Sensing and Bio-Sensing Research 28 (2020) 100332

Contents lists available at ScienceDirect

Sensing and Bio-Sensing Research

journal homepage: www.elsevier.com/locate/sbsr

Aluminum foil as a substrate for metal enhanced fluorescence of bacteria labelled with quantum dots, shows very large enhancement and high contrast

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ARTICLE INFO

Keywords:
Surface enhanced fluorescence
Metal enhanced fluorescence
Quantum dots
Aluminum foil
Enhancement facor
Bacteria
E. coli
QD toxicity
Contrast
Single bacterial cell detection

ABSTRACT

Very high surface/metal enhanced fluorescence was observed for *E. coli* single bacteria cells labeled with composite CdSeS/ZnS quantum dots (QDs) on three substrates: aluminum foil, aluminum film and gold film. The enhancement factors relative to maximum fluorescence intensity on glass for those substrates were in the range of several hundred (up to 500) for two-excitation wavelengths 532 and 633 nm. Contrast as a ratio of signals from QD labeled to signals of QD unlabeled (control) cells was also in the range of 100 s for those substrates and the highest contrast of 370 was observed on Al film. When CdTe QDs were used for labelling cells on all substrates or when fluorescence from cells with both QDs was measured on silver film, low or no enhancement was observed. Overall, untreated aluminum foil demonstrated great potential as low-cost substrate for surface/metal enhanced fluorescence, which delivers even more reproducible signal than gold film.

1. Introduction

Quantum dots (QDs) is an important class of semiconductor nanoparticles, which gained an enormous amount of attention in the last few decades since their discovery in the 1980s by A. Ekimov and L. Brus [1,2]. This attention can be explained by the excellent optical properties of the QDs in comparison with fluorescent dyes, such as higher quantum yield, higher extinction coefficient, and better resistance to photobleaching [3,4]. In addition, their size-dependent emission spectra and multiplexing capabilities make them a subject for a variety of applications. Some major applications of quantum dots include, but not limited to optoelectronics, biomolecular detection and in vitro assays as well as optosensing of drugs [5–7]. Among these applications, the whole-cell labeling is of particular interest as a pathogen detection technique and instrument for the cell study [8]. There are some reports of the use of quantum dots on the non-metalic surface for the cell detection, such as detection of *E. coli* cells labeled with streptavidin conjugated quantum dots on glass cappilar substrate by Ozcan research group [9]. Hovewer, as far as we know, there is hardly any report about QDs applied for cell detection/discrimination by fluorescence on metalic substrates.

Metal-enhanced fluorescence (MEF) is a phenomenon that enhances the fluorescence of fluorophores by utilizing surface plasmon resonance (SPR), which happens when the fluorophores are confined at a certain distance from metal nanostructure [10,11]. This phenomenon can be exploited to increase substantially sensitivity, quantum yield and improve photostability of the fluorescence-based detection techniques [12]. These benefits of MEF make it preferable in a number of applications in biodetection and bioimaging [13]. For instance, Liu's group showed the detection of DNA oligonucleotides with metal-enhanced QDs fluorescence and reported the limit of detection (LOD) of 50 nM [14].

One of the major figures of merit for MEF as an anlytical method is its enhancement factor. Geddes'research group reported the 30-fold enhancement of fluorescence emission for carbon quantum dots on silvered plates [15]. Recently, we investigated the MEF of C-dots on plane gold film and reported EF above 50 and signal-to-noise ratio of 200 [16]. Xie group reported the enhancement of fluorescence over 100 times for Ag_2S quantum dots on the Au nanostructured arrays for the excitation in second near-infrared window [17]. The same group reported even high MEF with EF up to 235 on gold nanodisc array. [18] Wang reported about the same 100-fold fluorescence enhancement of

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https://doi.org/10.1016/j.sbsr.2020.100332
Received 18 September 2019; Received in revised form 19 February 2020; Accepted 20 February 2020

CdSe/ZnS quantum dots on relatively expensive nonporous gold films [19]. Single nanoparticle imaging study of Fujii group demonstrated that hot spot enhancement as high as 700-fold is achievabe for some particular QD positions, especially when silicon QD is placed in the gap between Au nanoparticle and gold film [20]. Overall, the combination of metal-enhanced fluorescence technique with good fluorophores such as QDs is reported to achieve relatively good quantum efficiency, photostability, and sensitivity. [21,22]

However rare noble metal films are still relatively costly substrates and they may suffer from surface contamination with S-containing compounds or/and corrosion (especially Ag film) [23,24]. Therefore, our research group explored aluminum foil as a cost-effective, easily obtainable alternative for those noble metal plasmonic materials. Recently, we reported the application of Al foil as an inexpensive, versatile and sensitive substrate for Surface-enhanced Raman Spectroscopy (SERS), where sometimes it can compete with gold film in terms of limits of detection (LODs) and enhancement factors [25–27]. MEF of organic compounds on aluminum nanostructured surfaces were reported with moderate up to 9 fold increase in fluorescent intensity by Lakowicz group upon excitation with 285 and 405 nm laser light [28]. Prashant et al. observed about same enhancement in fluorescence for protein incubated on polyelectrolyte multilayer coated on aluminum mirror surface and Al foil relative to functionalized glass [29].

Hovewer, we have not found any reports about application of Al foil or Al film as a substrate for MEF of quantum dots or as a substrate for MEF of any cells labeled with QDs in literature yet. To explore Al foil as MEF substrate we used two kinds of commercially available QDs: CdSeS/ZnS and CdTe, which have been already applied in a few fluorescence based research publications. For instance, Jin et al. reported that CdTe quantum dots are capable of detecting low concentrations of uric acid down to 0.1 μM with a linear range of 0.22–6 μM [30]. Even though these quantum dots facilitate the detection of analytes, they might have toxic effects on the analytes. In this paper, we demonstrate that untreated Al foil is not just cost-effective, but also highly performing substrate for MEF of *E. coli* bacterial cells labeled with quantum dots, that may have advantages even over gold film, particulary in a bit higher reproducibility and contrast of MEF signal.

2. Experimental section

CdSeS/ZnS alloyed QDs (diameter 6 nm, λ emission = 665 nm), CdTe core-type QDs (diameter 3.47 nm, λ em = 610 nm), were purchased from Sigma-Aldrich. Gold, silver and aluminum films, 100 nm thickness, on microscope glass slides were purchased from EFM Corporation, aluminum foil was purchased at local supermarket and it was attached to microscope glass slides with double sided adhesive tape.

The procedure for bacteria labelling with C-dots, described by our group, was adopted for QDs with some changes [16]. *E. coli* bacteria (DH5α) were grown aerobically overnight on a solid sterilized Luria-Bertani agar medium at temperature 37 °C. After that, the bacterial colony was added to 10 mL of Lysogeny broth, where it was cultivated for 1.5 h at 37 °C (OD600 ~ 0.1). Meanwhile, 10 mM phosphate-buffered saline (pH = 7.4) was prepared in ultra-pure water and then it was sterilized. 0.5 mL of bacteria suspension was centrifuged at 6000*g* for 15 min. The pellet was resuspended in 1 mL of PBS solution, wchich was already used as a medium for modification of *E. coli* with QDs as reported in Zhu et al. publication [9]. This step was repeated two times, and after that the bacteria culture was reconstituted in following mixtures (1 mL each): CdSeS/ZnS QDs in PBS (1:50 and 1:25), CdTe QDs in PBS (1:50 and 1:25). The mixtures of QDs and bacteria were stirred at 100 rpm for 17 h at 37 °C. After constant stirring, the mixtures were centrifuged at 6000*g* for 15 min. Two more centrifugations+ resuspension cycles were done at the same rate for 10 min each. After the first two centrifugations, the pellets were resuspended in 1 mL of PBS but after third centrifugation, 400 μL of PBS was used. 10 μL of the bacteria/QDs suspension were drop-casted ono the surfaces of Au film, Al film, Ag film, Al foil, and glass substrates.

The fluorescence measurements were performed using a confocal HORIBA Labram Raman microscope with excitation wavelengths of 532 nm and 633 nm and objective x100 for imaging of single cells (Fig. S6A of Supporting Information). The measurement time of 1 s and the neutral density filter 1% was used for measurements with both lasers, which resulted in laser power at the sample of 0.13 mW and 0.05 mW respectively. The power was selected in order to minimize the maxing out of the detector (signal above 65,000 cps), which were frequent for cells labeled with CdSeS/ZnS QDs on gold and on Al foil (up to 30% of spectra) at higher laser power of 2.5%. More data about power optimization are available in Table S4 of the Supporting Information. The signals are averaged from 10 to 20 spectra of QD labeled single cells, 6–8 spectra of control cells and 6 spectra of the background taken with ×100 objective; MEF enhancement factors calculated as ratio of fluorescent signal (maximum average spectra intensity) on metal film to the same kind of signal on glass substrate. Contrast is calculated as a ratio of signal from QD labeled bacteria cell to signal from unlabeled (control) cell on the same substrate and same spectra acquisition parameters. For evaluation of QD toxicity standard zone of inhibition assay using Kirby-Bauer test was conducted [31]. The procedure and numerical results of the test are described in Supporting Information (on page 1). SEM maps were obtained on Carl Zeizz Crossbeam 540 SEM. EDS (Energy Dispersive X-ray spectroscopy) spectra obtained on the same SEM microscope with X-Max 150 detetor from Oxford Instruments. Smart SPM 1000 Scanning Probe Microscope system from AIST-NT was used to perform AFM measurements in tapping mode, using cantilevers: NSG10DLC (from Nanotunning.com) and NSG30 (from Tipsnano.com), frequency 240–440 kHz. The representative AFM Image of CdSeS/ZnS QDs on Si waffer is shown on Fig. S4 and Table S1 shows the hights of those QDs meassured by AFM with an average 6.3 nm and standard deviation 1.2 nm. The representative SEM images are shown on Fig. S4 in Supporting Information The representaive EDS spectra and elemental composition tables (print sceened) for cells labled with CdSeS/ZnS QDs and unlabled cell on two substrates (gold and aluminum films) are shown on Figs. S5 and S6, respectivly. While the EDS numerical results are shown in Table S3.

3. Results and discussion

3.1. Contrast in antimicrobial activity of QDs

Antibacterial activity of QDs is demonstrated on Fig. 1 Gray spots there show zones of inhibition of bacterial grows. Since there is no such spots for various concentrations (10, 20, 40, 100 ppm) of CdSeS/ZnS QDs (Fig. 1A) and only control spot (from penicillin) is visible on the dish. Therefore, we can conclude that those QDs are nontoxic not only at the concentration level used in the assay(40 ppm) but even at concentration of 100 ppm, which may produce even higher emission intensity and even higher MEF enhancement than 40 ppm CdSeS/ZnS QDs used in this experiment. The nontoxic properties of those QDs are in good agreement with literature about QD toxicity. CdSeS/ZnS quantum dots are core-shell structured QDs, where the core is CdSeS and shell is ZnS, which is non-toxic.

According to Li et al. and Fu et al., ZnS is chemically stable and has passivating properties that prevent decomposition and oxidation of the core [32]. Thus, it can block the release of toxic Cd^{2+} ions to the environment [33]. Jaiswal et al. labeled living cells using similar kind of CdSe/ZnS QDs and those did not affect the growth of normal cell and cell signaling [34].

CdTe QDs demonstrate cell toxicity since clear gray spot appears on Fig. 2B even for 10 ppm and larger spots appear for higher CdTe QD concentrations. The toxicity of those QDs is also reported in scientific literature. For instance, Li and co-workers determined the half

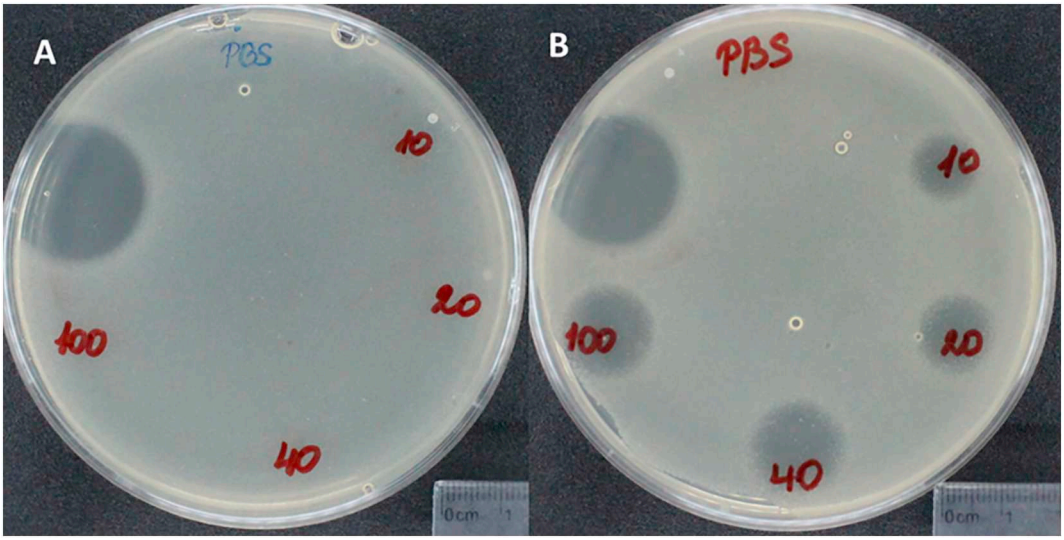

Fig. 1. Images of two bacterial plates in cyto-toxicity (antimicrobial activity) experiment:
A) 10, 20, 40, 100 ppm of CdSeS/ZnS QDs in PBS.
B) 10,20,40, 100 ppm of CdTe CDs in PBS.
Both plates have spot of PBS solution and penicillin solution (the largest spot) as controls.

inhibition concentration of QDs (IC50), the growth rate constant (k), and optical density (OD) of *E. coli* mixed with different concentrations of CdTe QDs They found that the higher the concentration of CdTe QDs, the lower the growth rate constant k and OD become. For concentrations 8×10^{-8} M and 12×10^{-8} M, k and OD decreased significantly, which showed the toxic effect of CdTe QDs [35].

CdTe QDs as bacteria cell markers and silver as a substrate make non-impressive if any MEF.

As can be observed from Fig. S1 in Supporting Information, CdTe QDs labeled bacteria do not demonstrate any significant enhancement factors, which are ratios of signal on metal substrates to signal on glass, for spectra taken from signal cells (×100) with both 533 and 632 nm excitation lasers. For instance, MEF EFs for 633 nm are at best in the range from 1.0 to 1.6. Those QDs also do not show any significant (e.g. much more than one) contrast, which is the ratio between average signal of QD labeled cell to average signal of control (unlabeled) cell.

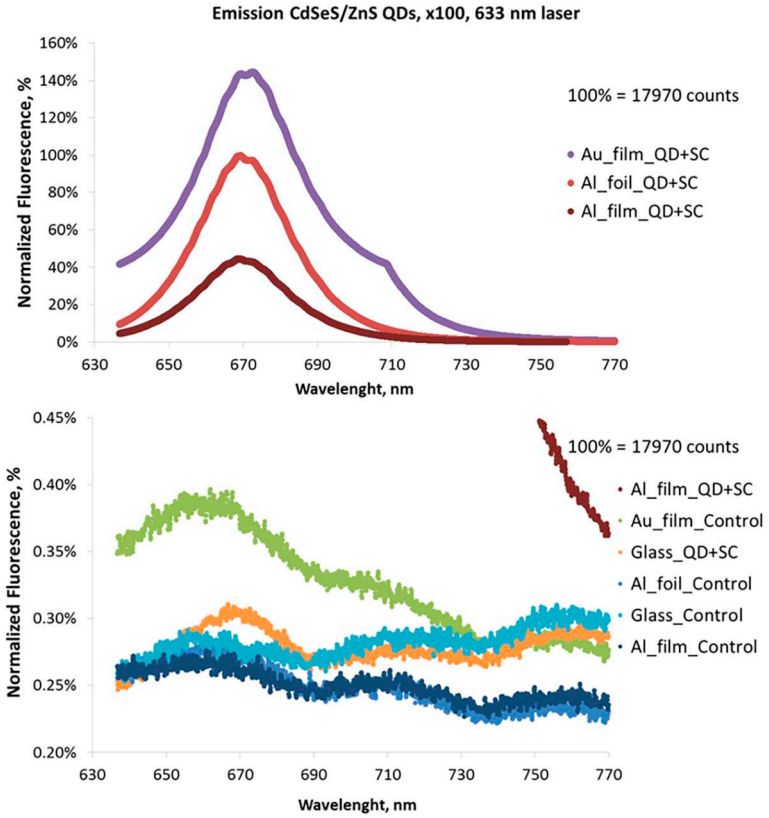

Fig. 2. Averaged Normalized Fluorescence Emission spectra of CdSeS/ZnS QDs with single bacterial cells (QD + SC) and of control (just SC) on various substrates, obtained with 633 nm laser excitation wavelength and ×100 objective. Maximum intensity for averaged spectrum QD + SC on Al foil is set as 100% (17,970 counts).

When contrast ratios were calculated from both 532 and 633 nm excitation, the contrast was about 1.0–1.2. Overall, likely due to their toxicity CdTe QDs have not shown any satisfactory MEF (or SEF) performance for labeling of bacteria cells.

CdSeS/ZnS and Cd/Te QDs in single bacteria cells (×100) on silver film substrate did not show any significant enhancement factors or significant contrast as can be seen on Fig. S2 in Supporting Information. Table S2 in Supporting Information, shows that the untreated silver film shows the maximum contrast observed for any QD at any imagimg condition is 1.6 and maximum enhancment factor is about 1.3. Those figure of merits (FOMs) of MEF on silver film are about 2 or even 3 orders lower than the same FOMs on three other metalic substrates. As shown on Fig. S2 in Supplimentary Materials, an absolute value of emission peak at 665–670 nm for CdSeS/ZnS QDs in single cells on silver film (or any other QD measurement done on silver) is less than 70 cps for 633 nm and less than 100 cps for 532 nm excitation wavelength. Since toxicity of silver ions to bacterial cells is widely reported in literature [36], we suspect that this antimicrobial activity, very beneficial for some silver applications, may not be beneficial in this case, preventing effective application of silver film as a substrate for MEF of bacteria with QDs. By the way, CdSeS/ZnS QDs on all substrates demonstrate emission peak around the same wavelength of 665–670 nm, as expected from the specification of those QDs.

CdSeS/ZnS QDs make great MEF in bacteria cell on Al foil, Au and Al films.

Figs. 2 and 3 show average emission spectra of a single bacteria cell labeled with CdSeS/ZnS QDs. Those demonstrate very high fluorescence signal on aluminum foil (here maximum is normalization factor 100% or 17,970 cps for 633 nm exitation and 18,103 cps for 532 nm exitation), on gold film and on aluminum film relativly to the same kind of signal on glass, while the later fluctuates near 0.3% of the signal on Al foil, when excited with any (633 or 532 nm) laser.

As shown in bar graph on Fig. 4 those QDs in bacteria demonstrate maximum MEF enhancement factor relative to glass above 450, when they emit on gold film with 633 nm excitation. Hovewer, EF for QD + cells MEF on Al foil is also very high, exceding 320. The maximum EF with 532 nm laser is observed among 3 substrates for MEF on Al foil and it is around 400. Overall, all 3 substrates (Au and Al films, Al foil) demonstrate MEF with the same 2+ order of magnitude enhamncement. Hovewer, the same Fig. 4 reveals that relative standard deviations in emission signal are on average about twice as high for MEF on gold film if compared to the relative standard deviations for MEF emission on Al foil and Al film.

This observation may give a sinificant edge to the application of Al foil as MEF substrate since it is not only many times more affordable substrate than gold film, but also as far as we can see more reproducible substrate.

As shown on Fig. 4, CdSeS/ZnS QDs with single bacterial cells demonstrate high fluorescece contrast ratio, or ratio of signal from QD labled cell(s) to signal from unlabeled/ control cell(s), wchich is at least two orders of magnitude for both excitation lasers 633 and 532 nm. The huge maximum contrast about 360–370 is observed on gold film and Al foil with 633 nm excitation, while maximum contrast on Al film with the same laser is about 130. Excitation with 532 nm laser gives the highest contrast for Al foil (317) and less for Al film (234) and gold (69). Overall the average maximum contrast for those 3 susbtrates is higher for 633 nm excitation (286), when compared to the average for 532 nm excitation (207). Average enhancement factor for those 3 metallic substrates is also a bit higher for 633 nm excitation than for 532 nm excitation (315 vs 261, respectivly). Therefore 633 nm laser may be preferable to 532 nm laser excitaion for this MEF method of single *cell* + QDs observation.

Clearly, using MEF of those non-toxic QDs, it should not be hard to distinguish QD labeled cells from unlabeled cells with very high degree of confidence, because of such high contrast on each of those 3 metallic substrates, but it is not the case when substrates are silver film or glass. Contrast and EF values for all five substrates are presented in the Table S1 of Suporting Information.

CdSeS/ZnS QD show good MEF just in dried PBS soluton on Al foil, Au and Al films.

When we probed just CdSeS/ZnS and CdTe QDs in dried PBS solution on the surface of the same substrates we observed spectra with lower, but still significant EFs and lower contrasts for CdSeS/ZnS relative to bacteria cells labled with the same QDs, as shown at Fig. S5

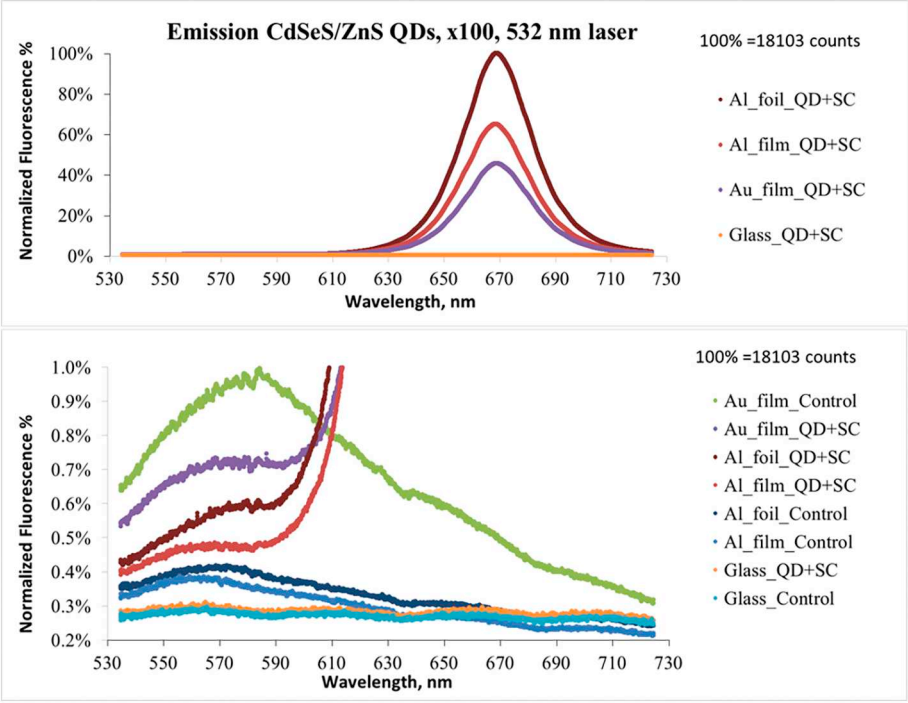

Fig. 3. Averaged Normalized Fluorescence Emission spectra of CdSeS/ZnS QDs with single bacterial cells (QD + SC) and of control (just SC) on various substrates, obtained with 532 nm laser excitation wavelength and ×100 objective. Maximum intensity for averaged spectrum QD + SC on Al foil is set as 100% (18,103 counts).

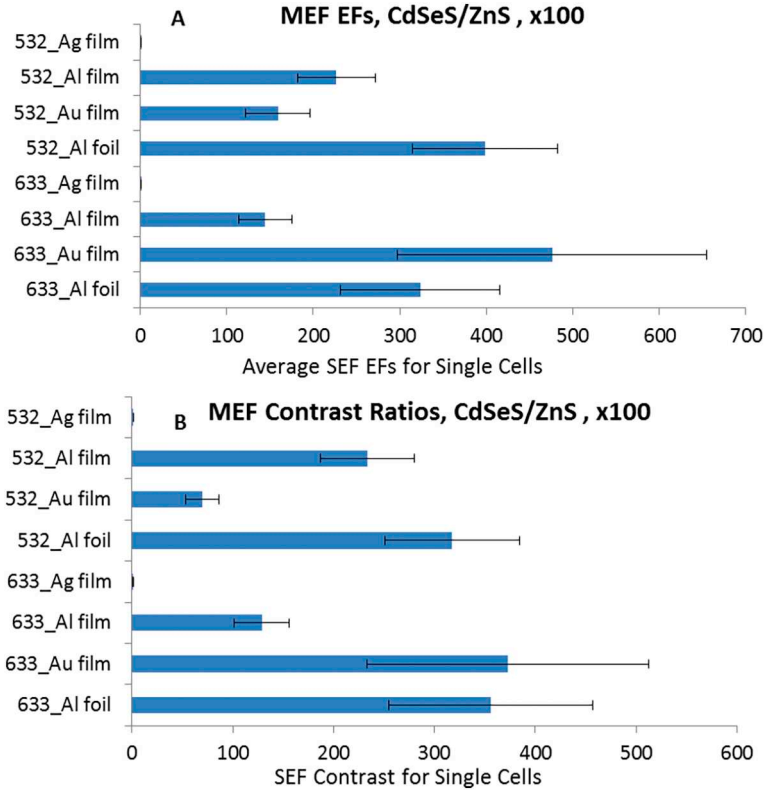

Fig. 4. A) MEF Enhanced Factors calculated for CdSeS/ZnS QDs with single cells (×100 objective); B) MEF Contrast Ratios calculated for CdSeS/ZnS QDs with single cells on four substrates, ×100 objective. All EFs calculated as ratio of fluorescent signal on each of four substrates (silver, aluminum, gold films and aluminum foil) to signal on glass substrate. 532 or 632 indicate excitation wavelength in nanometers, using maximum fluorescent intensities.at emission wavelength about 670 nm. Contrast ratio is the maximum fluorescence intensity of QD labeled bacteria cells divided by maximum intensity of unlabeled (control) cell(s).

and in Table S2. Hovewer, general trends for bare QDs remain the same: Al foil and Au film demonstrate about similar performance with significant EFs about 40 at 633 nm excitation and EFs about 20 at 532 nm excitation. It is likely that when those QDs penetarte the cell, their quenching by metal surface sinificantly decreases and emmission rate appears to be boosted by about one order of magitude.

Also we can conclude that EDS (Energy Dispersive X-ray spectroscopy) confirmed the presence of CdSeS/ZnS QDs in the cells with above LOD signal of 3 elements (Cd, S and particulary Zn), but no significant presence of these elements in control was detected… Hovewer, concentration of QDs in cells is still too low for exact quantification of QDs in the cell by this relativly semi-quantitative method. More details about EDS results are shown and described in Supporting Information.

4. Conclusions

Bacteria cells labeled with non-toxic CdSeS/ZnS QDs demonstrated same order of very high MEF enhancement up to 450–480 on three substrates Al foil, Al film and Au film. MEF (SEF) on those three substrates demonstrated about very high contrast up to 360, while MEF/SEF signal on Al foil and on Al film were nearly two times more reproducible than the signal observed on Au film.

However, CdSeS/ZnS QDs on silver film as well as CdTe QDs on all 4 tested metallic substrates have not demonstrated any significant efficiency as contrasting/ labelling agents for fluorescence mapping of bacterial cells on any tested substrate, which may be due to the significant bacterial toxicity of CdTe QDs, proven experimentally, as well as high antibacterial activity of silver. Overall, Al foil as a low cost efficient substrate and CdSeS/ZnS as non-toxic core–shell type of QDs emerge from this paper as a favorable combination for MEF bioimaging applications.

Author statement

This research is supported by the Nazarbayev University Faculty Development Competitive Research grant SST2018045 (Kazakhstan).

Alisher Sultangaziyev, Aktilek Akhmetova, Zhanar Kunushpayeva, Alisher Rapikov contributed equally as authors to this work. They performed data curation, including: sample preparation and measurements, raw data analysis, graph plotting.

Dr. Olena Filchakova did visualization and supervision of bacteria labelling with QDs and toxicity measurement experiments.

Corresponding Author: Rostislav Bukasov* did funding acquisition, visualization and supervision of all fluorescence measurements and data analysis, writing – original draft; writing second draft & editing.

Declaration of Competing Interest

The authors declare no conflict of interest and have no financial disclosures.

Acknowledgements

We would like to acknowledge NU students and RAs: Adil Les, Saida Zhunussova, Gulsamal Zhubanova. Also Alisher Sultangaziyev, Aktilek Akhmetova, Zhanar Kunushpayeva, Alisher Rapikov contributed equally as authors to this work.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sbsr.2020.100332.

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