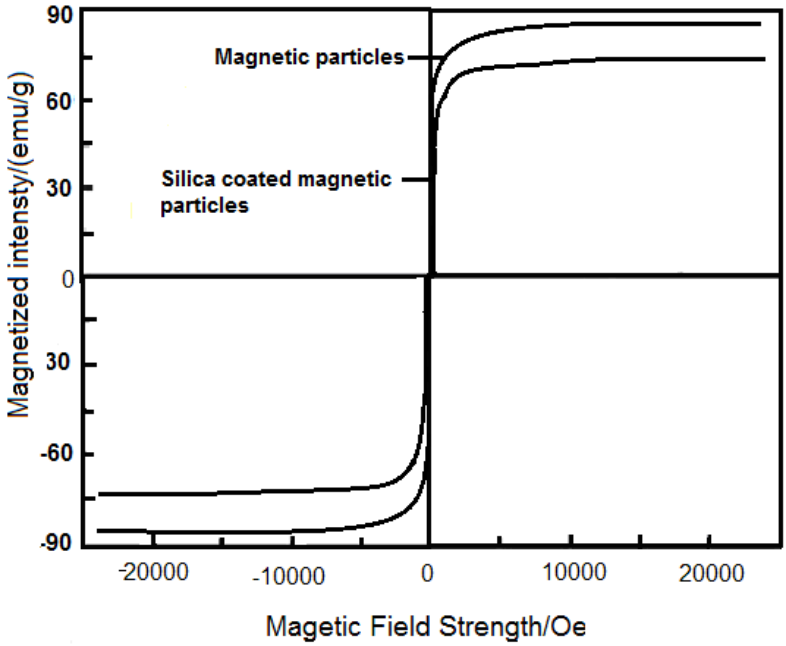

Figure 3 Magnetization curve of magnetic and silica coated magnetic particles as a function of the magnetic field.

3.2 Magnetic pull-down of bacterial cells

A series of experiments were carried out for determining capture efficiency of aptamer-nanoparticles quantitatively. Samples containing various numbers of bacterial cells were subjected to pre-concentration procedure by magnetic-aptamer nanoparticle pull-down. Then, the isolated cells were eluted and enumerated by plate culturing method (Figure 4). In these experiments, four other bacterial cell types (S. epidermis, E. coli, S. enterica and K. pneumonia) were used as control experiments to shown the specificity of the pull-down procedure. Therefore, the aptamer-magnetic pull-down purified the S. aureus cells from blood components and also from other potential bacterial cells to contribute further to the specificity of the detection in the second step. Aptamer-nanoparticles were able to capture more than 78% of cells from PBS buffer spiked with 10^2 CFU/mL (Figure 4A). The capture efficiency decreased gradually with the increasing number of cells in the samples, which should be resulting from the binding capacity of aptamer-nanoparticles since a fixed amount of particles at 0.1 mg/ml were included in all experiments. The same experiments were repeated with whole blood to obtain a very similar trend of decreasing capture efficiency with increasing number of bacterial cells, but reduced efficiency (Figure 4B). For example, about 61% of the cells were recovered after applying the pull-down procedure in the whole blood samples spiked with 10^2 S. aureus cells. This difference in captured cells by aptamer-nanoparticles might be originating from non-specific occupation of bacterial cell binding domains on aptamer molecules due to complexity of the blood. Otherwise, the efficiency results were similar to our previous magnetic pull-down assay with aptamers for other bacterial

cells in milk, using the magnetic p(HPMA/EGDMA)-g-p(GMA) beads (Ozalp et al. 2014).

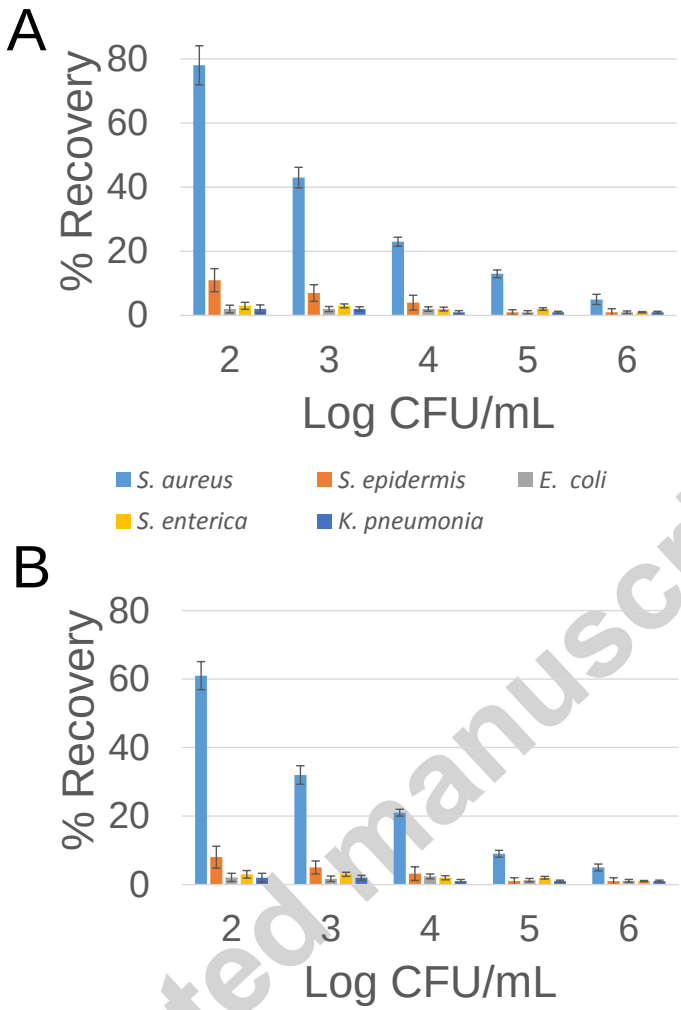

Figure 4. Determination of bacterial cells pulled-down by aptamer-nanoparticle system by culture counting method. About 104 CFU/ml of the indicated bacteria were added just before pull-down procedure either in PBS buffer (A) or whole blood (B). The labels for each bar was shown in the middle. The S. aureus was the first bar and then S. epidermis, E. coli, S. enterica and K. pneumonia were placed in this order for each experiment.

3.3 Synthesis and characterization of Nanokeepers

Previously we showed that modified hairpin sequence of the TT probe would foster complete detachment from the nanocapsule upon MNase actuation by using a molecular beacon form of NK01 sequence (FAM-TTmCmGmCmUmUmC mGmGmCmGmAmA–Quencher) (Hernandez et al. 2014a). Degradation of NK01 through nuclease activity and stability were comparable to those reported by the original TT probe (Hernandez et al. 2014b). Subsequently, the utility of NK01 as part of NanoKeepers approach were proved as a therapeutic antibiotic controlled release system. Figure 5A confirmed these results by using magnetically recovered S. aureus MNase activity. In order to verify in how far NK01 would block and retain a cargo

molecule inside the nanocapsules, we have used MCM-41 type silica particles. The nanoparticles had an average diameter of 187 nm with a uniform size dispersion and electron microscopy images confirmed the mesoporous structure (Supplementary data, Figure S1). The pores (average diameter 2.7 nm) were modified with a sulfo-linker for allowing subsequent amine coupling as the NK01 oligo had been functionalized with a 5'-amine group (NK01-NH_2) in order to facilitate the coupling to the capsule surface as previously described (Hernandez et al. 2013).

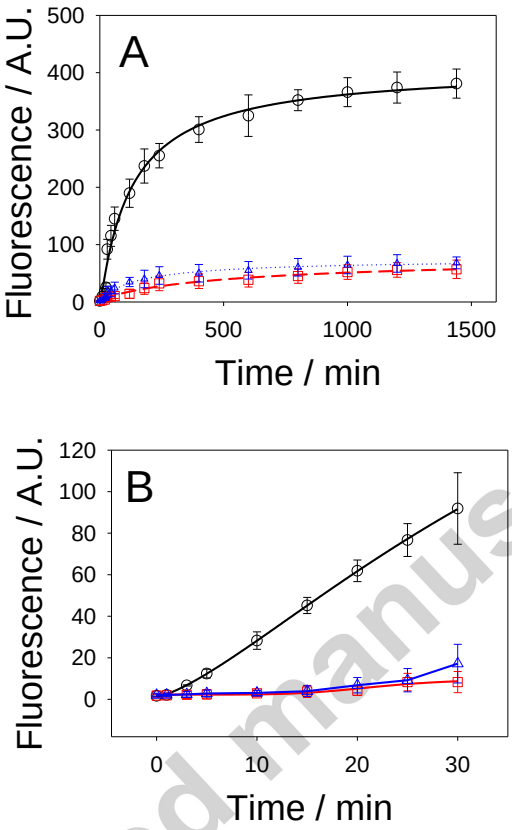

Figure 5 A) Evaluation of NanoKeepers in PBS buffer. NanoKeepers loaded with fluoroscein and capped with NK01 oligo (NanoKeepers-NK01) were incubated in the buffer, to determine their stability and cargo retention (red line). NanoKeepers-NK01 were incubated with buffer containing magnetically isolated S. aureus from blood to evaluate the release of cargo by the specific targeting of secreted MNase (black line). Non-specificity studies were carried out by S. epidermis on NK01 capped Nanokeepers (blue lines). B) The same data was blown-up for early time release up to 30 min. All the measurements were carried out in triplicate; the results show average fluorescence intensity and the error bars represent standard deviations.

As depicted in Figure 5, a release of fluorescein occurred upon addition of captured S. aureus cells, indicating specific release based on MNase activity. In samples containing S. epidermis, a minimal release of fluorescein was observed, revealing a favorable low-leakage capsule stability. These results proves that the concept of NanoKeepers can generate fluorescent signal based on nuclease activity, hence, its biosensor applications. To corroborate the specificity of NanoKeeper-NK01, a sub-optimal sequence was incorporated into the nanocapsules following the aforementioned approach, denoted as NK02 (NH_2-UmUmmCmGmCmUmUmCmGmGmCmGmAmA). In the NK02 oligonucleotide, the TT

DNA motif originally present in NK01 was replaced by two 2'-O-Methyl Uracils (UmUm) in an attempt to reduce the degradation efficiency of MNase. Figure 5A showed that fluorescent signal increased during the 24 hour measurements, which was in agreement with the previous study (Hernandez et al. 2014a). Any point in this graph could be selected for designing a sensor for the presence of S. aureus. However, a fast assay is highly desirable for real applications for S. aureus measurements in blood. For this reason we focused on the early time in the fluorescence signal. Figure 5B is a blow-up representation of early time course of release up to 30 min. The addition of captured S. aureus increases fluorescence signal in a linear fashion starting from 5 min. Thus, we chose 10 min as the assay time, which produce a distinctive signal compared to control experiments (Blue and red lines). In the following experiments, 10 min incubation time was used in all assays.

3.4 Nanokeeper assay on captured cells

The assay was developed by stepwise application of aptamer-magnetic capture of S. aureus cells from one ml blood samples, a brief heating of the captured cells to release maximum number of MNase and finally mixing with 0.1 mg of fluorescein-Nanokeepers for signal generation and amplification (Figure 1). A series of experiments with various number of S. aureus spiked whole blood samples resulted in fluorescent signals as presented in Figure 6. The fluorescence signal increased dramatically in the presence of different number of S. aureus cells up to 10^5 cells. The linear range is from 800 CFU/ml blood to 10^4 CFU/ml blood. The fluorescence intensity reaches a maximum at $2x10^5$ CFU/ml. The limit of detection (LOD) value was determined as 682 cells (Figure 6 inset). Thus, our results showed that micrococcal nuclease sensitive oligonucleotide Nanokeeper can be used as an assay system. In fact, a FRET-based MNase activity measuring ELISA method recently reported LOD value of 0.5 ng/ml enzyme, which corresponds to about 1500 CFU/ml (Chandan et al. 2016). The reported assay system achieved better LOD at 682 CFU/ml, which should be a result of amplication achieved by nanokeepers approach.

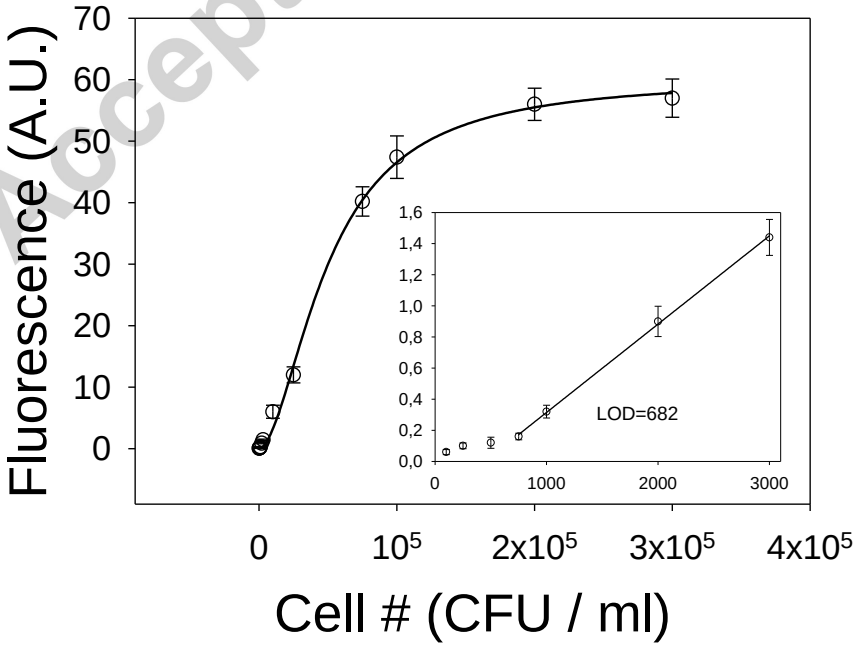

Figure 6 Fluorescence emission signal of assay mixture after 1 ml blood spiked with the indicated number of S. aureus cells and assay procedure applied.

4. Conclusions

In this study, a sensitive assay was developed for specific detection of Staphylococcus aureus, based on the triggered release of fluorophore molecules through nuclease resistant nucleic acid gates since specific degradation of oligonucleotides by micrococcal nuclease unblocks the pores of silica nanoparticles. The proposed assay system provides a new and effective strategy to monitor S. aureus infections directly in the blood samples. Another advantage of the method presented here is that magnetic separation and fluorescent reading could easily be incorporated into automated systems in portable devices.

Acknowledgements

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Highlights for review:

- Fast and specific solution type assay for on-site determination of S. aureus
- Determination of S. aureus from blood in a two-step procedure

- Micrococcal nuclease for specific and sensitive determination of S. aureus cells