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Quantum Dot FRET-Based Probes in Thin Films Grown in Microfluidic Channels

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The objective of this study was to fabricate new sensing thin films, which make use of luminescent quantum dots and take advantage of their unique photophysical properties while minimizing their potential toxicity. To realize this goal, CdSe/ZnS luminescent quantum dots were incorporated into the inner layers of polyelectrolyte thin films, and molecular fluorophores were attached to the film surface. This resulted in fluorescence resonance energy transfer (FRET) between the quantum dots, which served as fluorescent donors, and molecular fluorophores, which served as fluorescent acceptors. Attenuation of the FRET interactions in the presence of target analytes was used as the sensing principle of these unique sensing films.

The layer-by-layer (LbL) deposition technique, which is based on alternate deposition of oppositely charged polyelectrolytes, to fabricate a polyelectrolyte multilayer (PEM) film has been widely used due to the simplicity and effectiveness of this method in creating high-quality polymeric thin films.[1] PEMs have found use in many applications and devices, including light-emitting devices,[2,3] bioactive material coatings,[4] electrically conductive polymers,[2,5] and hollow polyelectrolyte capsules for drug delivery.[6] Fluorescent dyes were previously immobilized to PEMs in order to study their physical properties, morphology, organization, and molecular orientation. For example, Richter et al. studied the properties of poly(styrene sulfonate) (PSS) and poly(allylamine hydrochloride) (PAH) films doped with fluorescent dyes.[7] In their study they used FRET between the conjugated polymer poly(*p*-phenylene vinylene) (PPV), which served as a fluorescent donor, and acceptor molecules, which were embedded in the PEMs. They were able to find the distance dependence between the donor and acceptor molecules by varying the number of polyelectrolyte layers and found that the FRET efficiency depended on $1/[1 + (d_0/d)]^4$, where d was the distance and d_0 the critical energy transfer distance. A deviation from this distance dependence at short distance between the donors and acceptors was attributed to low lateral density of the acceptor molecules and their distribution. Caruso et al. carried out a similar study in which they assessed the FRET between 6-carboxyfluorescein (6-CF) molecules as donors and rhodamine B-labeled melamine formaldehyde (RhB-MF) particles as acceptors.[8] PEMs of varying thickness were adsorbed on the RhB-MF particles. The energy transfer took place when 6-CF was adsorbed onto the RhB-MF particles. More recently, Schneider et al. used gold nanoparticles as acceptors in FRET studies of PEMs. In their study, PEM films containing fluorescent donor molecules were formed on the surface of gold nanoparticles. FRET measurements were used to characterize the properties of these composite particles.[9]

Luminescent quantum dots have been largely used as donors in FRET solution assays.[10,11] These unique luminescent nanoparticles are characterized by a broad excitation wavelength range, high photostability, narrow emission peaks, and size-dependent tunability. These properties enable fine-tuning of FRET interactions between the quantum dot donors and the molecular acceptors linked to their surface to maximize FRET efficiency. We have previously shown that it is possible to covalently link molecular fluorescent acceptors to the surface of single quantum dots and utilize these FRET probes for real-time monitoring of protease activity in solution.[12] Recent data describing FRET interactions between luminescent quantum dots and molecular acceptors support a model for these interactions based on the Forster theory as long as the quantum dot donors can be approximated to behave like point dipoles.[11]

In the current study, PEM films were grown in microfluidic channels to facilitate FRET-based assays of volume-limited samples. The microfluidic channels were fabricated using standard microlithography on poly(dimethylsiloxane) (PDMS).[13] The quantum dots-embedded PEM film was grown through alternate deposition of positively charged PAH and negatively charged PSS layers on the PDMS surface. Mercaptoacetic acid-modified CdSe/ZnS quantum dots, which were prepared following previously established ligand exchange protocols,[14] were deposited on a positively charged PAH layer of the PEM film. This was followed by alternate deposition of oppositely charged polyelectrolyte layers. Rhodamine molecules were then covalently immobilized to a PAH layer on the film surface. Figure 1 describes the fluorescence properties of PEMs that contain quantum dots and molecular acceptors and the FRET interactions between the quantum dots and molecular acceptors when separated by varying numbers of polyelectrolyte layers.

Excitation of the quantum dot donors at 445 nm resulted in two clearly separated emission peaks of the quantum dots at 540 nm and of the rhodamine at 590 nm. The ratio between the fluorescence

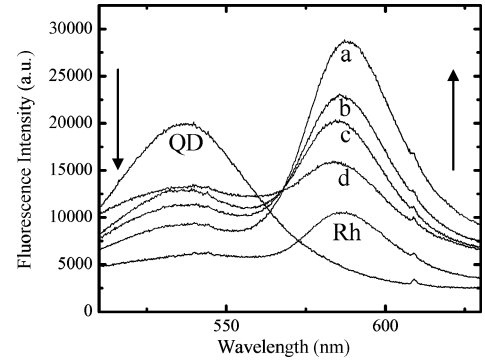

Figure 1. Fluorescence spectra of microfluidic channels containing control PEMs with quantum dots or rhodamine and microfluidic channels containing quantum dots and rhodamine, which are separated by 2 (a), 4 (b), 6 (c), and 8 (d) layers of polyelectrolytes.

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intensity of the quantum dots and the fluorescence intensity of the rhodamine acceptors (F_d/F_a) was found to be dependent on the number of polyelectrolyte layers separating the quantum dots from the rhodamine molecules. The maximum FRET efficiency was observed when the rhodamine molecules were separated from the quantum dots by two polyelectrolyte layers. Increasing the number of PEM layers resulted in lower FRET efficiency.

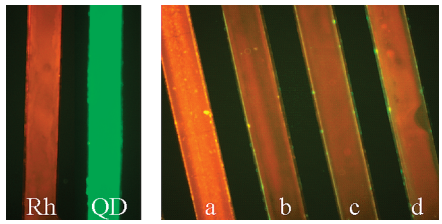

Figure 2. Fluoresence images of microfluidic channels (50 μm wide, 30 μm deep, and 3 cm long) containing control PEMs with rhodamine (Rh) or quantum dots (QD) and microfluidic channels containing quantum dots and rhodamine, which are separated by 2 (a), 4 (b), 6 (c), and 8 (d) layers of polyelectrolytes.

Fluorescence images of quantum dots (green emission) and rhodamine molecules (red emission) that were deposited in the PEMs (Figure 2) show that the emission color of quantum dot-containing PEM films was bright green in the absence of rhodamine. The emission color of the PEM films changed from green to orange in the presence of rhodamine due to FRET between the quantum dots and the rhodamine molecules. The FRET intensity reached a maximum when the quantum dots were separated from the rhodamine molecules by two layers of polyelectrolyes (channel a). The FRET intensity decreased with increasing number of polyelectrolyte layers separating the quantum dots from the rhodamine acceptors (channels b, c, and d).

The utility of the PEM-containing microfluidic channels as sensors was demonstrated through real-time monitoring of the enzymatic cleavage by trypsin of neurotensin, a peptide, which is localized in the gastrointestinal tract and the brain.[15,16] The FRET-sensing PEM film was fabricated as described above with one modification: rhodamine-labeled neurotensin molecules were conjugated to the PEM film surface. To carry out the enzymatic assay, the microfluidic channels were filled with a solution containing 0.25 mg/mL trypsin, and the flow was stopped to allow the cleavage of the rhodamine-labeled neurotensin molecules from the PEM surface. Figure 3 shows the effect of trypsin on the FRET signal between the quantum dots and the rhodamine-labeled neurotensin peptide molecules during trypsin proteolysis.

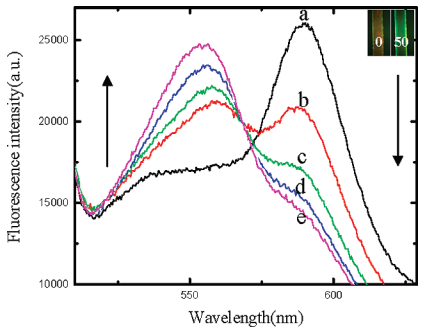

Figure 3. FRET spectra of the quantum dot FRET-based sensing PEM films prior to trypsin incubation (a) and following 10 (b), 20 (c), 30 (d), and 50 (e) min of incubation with 0.25 mg/mL trypsin at room temperature. The fluorescence intensity of rhodamine decreases while the emission intensity of the quantum dots increases. The inset box shows the FRET images of a PEM film prior to and following 50 min of incubation with trypsin.

Both spectra and image, shown as the inset, show that the fluorescence intensity of the green-emitting quantum increases while the orange emission due to FRET between the quantum dots and rhodamine molecules decreases as the proteolytic cleavage of neurotensin molecules removes the rhodamine acceptors from the PEM film surface. Control experiments with trypsin free solutions show no FRET signal change and confirm our conclusion that the observed FRET changes were due to enzymatic cleavage of neurotensin and not due to nonenzymatic modification of the PEM film.

In conclusion, we have successfully fabricated PEM films in microfluidic channels with FRET sensing capabilities and employed them in enzymatic assays. The employment of FRET sensors enables real-time monitoring of the assays since it eliminates the need to remove cleaved rhodamine molecules from the channels during the assays. The presence of the cleaved fluorescent rhodamine molecules in the channels precludes the use of conventional fluorescence techniques to monitor the enzyme assays. On the other hand, it is possible to monitor the assays by FRET since the cleaved rhodamine molecules do not affect the FRET properties of the film. Incorporating the quantum dots into sensing films enables us to take advantage of their photophysical properties and use them as luminescent probes while minimizing their toxicity. Since it has already been shown that PEM films are biocompatible and exhibit excellent adhesion properties suitable for cell growth,[13,17] we are currently pursuing this direction by employing the FRET-based sensing films introduced in this paper to monitor the proteolysis activity of CAPAN-2 pancreatic cancer cells. The ability to grow the PEM sensing films in microfluidic channels will also enable us to perform the assays on tissue cultures, taking advantage of the *in vivo*-like environment created when PEM films are grown in microfluidic platforms. It will also be possible to carry out cellular assays and study cell-substrate interactions at the single cell level.

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Supporting Information Available: Experimental procedures. This information is available free of charge via the Internet at http://pubs.acs.org.

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