Quantum Dot Dissemination and Behavior in Bacterial Biofilms

Jayne B. Morrow, R. David Holbrook, Cynthia J. Zeissler
*National Institute of Standards and Technology
100 Bureau Drive
Gaithersburg, MD 20878

ABSTRACT

Quantum dots (QDs) of two different surface chemistries (carboxyl (COOH) and poly ethylene glycol (PEG) modified) were utilized to determine surface chemistry impacts on QD mobility and distribution in bacterial biofilms. Epifluorescent and Confocal Laser Scanning Microscopy (CLSM) were used to evaluate QD association with biofilm components (proteins, cells and extracellular polymeric substances (EPS)). Bulk surface chemistry measurements, including electrophoretic mobility and contact angle, were used to predict the governing interfacial interactions between QDs and bacterial cells and biofilms upon initial contact. Interaction energies of COOH QDs with bacterial biofilms, governed by interfacial forces include van der Waals, electrostatics and hydrophobicity (including Lewis acid/base contributions), were only slightly attractive, approximately -2 kT for biofilm surfaces and a repulsive energy barrier was predicted for COOH QDs and *Pseudomonas aeruginosa* PAO1 bacterial cells. COOH QDs were found to be associated with bacterial cell surfaces, but not uniformly distributed in the biofilms, with epifluorescence and CLSM. COOH QD association is believed to be due to localized surface charge and hydrophobicity, In contrast, interaction energies are predicted to be largely attractive for 5 nm diameter PEG QDs with biofilms grown on PVC pipe (-135 kT) and to *P. aeruginosa* PAO1 cell (-73 kT) surfaces.

Keywords: quantum dots, biofilms, bacteria, adhesion, transport

1 INTRODUCTION

Biofilms are communities of microorganisms attached to surfaces that predominate in water/surface interfaces common to nearly all ecosystems [1]. The study of microorganisms at the aqueous/substratum interface is of great interest because bacterial attachment to surfaces is often associated with surface deterioration and corrosion. Extracellular polymeric substances anchor bacteria to the substratum [1] and serve as a survival mechanism in diverse environments including water distribution systems [2]. Decontamination of biofilms by dynamic changes in fluid shear resulting in biofilm deformation and detachment [3, 4] may be a viable alternative to chemical disinfection methods, however, detachment mechanisms have yet to be elucidated.

QDs have been utilized for fluorescence imaging in biological systems and perform better than standard fluorophores for biofilm imaging [7]. QDs are colloidal semiconductor nanocrystals whose photoluminescence emission wavelength is proportional to the dot size. Dots of two different surface chemistries (carboxyl and PEG modified) were utilized to measure solute mobility and distribution in bacterial biofilms as a function of fluid shear. PEG dots are commonly utilized to image tissue and cell samples and are considered relatively inert [8]. Bacterial EPS are for the most part carboxylated surface sugars [9]. Carboxylated latex microspheres are commonly used as microbial simulants in colloid stability and transport experiments [10].

Surface interaction forces can be estimated using colloid stability theory. Due to the small size of QDs (< 10 nm in this study) and bacteria (usually close to 1 μm in diameter), many forces can play a significant role in influencing initial attachment. Bacterial surface interactions are typically modeled by colloid stability theory [5, 6]. Traditional colloid stability theory developed by Derjaguin-Landau-Verwey-Overbeek (DLVO) is a continuum approach that attempts to describe colloid or bacterial surface association by accounting for van der Waals and electrostatics forces that can attract or repel a cell from a substratum. However, Extended DLVO (ExDLVO) theory takes into account Lewis acid/base (A/B) interactions to that may more accurately predict the initial attachment of bacteria to surfaces [6]. Additionally, Lewis A/B and van der Waals surface tension parameters provide estimations of surface hydrophobicity [6], which may be important for predicting QD behavior. Bulk surface chemistry measurements, including electrophoretic mobility and contact angle, can be used to estimate the governing interfacial interactions between QDs and bacterial cells/biofilms upon initial contact.

The objective of this work was to determine the impact of QD surface chemistry on their mobility and behavior in bacterial biofilm communities. *Pseudomonas aeruginosa* PAO1, a commonly studied biofilm forming organism, and native drinking water (DW) bacterial biofilms were subjected to carboxyl and PEG modified QDs. Shear induced QD penetration was observation with Confocal Laser Scanning Microscopy (CLSM) and epifluorescent microscopy. Fluorescent and fluor-conjugated probes were used to determine what biofilm component the QD were adhering to.

2 METHODS

2.1 Biofilm Growth and Accumulation

DW biofilms were grown on PVC coupons (1/2" diameter) with synthetic water (1.2 mM $NaHCO_3$, 0.54 mM $MgSO_4 \cdot 7H_2O$, 0.2 mM $CaSO_4 \cdot 2H_2O$, 0.004 mM K_2HPO_4, 0.002 mM KH_2PO_4, 0.08 μM $(NH_4)2SO_4$, 0.17 μM NaCl, 36 nM $FeSO_4 \cdot 7H_2O$, 0.011 mM $NaNO_3$, 0.2 mM $CaCO_3$, 24 mg/L humic acid, average pH of 7.9) at a rate of 1mL/min for 2 weeks followed by a 3 day starvation period (synthetic water without humic acids). *Pseudomonas aeruginosa* PAO1 provided by Tim Tolker-Nielsen and Mikkel Klausen (Danish Technical University, Lyngby, Denmark) were grown in liquid culture or on PVC coupons with ABT media plus 10 mM Sodium Citrate and 30 μg/mL gentimicin as the antibiotic resistance marker for the constitutive chromosomal green fluorescent protein (gfp) [11] at a flowrate of 0.3 ml/min for 1-5 days. Fluorescent and fluor-conjugated probes (Table 1) were used to identify specific biofilm components and QD locations. Biofilms and quantum dot dissemination were imaged with CLSM (Zeiss LSM 510)[1] and epifluorescent microscopy (Olympus AX-70), respectively.

2.2 Bulk Surface Chemistry Measurements

Bulk surface properties of biofilms were determined with capillary electrophoresis, a Zeta PALS zeta potential analyzer (Brookhaven Instruments) and by sessile drop contact angle measurements (van Oss 1994). Surface properties were utilized to model interaction phenomena using ExDLVO colloid stability theory [6].

Table 1. Fluorescent and fluor-conjugated probes applied in this study

Probe	Supplier	Ex/Em (nm)	Target
COOH Quantum dot	Invitrogen	488/655	
PEG Quantum dot	Invitrogen	488/655	nonspecific
Syto9	Molecular Probes	488/522	Viable cells, nucleic acids
Sypro Orange	Molecular Probes	470/570	Protein
Nile Red	Molecular Probes	552/636	Hydrophobic lipids
Arachis hypogaea lectin TRITC conjugated	Sigma	554/576	β-Gal (1-3)galNAc

[1] Certain commercial equipment, instruments or materials are identified in this paper to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

3 RESULTS AND DISCUSSION

Particle or colloid stability theory is used to understand particle interactions with surfaces in varied environmental conditions. Extended DLVO theory was utilized to determine what forces govern QD association with bacterial biofilms relevant to drinking water environments. ExDLVO interaction energy predictions suggest COOH QDs should weakly associate with biofilm surfaces as indicated by a small attractive energy minimum (-2.3 kT) (Figure 1). COOH QD attractive association with biofilm surfaces is predicted to occur approximately 1 nm from direct contact and is only slightly larger than dispersive Brownian motion forces (1-1.5 kT). To determine if COOH QDs were more likely to attach directly to cell surfaces, ExDLVO predictions were made using bulk culture surface property measurements (Table 2) of *P. aeruginosa* PAO1 cells. ExDLVO predicted a repulsive energy barrier (≈120 kT) due to high negative surface charge on both *P. aeruginosa* PAO1 cells and COOH QDs, preventing direct COOH QD attachment to cell surfaces (Figure 1).

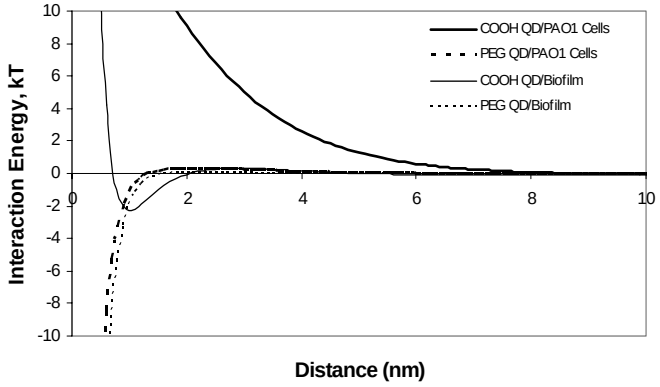

Figure 1. ExDLVO Interaction energy profiles for QDs, bacterial cells and biofilms. Sphere/plate interactions in low IS buffer, for COOH QDs and PAO1 cells (ΔG^{AB}_{do} = 19.8 mJ/m^2, A_{132} = 4.4 x 10^{-21} J) and PEG QDS and PAO1 cells (ΔG^{AB}_{do} = -10.7 mJ/m^2, A_{132} = 2.8 x 10^{-21} J), COOH QDs and DW biofilm (ΔG^{AB}_{do} = 22.9 mJ/m^2, A_{132} = 5.4 x 10^{-21} J) and PEG QDS and DW biofilm (ΔG^{AB}_{do} = 21.4 mJ/m^2, A_{132} = 3.4 x 10^{-22} J). ΔG^{AB}_{do} is the Gibbs Free Energy and A_{132} is the effective Hamaker constant between the QD (1) and bacterial cells or biofilm (2) in low IS buffer (3), respectively.

Epifluorescence and CLS microscopy images revealed COOH QDs associate with DW and *P. aeruginosa* biofilms (Figures 2 and 3). Contrary to ExDLVO predictions, COOH QD association was localized on cell surfaces and not evenly or randomly dispersed in the *P. aeruginosa* or DW biofilms. Fluorescent probes specific for polysaccharides, protein and hydrophobic lipid domains were utilized to determine COOH QD association sites.

CLSM images revealed only limited COOH QD association with β-Gal (1-3)galNAc, a polysaccharide component commonly found in bacterial biofilms (shown as blue signal in Figure 3). ExDLVO predictions of COOH QD and P. aeruginosa cell surfaces were repulsive suggesting COOH QD association with bacterial is occurring at localized positive surface charge and hydrophobic domains such as those presented on bacterial surface proteins. Localized surface properties, obscured in bulk surface measurements, can significantly impact attachment of small particles [6]. Positively charged or hydrophobic domains would act as preferential sorption sites for the COOH QDs, resulting in a higher localized fluorescence signal as observed with Epiflourescence and CLS microscopy.

Table 2. Surface Property Summary

	Zeta-potential, mV	ΔG_{iwi}, mJ/m^2	Hamaker Constant, A, mJ[a]
PVC biofilms	-7[b]	13.3[c]	7.36×10^{-17}
PAO1 cells	-28[d]	19.0[c]	6.67×10^{-17}
COOH QD	-60[e]	7.4[f]	7.78×10^{-17}
PEG QD	-4[g]	-74.2[h]	4.25×10^{-17}

[a] Surface tension value for van der Waals attraction, γ^{LW}, determined with Young Equation from sessile drop contact angle measurements with diiodomethane, formamide and water, surface tension values used to calculate the Hamaker constant for the surface [6], [b] [12]
[c] Surface tension values determined with Young Equation from sessile drop contact angle measurements with diiodomethane, formamide and water. Surface tension values used to calculate surface (subscript i) interaction energy in water (subscript w) [6], $\Delta G_{iwi} < 0$ is hydrophobic
[d] Electrophoretic mobility determined by measurements with ZetaPALS (Brookhaven Instruments Corp.) converted to zeta-potential with Smoluchowski Equation [13], n = 30
[e] [10], [f] [14], [g] [15], [h] [16]

In contrast to the COOH QDs, a significant attraction is predicted for PEG functionalized dots for both DW biofilm (-135 kT) and PAO1 cell (-73 kT) surfaces. Highly hydrophobic PEG quantum dots (Table 2) were predicted to adhere to bacterial biofilms. PEG polymer brushes are commonly used to condition surfaces rendering them relatively uncharged and inert to protein accumulation and reaction with the mammalian systems [8]. Surface charge estimates suggest PEG coated surfaces have a near neutral surface charge at neutral pH (~ -4 mV), therefore, interaction energy predictions indicate PEG functionalized QDs may bind to biological surfaces due to van der Waals attractive interaction energy and hydrophobic forces, not surface charge.

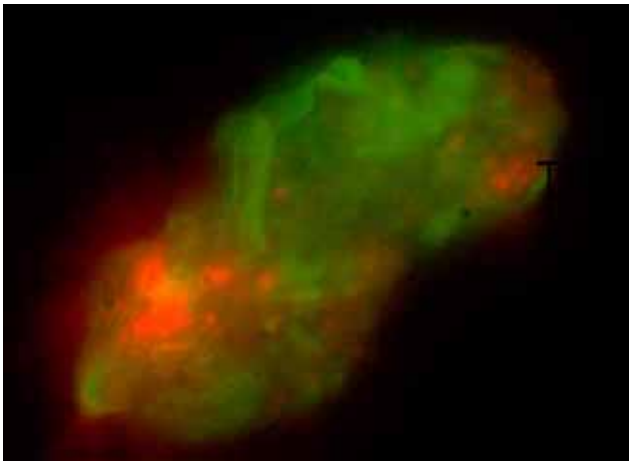

Figure 2. Epifluorescence image of COOH QD distribution in drinking water biofilms. COOH QDs (488 nm/635 nm LP) are shown in red and the DW biofilm components are shown in green.

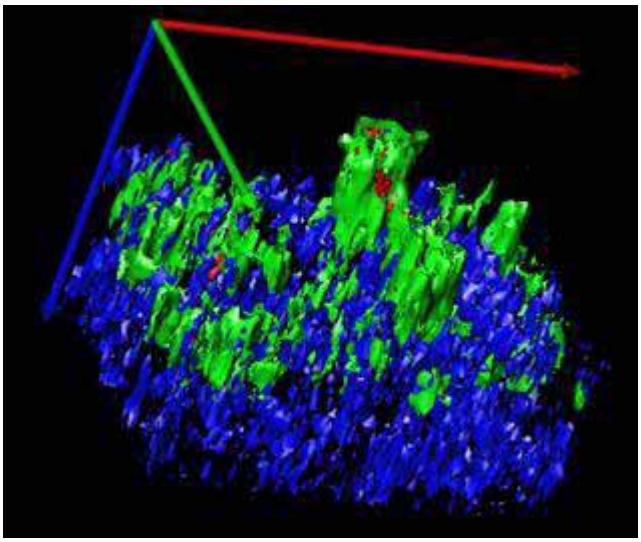

Figure 3. 3D reconstruction of CLSM image stacks showing distribution of COOH QD in PAO1 biofilms. COOH QDs (488 nm/635 nm LP, shown in red) are localized on both P. aeruginosa PAO1 biofilm cells surfaces labeled with gfp (488 nm/505-530 nm, shown in green) and TRITC conjugated Arachis hypogaea lectin (543 nm/530 – 600 nm, shown in blue) specific for biofilm EPS.

Epifluorescence and CLSM images suggest that COOH QD behavior in biofilm systems may be only limitedly predicted by ExDLVO theory, which relies on bulk measurements. This research reflects the need for comprehensive measurements of surface properties at the nano and macroscales in order to determine the fate and behavior of nanoparticles in environmental systems.

REFERENCES

1. Costerton, J.W., et al., *Microbial biofilms.* Annual Reviews Microbiology, 1995. **49**: p. 711-745.
2. Hall-Stoodley, L., J.W. Costerton, and P. Stoodley, *Bacterial biofilms: from the natural environment to infectious disease.* National Reviews in Microbiology, 2004. **2**(2): p. 95-108.
3. Choi, Y.C. and E. Morgenroth, *Monitoring biofilm detachment under dynamic changes in shear stress using laser-based particle size analysis and mass fractionation.* Water Science and Technology, 2003. **47**(5): p. 69-76.
4. Stoodley, P., et al., *Biofilm material properties as related to shear-induced deformation and detachment phenomena.* Journal of Industrial Microbiology and Biotechnology, 2002. **29**(6): p. 361-367.
5. Israelachvili, J., *Intermolecular and surface forces*. 2 nd ed. 1992, San Diego, CA: Academic Press Inc.
6. van Oss, C.J., *Interfacial Forces in Aqueous Media*. 1994, New York, New York: Marcel Dekker, Inc. 440.
7. Chalmers, N.I., et al. *Comparison of quantum dot- and fluorophore-conjugated antibodies for bacterial biofilm research*. in *Focus on Microscopy* 2005. Friedrich Schiller University, Jena, Germany.
8. Moghimi, S.M., *Chemical camouflage of nanospheres with a poorly reactive surface: towards development of stealth and target-specific nanocarriers.* Biochimica et Biophysica Acta (BBA) - Molecular Cell Research, 2002. **1590**(1-3): p. 131-139.
9. Bhaskar, P.V. and N.B. Bhosle, *Microbial extracellular polymeric substances in marine biogeochemical processes.* Current Science, 2005. **88**(1): p. 45-53.
10. Chen, J.Y., et al., *Role of spatial distribution of porous medium surface charge heterogeneity in colloid transport.* Colloids and Surfaces A: Physicochemical Engineering Aspects, 2001. **191**: p. 3-15.
11. Klausen, M., et al., *Biofilm formation by Pseudomonas aeruginosa wildtype, flagella, and type IV pili mutants.* Molecular Microbiology, 2003. **48**(6): p. 1511-1524.
12. Tsuneda, S., et al., *Extracellular polymeric substances responsible for bacterial adhesion onto solid surface.* FEMS Microbiology Letters, 2003. **223**(2): p. 287-292.
13. Hiemenz, P. and R. Rajagopalan, *Principles of Colloid and Surface Chemistry*. 3rd ed. 1997, New York, NY: Marcel Dekker, Inc. 650.
14. Drelich, J., et al., *Contact angles for liquid drops at a model heterogeneous surface consisting of alternating and parallel hydrophobic/hydrophilic strips.* Langmuir, 1996. **12**: p. 1913-1922.
15. Malvern, I., *The use of Zeta Potential Measurements to Study Sterically Stabilized Liposomes.* Application Note by Malvern Instruments, 2006.
16. Gudipati, C.S., et al., *The Antifouling and Fouling-Release Perfomance of Hyperbranched Fluoropolymer (HBFP)-Poly(ethylene glycol) (PEG) Composite Coatings Evaluated by Adsorption of Biomacromolecules and the Green Fouling Alga.* Langmuir, 2005. **21**(7): p. 3044-3053.