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Lab-in-fiber Platform for Plasmonic Photothermal Study

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ABSTRACT

A lab-in-fiber platform, comprising a photonic crystal fiber component for light-sample interaction, was experimentally demonstrated to be effective as a sensor and micro-reactor. Specifically, it enabled the discrimination between free and liposome-encapsulated fluorophores as well as allowed for the excitation of in-fiber plasmonic photothermal effects, by alternating between different fiber-coupled inputs. The significant increase in fluorescence emissions upon release of fluorophores, encapsulated within liposomes at self-quenching concentrations, was perceived as a shoulder in the device's spectral output that otherwise only comprises the input excitation. Markedly, the observed shoulder was only discernible when the photonic crystal fiber was placed in a bent orientation. This was explained to be associated with the bending-induced refractive index profile changes in the fiber cross section that led to increased amounts of evanescent fields for light-sample interactions. Results highlighted the viability of the lab-in-fiber platform as an alternative to current lab-on-a-chip devices.

Keywords: Photonic crystal fiber, plasmonic photothermal, liposome, fluorescein, lab-on-a-chip, opto-fluidics.

1. INTRODUCTION

Photonic crystal fibers (PCFs) or microstructured optical fibers have been widely explored since its devise by Knight *et al.* in 1996 [1]. The periodic array of air holes that run laterally along the lengths of PCFs enable light guidance via modified total internal reflection or photonic bandgap guidance [2, 3], while doubling as microfluidic channels for sample infiltration and thus facilitating light-sample interactions. This unique property has seen to the numerous applications of PCFs in refractive index sensing [4-6] as well as absorption [7] and fluorescence [8, 9] spectroscopy. In addition, this trait renders it notably similar to lab-on-a-chip devices, which unfortunately require sophisticated waveguide incorporation processes onto polymer chips, in order to enable optics-based detection regimes [10-12]. Moreover, since lab-on-a-chip devices also serve as micro-reactors, PCFs seem to be a viable alternative for similar processes.

Utilizing the PCF platform described in our previous work [6], a photo-inducible reaction study is proposed to highlight the optical cum microfluidic aspects of the lab-in-fiber platform. In particular, a recent area of interest is the use of metal nanoparticles in the enhancement of photothermal effects (or plasmonic photothermal effect) for therapeutic applications [13-15]. These metal nanoparticles resonate under specific excitation wavelengths, generating localized surfaced plasmons and which subsequently elicits an extremely localized heating effect capable of disruption cellular membranes, ideal for the specific targeting of tumors or bacteria. A simplified membrane model for such a study is liposomes [16, 17] – closed vesicular structures comprising self-assembled lipid bilayers, first described by Bangham *et al.* [18] in 1964. Studies have demonstrated the light-controlled release of liposomal contents upon the optical excitation of adjacent or membrane-bound metal nanoparticles [19-22]. These studies exploit the ability of liposomes in encapsulating water-soluble compounds within their aqueous cores and depend on the consequent release of these contents upon a gel-to-liquid phase transition of their lipid membranes. Often in such studies, fluorescent dyes are employed for ease in detection and quantification. Specifically, fluorescent dyes are encapsulated at self-quenching concentrations within liposomes [23], and only upon reaching phase transition temperatures [24] would pores form in the liposomal membrane enabling release of the trapped dye and hence produce detectable fluorescence signals.

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This paper hence focuses on the employment of the lab-in-fiber platform as a sensor for the optical detection of fluorescence emissions upon fluorophore release from liposomes. It further analyses the viability of the platform as a micro-reactor for in-fiber excitation of plasmonic photothermal effects in the similar release of liposome-encapsulated fluorophores.

2. MATERIALS AND METHODS

2.1 Chemicals and other materials

1,2-dipentadecanoyl-sn-glycero-3-phosphocholine (15:0 PC) was purchased from Avanti Polar Lipids Inc. (Alabaster, Alabama). Whatman NucleoporeTM track-etched polycarbonate membrane filters with pore size of 0.2 μ m and PD-10 desalting columns (packed with SephadexTM G-25M) were obtained from GE Healthcare (Singapore). 5(6)-carboxyfluorescein was purchased from Sigma-Aldrich (Singapore). 1M Tris buffer at pH8.8 was acquired from 1st Base (Singapore). 53nm gold nanoparticles were obtained from Nanopartz (Loveland, Colorado). Thiol polyethylene glycerol MW 5000 was acquired from Nanocs (New York, New York). All other chemicals were of reagent grade.

2.2 Liposome synthesis

Liposomes were synthesized via a procedure based upon the preparation of reverse-phase evaporation vesicles (REV) previously described by Szoka *et al.* [25] and Pidgeon *et al.* [26, 27]. The aqueous and organic phases comprised 100mM 5(6)-carboxyfluorescein (CF) tuned to pH12 and 15:0 PC dissolved in diethyl ether, correspondingly. Synthesized liposomes were extruded 10 to 15 times through a polycarbonate membrane of 0.2 μ m pore size. Aliquots of this liposome suspension were then passed through a PD-10 desalting column (equilibrated with Tris buffer) to remove non-encapsulated CF. Control samples of CF were prepared in an identical manner, in the absence of lipids.

2.3 PEGylating gold nanoparticles

A thiol polyethylene glycerol (PEG-SH) solution was first sonicated before being added dropwise to a solution of gold nanoparticles (AuNPs) under vigorous stirring. The mixture was then allowed to stir overnight before it was centrifuged at, decanted of its supernatant and resuspended in Tris buffer. The centrifugation and resuspension process was repeated to ensure maximal removal of unattached PEG and original AuNP capping molecules. The resulting AuNPs were coated with PEG at a density of approximately 2 particles/nm².

2.4 Absorbance and fluorescence measurements

Aliquots of samples were diluted prior to measurements. Absorbance measurements were made with a UV-Vis-NIR scanning spectrophotometer (Shimadzu) in 1cm path length quartz cells over the wavelength ranges of 450-600nm for AuNP samples respectively. Fluorescence measurements were recorded with a fluorescence spectrometer (Perkin Elmer) in low-volume disposable cuvettes under 480nm excitation, over the wavelength range of 500-600nm for lipo-CF samples containing the fluorophore, CF.

2.5 Size and concentration determination

The average size and poly-dispersion index (PDI) of the liposome suspensions were determined by dynamic light scattering using a Malvern Zetasizer Nano ZS. All readings were performed with approximately 0.5ml of samples in low-volume disposable cuvettes at 25°C.

2.6 Lab-in-fiber platform

Figure 1. Schematics of lab-in-fiber platform. Inset: Scanning electron micrograph of utilized PCF's cross-section.

Details of the lab-in-fiber platform can be found in [6]. In brief, the treated lipo-CF samples were infiltrated into the PCF component and illuminated via a fiber-coupled blue LED (ThorLabs; 2.3mW; $\lambda = 490\text{nm}$ – corresponding to the peak absorbance of CF) at the device input. The resultant spectra were collected at the device output. For plasmonic photothermal studies, where samples comprised liposome-AuNP mixtures, the input source was switched between the blue LED and a green DPSS laser (Lambda Pro DPSS Laser; 20mW; $\lambda = 532\text{nm}$ – corresponding to the peak absorbance of the AuNPs) for excitation of CF and the gold nanoparticles, correspondingly. Specifically, each cycle involved the collection of 2 spectra, namely before and after the plasmonic photothermal treatment – a 15min illumination of the infiltrated sample with the green DPSS laser.

3. RESULTS AND DISCUSSION

3.1 Characterization of synthesized liposomes and AuNP

Figure 2. Emission spectra of lipo-CF and CF with (heated) and without (control) heating in 50°C water bath. Inset: Schematics of lipo-CF before and after heating, illustrating efflux of encapsulated CF in the latter, accompanied with respective images of lipo-CF samples in cuvettes.

Identical amounts of lipo-CF were subjected to 2 different temperature treatments – Control: placed in iced bath; Heated: placed in 50°C water bath. Their emission spectra, as shown in Figure 2, highlighted a significant difference in the emission intensities with that of heated samples being approximately 13.5-fold that of control samples. This massive increase in emission can be attributed to the huge drop in concentration self-quenching effects upon the efflux of encapsulated CF facilitated by the gel-to-liquid phase transition of the liposome membrane. Specifically, under strong alkali conditions, CF predominantly exists in its dianionic state (CF^{2-}) [28], which although possessing the highest quantum yield amongst its various protolytic states [29] is also the composite of non-fluorescent CF aggregates (dimers and trimers) [30, 31]. On top of being non-fluorescent, these aggregates are also responsible for quenching elicited by Förster's energy transfers from monomers [23]. Using the dimerization parameters described in [23], the calculated inter-fluorophore (mixture of monomer and dimers) distance for 100mM of CF is approximately 33.5Å, which is very much within Förster's critical transfer distances (51Å and 57Å for monomer-monomer and monomer-dimer, correspondingly). This migration of energy to non-fluorescent aggregates thus results in the observed concentration self-quenching effect in lipo-CF control samples with unperturbed lipid bilayers (i.e. no gel-to-liquid phase transition). Upon heating to T_m , however, thermodynamics describes large spikes in the total transient membrane pore area, which implies maximum membrane permeability and hence maximum leakage [32]. Since, doubly charged CF^{2-} molecules are only unable to permeate gel-phased lipid bilayers, the formation of transient pores provides channels that enable their diffusion-facilitated efflux. This efflux thus results in a drop in encapsulated concentrations of CF, drastically reducing the effects of concentration self-quenching, and therefore eliciting a detectable fluorescence emission peak. Notably, this change is even observable by the naked eye, from dull orangey-brown to bright greenish-yellow, as shown in the insets of Figure 2. It is also noteworthy that heating does induce deaggregation [30], but this effect was accounted for by control samples of CF, revealing a less than 1% difference.

Figure 3. Normalized absorbance spectra of AuNP before and after PEGylation.

Absorbance spectra of AuNP were recorded before and after PEGylation to ensure minimal change in their absorbance spectra, particularly at the peak absorbance wavelength. As illustrated in Figure 3, a red-shift of 3nm occurs at the peak absorbance wavelength, concurring with the increased particle size upon PEGylation. Furthermore, slight broadening of the absorbance band was also observed, implying some presence of particle aggregation possibly occurring during the PEG-capping process. A drop in peak absorbance was also noted (results not shown here), attributed by the loss in AuNPs during the centrifugation and resuspension process. The resultant PEGylated AuNPs were further concentrated by 10-times by centrifugation before use.

Table 1. Size distribution of lipo-CF and AuNPs.

Sample	Average size (nm)	PDI
Lipo-CF (Control)	172.13 ± 0.75	0.056 ± 0.029
Lipo-CF (Heated)	135.50 ± 8.24	0.073 ± 0.019
AuNP	60.89 ± 1.60	0.168 ± 0.017
PEGylated AuNP	85.06 ± 0.80	0.114 ± 0.005

The average particle sizes and PDI for the lipo-CF samples and AuNPs are listed in Table 1. The observed drop in hydrodynamic diameter for heated Lipo-CF samples implies a possible rupture of the lipid bilayers that resulted in liposomal splitting or budding of smaller vesicles. This has likely occurred due to the high temperatures used, which on top of inducing pore formation has provided sufficient energy for further organizational changes. The increase in AuNP size, on the other hand, corresponds to the addition of two PEG molecules adhered on both sides of the AuNP diameter, totaling approximately 20nm based on the Gaussian random coil model [33].

3.2 Lab-in-fiber spectroscopic analysis of lipo-CF

Figure 4. Normalized output spectra of lab-in-fiber platform with 490nm blue LED input for various infiltrations of lipo-CF samples, under straight and bent PCF orientations.

The output spectra of the device were collected upon illumination of the infiltrated lipo-CF samples with the fiber-coupled blue LED. As shown in Figure 4, a discernible difference is observed between the control and heated lipo-CF samples beyond 500nm. In particular, a shoulder comprising of regions residing below and above the control spectrum (separated by an inflection point at approximately 500nm) spans the right tail of the spectrum, corresponding to the absorbance and emission of CF. Results therefore concurred with that obtained from the fluorescence spectrometer in Figure 2, where a distinct fluorescence emission was observed only for heated lipo-CF samples. Interestingly, this shoulder was not observed when the PCF component in the device was straightened, eliciting no difference between the heated and control lipo-CF samples. This phenomenon could however be attributed to the increased amount of evanescent fields penetrating the air holes of the photonic crystal structure enhancing the extent of light-sample interaction. As reported in our previous work [6], bending induces an increase in the cross-sectional refractive index of the PCF towards the outside of the bend, this induces a same-direction displacement of the core mode as well as allows for core-cladding coupling, both of which contribute to the increased evanescent fields within the PCF's air holes. To further eliminate the possibility of the spectral differences occurring due to refractive index differences, the refractive indices of the control and heated lipo-CF samples were measured with a pocket refractometer (Atago) at 22°C, reading

identical refractive indices of 1.337. It is also appropriate to highlight that the lipo-CF control output spectrum closely resembles that of water (results not shown here), which mainly constitutes the excitation spectrum.

Figure 5. Output spectra of lab-in-fiber platform with 490nm blue LED input for various infiltrations of lipo-CF samples, under bent PCF orientation. Lasing was also performed in the bent PCF orientation at 532nm excitation by a fiber-coupled green DPSS laser.

Plasmonic photothermal studies were subsequently performed with the lab-in-fiber platform. Briefly, upon infiltration of the lipo-CF and AuNP mix at an approximate 1:1 particle-count ratio, output spectra were collected before and after a 15min period of continuous excitation with the fiber-coupled green DPSS laser. It is to be noted that the excitation of plasmonics was performed with the PCF in the bent orientation, as it is expected to result in a better light-sample interaction. As shown in Figure 5, a shoulder does appear to emerge beyond 500nm for the lased lipo-CF and AuNP mix. Admittedly, the significance of this shoulder pales in comparison to that illustrated in Figure 4, which could imply the inefficiency of plasmonic photothermal effects in triggering complete liposomal release. These results, however, could also be coherent with literature depicting metal nanoparticle elicited quenching due to a dominance of absorbance over scattering components for AuNPs smaller than 80nm [34], larger than the AuNPs used in this study. The prominence of the shoulder is therefore reduced due to afore described quenching effects on the released fluorophores' emission. Further studies involving different AuNP sizes would be performed to verify and account for the fluorescence quenching and enhancement effects of metal nanoparticle. In addition, the ratio between lipo-CF and AuNP would be varied to analyze the effects of fluorophore-metal distance on fluorescence. Lastly, to contrast the extent of this in-fiber plasmonic photothermal triggered release with that elicited by heat, subsequent work would also include a comparison with heated mixtures of lipo-CF and AuNPs, accounting for fluorophore-metal interactions upon a substantial release trigger by heat.

4. CONCLUSION

In summary, a lab-in-fiber platform is experimentally demonstrated to be effective in distinguishing between fluorescence emission of free and liposome-encapsulated CF. This observable difference was explained to be due to the enormous drop in concentration self-quenching effects when fluorophores, encapsulated at high concentrations within liposomes, are released into the liposome exterior upon heat-elicited pore formation in the lipid bilayer. Within the lab-in-fiber device, this difference was detected as a shoulder or point of inflection on the right tail of the spectrum, corresponding to CF absorbance and emission. Notably, this phenomenon was only observed when the PCF component was in the bent orientation, and was explained to be due to bending-induced refractive index profile change across the PCF cross section that increased the penetration of evanescent fields. This rise in the amount of evanescent fields residing within the air holes, thus enhanced the extent of light-sample interactions. Subsequent study on the in-fiber-excitation of plasmonic photothermal effects, conducted on mixtures of liposomes and AuNPs, highlight a similar presence of a shoulder that was undeniably less significant than heat-elicited release of CF. This was attributed to the efficiency of the in-fiber plasmonic photothermal effect as well as fluorophore-metal interactions – quenching. Hence,

subsequent work would focus on addressing the aforementioned concerns. Nevertheless, the lab-in-fiber platform has exhibited viability for application as micro-reactors, highlighting its potential as an alternative to lab-on-a-chip devices.

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REFERENCES

- [1] Knight, J. C., Birks, T. A., Russell, P. S. J., and Atkin, D. M., "All-silica single-mode optical fiber with photonic crystal cladding," *Opt. Lett.*, 21(19), 1547-1549 (1996).
- [2] Knight, J. C., "Photonic crystal fibres," *Nature*, 424(6950), 847-851 (2003).
- [3] Russell, P., "Photonic crystal fibers," *Science*, 299(5605), 358-362 (2003).
- [4] Yu, X., Shum, P., Ren, G. B., and Ngo, N. Q., "Photonic crystal fibers with high index infiltrations for refractive index sensing," *Optics Communications*, 281(18), 4555-4559 (2008).
- [5] Zhang, Y. F., Chan, C. C., and Sun, J., "Enhancement of temperature measurement by using photonic bandgap effect," *Sensors and Actuators A: Physical*, 157(2), 276-279 (2010).
- [6] Yong, D., Ng, W. L., Yu, X., and Chan, C. C., "A compact opto-fluidic platform for chemical sensing with photonic crystal fibers," *Sensors and Actuators A: Physical*, 191(0), 22-26 (2013).
- [7] Yu, X., Zhang, Y., Kwok, Y. C., and Shum, P., "Highly sensitive photonic crystal fiber based absorption spectroscopy," *Sensors and Actuators B: Chemical*, 145(1), 110-113 (2010).
- [8] Williams, G. O. S., Euser, T. G., Chen, J. S. Y., Russell, P. S. J., and Jones, A. C., "Fluorescence spectroscopy in photonic crystal fiber." 1-1.
- [9] Smolka, S., Barth, M., and Benson, O., "Selectively coated photonic crystal fiber for highly sensitive fluorescence detection," *Applied Physics Letters*, 90(11), 111101-3 (2007).
- [10] Bilenberg, B., Nielsen, T., Clausen, B., and Kristensen, A., "PMMA to SU-8 bonding for polymer based lab-on-a-chip systems with integrated optics," *Journal of Micromechanics and Microengineering*, 14(6), 814 (2004).
- [11] Mazurczyk, R., Vieillard, J., Bouchard, A., Hannes, B., and Krawczyk, S., "A novel concept of the integrated fluorescence detection system and its application in a lab-on-a-chip microdevice," *Sensors and Actuators B: Chemical*, 118(1-2), 11-19 (2006).
- [12] Balslev, S., Jorgensen, A. M., Bilenberg, B., Mogensen, K. B., Snakenborg, D., Geschke, O., Kutter, J. P., and Kristensen, A., "Lab-on-a-chip with integrated optical transducers," *Lab on a Chip*, 6(2), 213-217 (2006).
- [13] Huang, X., El-Sayed, I. H., and El-Sayed, M. A., [Applications of Gold Nanorods for Cancer Imaging and Photothermal Therapy] Humana Press, (2010).
- [14] Khlebtsov, B., Zharov, V., Melnikov, A., Tuchin, V., and Khlebtsov, N., "Optical amplification of photothermal therapy with gold nanoparticles and nanoclusters," *Nanotechnology*, 17(20), 5167 (2006).
- [15] Morton, J. G., Day, E. S., Halas, N. J., and West, J. L., [Nanoshells for Photothermal Cancer Therapy] Humana Press, (2010).
- [16] Sessa, G., and Weissmann, G., "Phospholipid spherules (liposomes) as a model for biological membranes," *Journal of Lipid Research*, 9(3), 310-318 (1968).
- [17] Kaufmann, S., Kumar, K., and Reimhult, E., [Preparation and Dynamic Patterning of Supported Lipid Membranes Mimicking Cell Membranes] Humana Press, 28 (2011).
- [18] Bangham, A. D., and Horne, R. W., "Negative staining of phospholipids and their structural modification by surface-active agents as observed in the electron microscope," *Journal of Molecular Biology*, 8(5), 660-IN10 (1964).
- [19] Paasonen, L., Laaksonen, T., Johans, C., Yliperttula, M., Kontturi, K. s., and Urtti, A., "Gold nanoparticles enable selective light-induced contents release from liposomes," *Journal of Controlled Release*, 122(1), 86-93 (2007).
- [20] Volodkin, D. V., Skirtach, A. G., and Möhwald, H., "Near-IR Remote Release from Assemblies of Liposomes and Nanoparticles," *Angewandte Chemie International Edition*, 48(10), 1807-1809 (2009).
- [21] Troutman, T. S., Leung, S. J., and Romanowski, M., "Light-Induced Content Release from Plasmon-Resonant Liposomes," *Advanced Materials*, 21(22), 2334-2338 (2009).

- [22] Wu, G., Mikhailovsky, A., Khant, H. A., Fu, C., Chiu, W., and Zasadzinski, J. A., "Remotely Triggered Liposome Release by Near-Infrared Light Absorption via Hollow Gold Nanoshells," *Journal of the American Chemical Society*, 130(26), 8175-8177 (2008).
- [23] Chen, R. F., and Knutson, J. R., "Mechanism of fluorescence concentration quenching of carboxyfluorescein in liposomes: Energy transfer to nonfluorescent dimers," *Analytical Biochemistry*, 172(1), 61-77 (1988).
- [24] Heimburg, T., [Lipid Melting] Wiley-VCH Verlag GmbH & Co. KGaA, (2007).
- [25] Szoka, F., and Papahadjopoulos, D., "Procedure for preparation of liposomes with large internal aqueous space and high capture by reverse-phase evaporation," *Proceedings of the National Academy of Sciences*, 75(9), 4194-4198 (1978).
- [26] Pidgeon, C., Hunt, A. H., and Dittrich, K., "Formation of Multilayered Vesicles from Water/Organic-Solvent (W/O) Emulsions: Theory and Practice," *Pharmaceutical Research*, 3(1), 23-34 (1986).
- [27] Pidgeon, C., McNeely, S., Schmidt, T., and Johnson, J. E., "Multilayered vesicles prepared by reverse-phase evaporation: liposome structure and optimum solute entrapment," *Biochemistry*, 26(1), 17-29 (1987).
- [28] De, S., and Kundu, R., "Spectroscopic studies with fluorescein dye - Protonation, aggregation and interaction with nanoparticles," *Journal of Photochemistry and Photobiology A: Chemistry*, 223, 71-81 (2011).
- [29] Klonis, N., and Sawyer, W., "Spectral properties of the prototropic forms of fluorescein in aqueous solution," *Journal of Fluorescence*, 6(3), 147-157 (1996).
- [30] Rohatgi, K. K., and Singhal, G. S., "Nature of Bonding in Dye Aggregates," *The Journal of Physical Chemistry*, 70(6), 1695-1701 (1966).
- [31] Arbeloa, I. L., "Dimeric and trimeric states of the fluorescein dianion. Part 2.-Effects on fluorescence characteristics," *Journal of the Chemical Society, Faraday Transactions 2: Molecular and Chemical Physics*, 77(10), 1735-1742 (1981).
- [32] Blicher, A., Wodzinska, K., Fidorra, M., Winterhalter, M., and Heimburg, T., "The Temperature Dependence of Lipid Membrane Permeability, its Quantized Nature, and the Influence of Anesthetics," *Biophysical Journal*, 96(11), 4581-4591 (2009).
- [33] Manson, J., Kumar, D., Meenan, B., and Dixon, D., "Polyethylene glycol functionalized gold nanoparticles: the influence of capping density on stability in various media," *Gold Bulletin*, 44(2), 99-105 (2011).
- [34] Lakowicz, J. R., "Radiative decay engineering 5: metal-enhanced fluorescence and plasmon emission," *Analytical Biochemistry*, 337(2), 171-194 (2005).