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Anila Hoskere Ashoka, Andrey Klymchenko. Ultrabright Fluorescent Polymeric Nanofibers and Coatings Based on Ionic Dye Insulation with Bulky Counterions. ACS Applied Materials & Interfaces, 2021, 13 (24), pp.28889-28898. 10.1021/acsami.1c06436 . hal-03452589

HAL Id: hal-03452589

<https://hal.science/hal-03452589>

Submitted on 27 Nov 2021

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Ultrabright Fluorescent Polymeric Nanofibers and Coatings Based on Ionic Dye Insulation with Bulky Counterions

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KEYWORDS: fluorescent polymeric materials, bulky counterions, thin films, nanofibers, coatings.

ABSTRACT. Preparation of bright fluorescent materials based on polymers is hampered by a fundamental problem of aggregation-caused quenching (ACQ) of encapsulated dyes. Here, ultrabright fluorescent polymeric nanofibers and coatings are prepared based on a concept of ionic dye insulation with bulky hydrophobic counterions that overcomes the ACQ problem. It is found that bulky hydrophobic counterion perfluorinated tetraphenyl borate can boost >100-fold the fluorescence quantum yields of cationic dye octadecyl rhodamine B at high loading (30 wt%) in a biocompatible poly(methyl methacrylate) (PMMA). The concept is applicable to both rhodamine

and cyanine dyes, which results in bright fluorescent polymeric materials of four different colors spanning from blue to near-infrared. It allows preparation of electrospun polymeric fibers with nearly 100-fold higher dye loading (30 wt%), while preserving good fluorescence quantum yields (31 %), which implies drastic improvement in fluorescence brightness. The counterion-based polymeric materials are also validated as coatings of model medical devices, such as stainless steel fiducials and 3D printed stents of complex geometry. Spin-coated fluorescent polymeric films loaded with a dye paired with the bulky counterion exhibit excellent biocompatibility and low toxicity. Moreover, counterion modified materials show much better stability against dye leakage in the presence of living cells and serum containing medium, compared to materials based on the dye with a small inorganic anion. Overall, by pushing the barriers of ACQ, our counterion approach emerges as a powerful tool to develop ultrabright fluorescent polymeric materials.

INTRODUCTION

Organic luminescent materials are used in many applications such as organic light emitting diodes¹ (OLEDs), security inks,² waveguides^{3,4}, sensing and imaging,⁵⁻⁸ etc. Efficient solid-state emission is strongly preferred for most of these applications.^{9,10} In this respect luminescent polymeric thin films, coatings and nanofibers are of particular interest. Thin films have several advantages due to the ease of handling, higher stability and suitability for device-based applications.¹¹ Fluorescent polymeric coatings can transform a non-fluorescent material into a fluorescent one simply depositing a micro-thin shell.¹² On the other hand, nanofibers, generally prepared by electrospinning method,¹³ is one of the promising materials for variety of applications. Electrospun fibers possess large surface area, small diameter and a wide variety of polymers,

ceramics can be electrospun into fibers.¹⁴⁻¹⁶ Electrospun fibers widely used in sensors,¹⁷⁻¹⁹ tissue engineering and drug delivery,²⁰ wound healing²¹ and other medical applications.²²

To implement luminescent capabilities, these materials are generally blended or conjugated with suitable dyes. However, organic dyes that are highly luminescent in dilute solutions often show poor emission in the solid state because of so-called aggregation-caused quenching (ACQ).^{9,10,23,24} Indeed, flat aromatic structure of organic dyes favors strong π - π stacking interaction forming weakly emissive or non-emissive H-aggregates.²⁵ Due to ACQ effect, organic dyes are used in small concentrations as dopants or as dilute solutions which often leads to limited brightness,^{26,27} especially in case of thin films, coatings and nanofibers. Indeed, according to Lambert-Beer equation, for 10 μ m polymeric film loaded at 1 mM concentration with a typical dye with extinction coefficient of 100,000 $M^{-1}cm^{-1}$ would exhibit an absorbance of 0.1, which implies only 21 % of light absorption, whereas for nanofibers of 100-500 nm diameter the absorbance would be 20-100 times lower.

Several approaches such as introduction of spiro-framework^{28,29}, bulky group substitution³⁰⁻³², J-aggregate formation³³, introduction of non-planar groups^{34,35}, supramolecular threading,³⁶ etc., have been put forward to attenuate the ACQ phenomena and to improve the brightness of the luminescent materials both in the case of molecular dyes as well as in polymeric nanomaterials. Among the various approaches, aggregation induced emission (AIE), introduced by Tang and coworkers is most popular³⁷. AIE molecules are poorly emissive in the solution state, whereas they show intense emission in the solid state due to phenomenon of restricted intramolecular rotation and proper dye-dye spatial arrangement^{27,38}. AIE approach was successfully applied for development of a large variety of fluorescent materials^{26,35,38-40}. Nevertheless, most of the reported modifications to achieve bright solid-state fluorescence are dye specific and require intense

organic synthesis to alter the emission properties. Moreover, AIE approach is not well adapted for blending with polymers, which is important for fabrication of functional materials and coatings.⁴¹ A universal approach to transform the luminescence properties of solutions to solids is still lacking. Previously, we introduced hydrophobic counterions as an effective tool to minimize ACQ and improve the brightness of the dyes inside the polymeric nanoparticles.⁴²⁻⁴⁵ Bulky counterions play a role of a spacer between the dye molecules inside the nanoparticles and reduce the dye aggregation, whereas the hydrophobicity of the counterions helps in better dye encapsulation.^{42,43,46} Indeed, the quantum yield of the encapsulated dyes in polymeric NPs improved with the size and fluorination level of the counterions,^{42,46} providing proper dye-dye spacing for preventing ACQ. The bulky counterions ensured high fluorescence quantum yield of dyes (20-50 %) for relatively high dye loading inside polymeric NPs (>200 mM vs polymer).⁴⁷⁻⁴⁹ Counterion approach was applied to develop NIR polymeric coatings with relatively low dye loading, which were validated for a various image-guided surgical applications.^{12,50} However, counterion approach has not been explored for development of bright fluorescent solid materials at high dye loading. Recently, Flood and co-workers proposed a supramolecular counterion approach coined as SMILES, where a cyanostar macrocycle in complex with an anion minimizes ACQ of dyes in pure salts and polymer blends.⁵¹ We asked a question whether counterion approach could be a universal tool to generate bright polymeric materials of any desired shape (e.g. films, coatings and nanofibers). In the present work, we introduce ultrabright fluorescent polymeric films, coatings and nanofibers based on a concept of ionic dye insulation with bulky hydrophobic counterions. Here, we address a fundamental problem of ACQ of organic dyes in polymeric materials at high loading. We show that bulky hydrophobic counterion can drastically improve brightness of cationic dyes in different bulk materials made of a biocompatible polymer in form

of thin films, electrospun nanofibers and coatings. Counterion modified materials exhibited outstanding optical properties characterized by high brightness and quantum yield when compared to similar materials prepared with the parent dye with small inorganic counterion. Bulky counterion also allowed better dye encapsulation and higher dye loading in different material forms. New materials are biocompatible, non-toxic and stable in biological fluids. Finally, we demonstrated a proof-of-concept to fabricate bright electrospun with exceptional dye loading and fluorescent coatings on 3D printed medical objects.

MATERIALS AND METHODS

All the chemicals and solvents were purchased from either Sigma Aldrich or TCI or Carlo Erba and used as received without further purification. Membright 488 was prepared as previously described.⁵² 3,3'-Dioctadecyloxacarbocyanine perchlorate (DiO/ClO₄) was from Sigma Aldrich. (1,1'-dioctadecyl-3,3,3',3'-tetramethylindodicarbocyanine chloride (DiD/Cl or F5-TPB) and blue cyanine iodide (BlueCy/I or F5-TPB) were prepared by following our previous reports.^{7,53} Fluorescence spectra were recorded on an Edinburg FS5 spectrofluorometer. All the spectra were corrected from the fluorescence of the corresponding blank (thin films or the fibers containing the PMMA polymer without dyes) and for the wavelength-dependent response function of the detector. Emission measurements were systematically done at room temperature, unless indicated otherwise. Absolute fluorescence quantum yields of the films and fibers were determined by integrating sphere method (SC30 integrating sphere, Edinburg Instruments). Scanning electron microscopy (SEM) images were acquired on Quanta TM 250 FEG scanning electron microscope (FEI, Edinhoven, Netherlands). Fluorescence microscopy images were acquired on Nikon Ti-E inverted microscope. Spin coating was performed on Laurell-WS-650 spin coater (Laurell

Technologies). Electrospinning was performed on FLUIDNATEK LE-10 electrospinning equipment (Bioinicia). 3D printing was done with Form 3, 3D printer (Formlabs).

Spin coating. Spin coating was performed on Laurell spin coater. Stock solution of PMMA (150 mg/mL, $M_w = 120,000$) was prepared by dissolving PMMA in acetonitrile for 18 hours. Coating solution was prepared by mixing PMMA (final concentration in solution: 1/5/10 mg/mL) with dye salts such as **R18/F5-TPB**, **R18/ClO₄** (1 to 30 wt% with respect to the polymer) or **BlueCy**, **DiO** and **DiD** (with I⁻/ClO₄⁻/Cl or F5-TPB counterions) 5 wt% each in acetonitrile solvent. Spin coating was carried out in dynamic mode by depositing 30/50 μ L of the above solution on a clean circular glass substrate having the diameter 12 mm and 18 mm (for cell experiments). Spin coating was also carried out on quartz-coated glass substrates (1cm x 1.5 cm) by depositing 100 μ L of the above solution. Rotation speeds were maintained at 2000 rpm for 30 seconds and further reduced to 500 rpm for another 30 seconds. Emission response of the films were measured either by directly placing them in a solid state sample holder or by placing the coated glass substrates inside a 3 cm cuvette and the film was illuminated at the middle portion.

Electrospinning. Polymer solution for electrospinning was prepared by dissolving 500 mg of PMMA (25 wt%, $M_w = 120,000$) in 2 mL of DMF overnight. The resulting viscous solution was mixed with various concentration of **R18/F5-TPB** and **R18/ClO₄** (0.5 to 30 wt% with respect to the polymer concentration). Final concentration of the polymer in DMF was adjusted to 22 wt%. Electrospinning was performed on a FLUIDNATEK LE-10 electrospinning equipment (Bioinicia). 1.5 mL of the above solution was loaded into a 5 mL syringe having 16 G needle. A constant flow rate of 1.5 mL/hour was maintained throughout with the help of a syringe pump. A voltage of 18 KV was applied to the syringe needle and an aluminum foil was used as the collector. The distance between the needle tip to the collector was adjusted to 15 cm. The fiber mat obtained

after the electrospinning was dried in an oven at 80° C for 2 hours. It was further dried under high vacuum overnight.

Absolute quantum yields measurements. Absolute quantum yields of the thin films and electrospun nanofibers were determined using an integration sphere (SC-30 Module, Edinburg Instruments). Thin films containing dyes such as **R18/F5-TPB**, **R18/CIO₄**, **BlueCy/F5-TPB**, **BlueCy/I**, **DiO/CIO₄**, **DiO/F5-TPB**, **DiD/Cl**, **DiD/F5-TPB** were prepared on a 12 mm glass substrate by spin-coating method. PMMA film without the dye was used as blank. Electrospun fiber mats were cut into 10 mm pieces and placed inside the sample holder. PMMA fibers without dye was used as reference. Absolute quantum yields were measured by direct excitation method with 5 and 0.7 nm excitation and emission slit widths respectively.

Fluorescence imager setup. A home-built fluorescence imaging system was utilized to capture the fluorescence images of DiD/Cl, DiD/F5-TPB thin films and electrospun fiber mats. Imaging setup consists of a 530/630 nm LED array lamp (ThorLabs/ ProPhotonix) as excitation source and a motorized filter wheel (ThorLabs) equipped with 600/52 and 650/90 band-pass filters (BrightLine HC, AHF) to collect the emitted light. Scientific CMOS (sCMOS) camera (ThorLabs) was used to capture the images. All the images were acquired from the camera at room temperature with 50/150 ms exposure time using ThorCam software. ImageJ software was utilized to analyze the images.

SEM imaging. For SEM imaging, PMMA electrospun mats loaded either with 5 wt% **R18/F5-TPB** or **R18/CIO₄** were used. Samples were sputter-coated with Palladium under Argon atmosphere for 15 minutes. SEM images were acquired by Quanta TM 250 FEG scanning electron microscope (FEI, Edinhoven, Netherlands) using 7.5 KV beam voltage. Fiber diameters were

determined by *ImageJ* software. 150 individual fibers were analyzed for each sample to determine the average diameter of electrospun fibers.

Biocompatibility assessment and fluorescence microscopy. Biocompatibility of the thin films were evaluated by culturing the HeLa cells on the spin-coated glass substrate. First, the 18 mm circular glass substrates were spin-coated with PMMA polymer having either and **R18/F5-TPB** or **R18/CIO₄**. An empty glass coverslip without the coating was used as the control. Thin films were sterilized by washing two times with 70% ethanol and dried. HeLa cells (ATCC[®] CCL-2) were grown in Dulbecco's modified Eagle medium (DMEM, Gibco-Invitrogen), supplemented with 10% fetal bovine serum (FBS, Lonza) and 1% antibiotic solution (penicillin-streptomycin, Gibco-Invitrogen) at 37°C in humidified atmosphere containing 5% CO₂. The films were placed on to a chambered cover glass (IBiDi[®]) and the cells were seeded on the films at a density of 1×10^5 cells/well, 24 h before the microscopy measurement. For imaging, the medium was removed and the remaining attached cells were washed 3 times with PBS (Gibco-Invitrogen) and the cells were stained with membrane marker Membright-488 (50 nM) in HBSS for 5 minutes in dark. Fluorescence imaging was performed by Nikon Ti-E inverted microscope with 60x in spinning disk mode using X-light (CrestOptics) module and laser excitation (Oxxius). Membright-488 was excited with 488 nm laser and the emission was collected in the green channel with 531/40 band-pass filter. and **R18/F5-TPB** and **R18/CIO₄** films were excited with 532 nm laser and the emission was collected in red channel with 600/50 band-pass filter. *ImageJ* software was used to process the fluorescence images.

3D-printing. Form 3, 3D printer (Formlabs) was used for 3D printing. Stent prototype (1cm width, 3 cm height) that mimics the vascular medical stents was designed with FreeCAD parametric 3D modeler software (STL file is available upon request). Clear V4 resin (Formlabs)

was used for 3D printing. After the 3D printing, polymer stents were rinsed with isopropyl alcohol to get rid of the excess resin and cured under UV light for 30 minutes.

Method for coating steel wires and 3D-printed stents. Coating formulation was prepared by mixing the PMMA polymer, $M_w = 120,000$ (100/30 mg/mL for steel wires and polymer stents respectively) and **R18/F5-TPB** or **R18/CIO₄** (0.1 or 5 wt % with respect to polymer) in acetonitrile solvent. Stainless steel wires having a diameter of 0.1 mm was cut into 1 cm pieces. Steel wires were cleaned with water and ethanol by sonication for 15 minutes and dried. Wires were dip-coated in the formulation and dried under fume hood for 15 minutes. Dip-coating step was repeated 2 times in order to have sufficiently thick and bright fluorescent coating layer. 3D-printed polymer stents were dip-coated in the same manner as explained above. Fluorescence images of the steel wires were captured in a home-built imaging set up. Images were analyzed with *ImageJ* software. Coated stents were observed under 365 nm UV lamp and images were captured with a camera.

Dye leakage study in serum. For dye leakage studies in serum, spin-coated thin films containing **R18/CIO₄** and **R18/F5-TPB** were placed in 1 mL of water containing 10 vol% fetal bovine serum (FBS). The samples were incubated at 37 °C for 2 or 24 hours. Absorption and emission spectra of the serum samples were measured after 2 and 24 h incubation. Serum sample without coating film was used as a control. Absorption and emission spectra of blank serum sample was compared with those incubated with **R18/CIO₄** or **R18/F5-TPB** films to determine the dye leakage.

Toxicity assessment of the films by WST-1 assay. Cytotoxicity of the **R18/CIO₄** and **R18/F5-TPB** spin coated films were determined by WST-1 assay. This assay is based on the cleavage of tetrazolium salt of WST-1 in to water soluble formazan by mitochondrial dehydrogenases present in the living cells. The formazan dye produced by the activity of the living cells can be quantified

by measuring the absorbance at 440 nm. First, the thin films were prepared by spin-coating on 12 mm glass substrates. Films were sterilized by washing two times with 70% ethanol. After drying, the substrates were placed in a 24 well plate. A glass substrate without any coating and the substrate coated with only the polymer was used as control. HeLa cells were seeded on the substrates (6 repeats per each dye) at a density of 3×10^4 cells/well and incubated for 24 h at 37°C in DMEM medium. Cells were also treated with DMEM containing 0.1 % Triton-X 100 as a positive control of cytotoxicity. After 24 h, WST-1 reagent (5 μ L/well) was added to each well containing 200 μ L DMEM medium. The plates were maintained at 37 °C for 4 h. Then, 200 μ L of DMEM from each well was transferred to a 96-well plate and the absorbance was measured at 440 nm and 630 nm. Percentage of viable cells with respect to control cells was calculated.

RESULTS AND DISCUSSION

Material fabrication and photophysical evaluation. As a control dye, we used commercial rhodamine octadecyl ester perchlorate (**R18/CIO₄**), which was synthesized as reported elsewhere.⁵⁴ Tetrakis (pentafluorophenyl)borate (F5-TPB) was selected as a bulky hydrophobic counterion, because it has shown effective to prevent ACQ in polymeric NPs.^{24,42,44} The perchlorate counterion in **R18/CIO₄** was replaced with F5-TPB by an ion-exchange method⁴² followed by purification by chromatography to yield **R18/F5-TPB** (Scheme 1 and SI). Rhodamine dyes (both **R18/CIO₄** and **R18/F5-TPB**) were mixed separately with a biocompatible polymer, poly(methyl methacrylate) (PMMA) in acetonitrile solvent and transformed into different material forms such as thin films, nanofibers and coatings by various techniques (Scheme 1).

Scheme 1. a) Dye salts: dye is shown in red, while the counterion is shown in blue. b) Schematic overview of the transformation of dyes with a biocompatible polymer into various material forms such as films, fibers and coatings, their corresponding fluorescence images.

Thin films of **R18/C104** and **R18/F5-TPB** were prepared by spin coating on the glass substrate. Initially, different concentrations of the PMMA (1/5/10 mg/mL) were tested by keeping the dye loading constant (5 wt% with respect to PMMA). Fluorescence intensity from the films increased with polymer concentration (Figure S1), which was clearly related to the increase in the film thickness. Therefore, we selected an intermediate polymer concentration (5 mg/mL) for further studies to avoid any potential inner filter effect caused by thick film at high dye loading. Although the absorption maxima for **R18/C104** and **R18/F5-TPB** films were similar (Figure 1a and Figure S1a-c), **R18/F5-TPB** films showed narrower absorption band with less intense short-wavelength shoulder (Figure 1a and Figure S1a-c), indicating less pronounced dye aggregation. Thin films containing **R18/F5-TPB** showed much higher fluorescence intensity compared to **R18/C104** films

at all the tested polymer concentrations (Figure 1b, S1(d-f) and S2). Photos of representative spin-coated thin films confirmed the striking effect of the counterion: **R18/F5-TPB** films displayed a bright red emission, whereas **R18/CIO₄** film appeared dark (Figure 1b inset). Then, the PMMA concentration was fixed to optimum 5 mg/mL and the effect of dye loading (0.1-20 wt%) on the fluorescence intensity was studied (Figure 1c). **R18/CIO₄** showed weak fluorescence at low loading (0.1 to 1 wt%) and its fluorescence was almost completely quenched at higher loadings (>5 wt%) (Figure 1c), suggesting the strong ACQ effect in thin films containing **R18/CIO₄**. On the other hand, **R18/F5-TPB** films systematically showed very high fluorescence intensity at different loadings and their fluorescence intensity increased with increase in the dye content. The absolute quantum yield (QY) values, measured by an integration sphere method (see SI for details), revealed remarkably higher values for **R18/F5-TPB** films compared to **R18/CIO₄** at all the tested dye loadings (Figure 1d and Table S1). At 20 wt% dye loading, **R18/F5-TPB** thin films showed more than 130-fold higher QY ($38 \pm 4\%$) than **R18/CIO₄** ($0.29 \pm 0.09\%$). Moreover, **R18/F5-TPB** maintained a high QY of $33 \pm 2\%$ even at 30 wt% loading (Figure 1d and Table S1), whereas for **R18/CIO₄** the fluorescence signal was no more detectable. These studies clearly suggest that in **R18/F5-TPB** thin films, bulky counterions play a major role in separating the dyes from π - π stacking interaction and preventing them from ACQ effect, whereas in the case of **R18/CIO₄** thin films, small perchlorate counterions cannot prevent dye aggregation, thus leading to fluorescence quenching. Moreover, bulky counterions allows higher dye loading in polymer films which otherwise, is difficult to achieve with the dyes having smaller counterions (like -ClO₄) without altering the optical properties. This material is expected to present an outstanding brightness, because here we achieved unprecedented dye loading in a polymer film while preserving high QY

values. In one of the brightest fluorescent materials recently reported by Flood and coworkers using SMILES method, a maximum of 10 wt% dye loading was attained.⁵¹

Figure 1. Photophysical properties of the thin films. (a) Absorption spectra of PMMA thin films containing **R18/C1O₄** and **R18/F5-TPB** (5 wt% each). (b) Corresponding emission spectra of thin films. Dotted lines in a and b represent the absorption and emission spectra, respectively, of **R18/C1O₄** and **R18/F5-TPB** (1 μ M) in Methanol. Inset in figure b are the representative fluorescence images of the spin-coated thin films captured with a camera under UV light illumination and processed with ImageJ software. (c) Fluorescence intensity of the thin films at different dye loading. (d) Absolute quantum yield values of thin films at different dye loading. Note: Absolute quantum yield for **R18/C1O₄** at 30 wt% loading was not determined because of the very low signal to noise ratio.

To verify the universality of this approach, we selected a series of cationic cyanine dyes having fluorescence emission from blue to far-red spectral region. PMMA thin films containing BlueCy, DiO and DiD dyes (5 wt% each) with small counterions ($I^-/ClO_4^-/Cl^-$) and bulky **F5-TPB** counterion were prepared by spin coating on glass substrate. Thin films containing dyes with **F5-TPB** counterion showed systematically higher fluorescence intensity compared to the respective thin films with smaller counterions (Figure 2 a-c). Images of the thin films containing BlueCy, DiO and DiD with **F5-TPB** displayed bright blue, green and red emission, respectively (insets in

Figure 2 a-c), whereas the films with small counterions were mostly non-emissive suggesting the strong ACQ effect. It should be noted that, a white tinge in the thin film of DiO/ ClO_4^- could be due to the formation of aggregates with broad emission band, Figure 2b. In case of DiD, the emission maximum was observed in the near-infrared region (711 nm), which was significantly redshifted compared to that in methanol (667 nm, Figure S3). Moreover, absolute QY values for thin films containing dyes with **F5-TPB** were much higher compared to those with small counterions (Figure 2d and Table S2). These studies confirm that the counterion approach can be extended to other cationic dyes to produce bright fluorescent materials of any color from blue to near-infrared.

Figure 2. Photophysical properties of PMMA thin films containing different dyes (5 wt% each) with or without bulky counterion. Emission spectra of thin films containing (a) BlueCy, (b) DiO and (c) DiD with small counterions ($\text{I}^-/\text{ClO}_4^-/\text{Cl}^-$) or with **F5-TPB**. Insets in panels (a) and (b) are the representative fluorescence images (photos) of the spin-coated thin films under UV light illumination. In panel (c) DiD-based thin films were imaged with a home-built imager setup (630 nm excitation with 690/50 nm band pass emission filter). (d) Absolute quantum yield values of thin films containing different dyes with small counterions ($\text{I}^-/\text{ClO}_4^-/\text{Cl}^-$) or with **F5-TPB**. Error bars are standard deviation of the mean (n = 2).

Biocompatibility, dye leakage studies and toxicity assessment. We have further evaluated the biocompatibility of the thin films by culturing HeLa cells on the spin-coated PMMA films containing 5 wt% **R18/F5-TPB** and **R18/CIO₄**. After 24 hours of incubation, the plasma membrane of HeLa cells was labelled with membrane marker, Membright-488. Fluorescence microscopy images of HeLa cells suggested that the cells were nicely adhered and proliferated on thin films containing **R18/F5-TPB** and **R18/CIO₄** (Figure 3), similar to the cells grown on the plain glass coverslips (Figure S4).

Figure 3. Fluorescence microscopy images of HeLa cells cultured on spin-coated PMMA thin films containing **R18/F5-TPB** (a) and **R18/CIO₄** (b) (5 wt% each). Plasma membrane was stained with Membright-488 (100 nM). Scale bar 20 μ m.

Moreover, HeLa cells showed prominent plasma membrane (stained in green) and regular morphology. These observations suggest that the cells are healthy on thin films without any signs of toxicity. However, the analysis of the rhodamine channel and overlay images clearly indicates the dye leakage from the thin films containing **R18/CIO₄** (Figure 3b, indicated by the white

arrows). In the case of **R18/F5-TPB** thin films, the red fluorescence from cells was almost negligible (Figure 3a), suggesting negligible dye leakage. The observed faint cell structures in the red channel in this case looked rather like optical effect because it reproduced the shape of the cells, being different from the characteristic intracellular staining observed for leaked R18 shown in Figure 3b. Dyes with smaller hydrophilic counterions (like ClO_4^-) tend to leach out from the surface in aqueous medium whereas the bulky hydrophobic counterions helps in better encapsulation and hence, no leakage was observed. This is similar to our previous observations in nanoparticles and NIR coatings.^{12,44} Additionally, we have evaluated the dye leakage from PMMA films loaded with **R18/F5-TPB** and **R18/ ClO_4** (5 wt%) in model biological medium containing fetal bovine serum (FBS) by recording absorption and emission spectra after 2 h and 24 h incubation at 37°C. Interestingly, when compared to the blank serum samples, samples that were incubated with **R18/ ClO_4** films showed significant enhancement in the dye absorption already within 2 h incubation (Figure 4a), suggesting the dye leakage into the serum. By contrast, serum samples containing **R18/F5-TPB** did not show any significant change in the absorption (Figure 4a). This observation was further supported by the remarkable increase in the fluorescence intensity from serum samples incubated with **R18/ ClO_4** films but no such change was observed for the serum samples incubated with **R18/F5-TPB** films for both 2 and 24h (Figure 4b). Much higher stability of **R18/F5-TPB** thin films compared to **R18/ ClO_4** films was confirmed for preparations with two different polymer concentrations (1 mg/mL and 5 mg/mL PMMA) by maintaining the constant dye loading (Figure S5). These studies further confirm that the bulky hydrophobic counterion prevents cationic dyes from leaching into biological medium. Additionally, we have performed the cytotoxicity assay by WST-1 reagent (2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulphophenyl)-2 H-tetrazolium salt) to assess the potential toxicity of the

thin films containing **R18/F5-TPB** and **R18/CIO₄** along with bare PMMA films. Thin films with different dye loadings were prepared and HeLa cells were cultured on it for 24 hours (see SI for details). More than 90 % cell survival was observed for all the tested conditions, similar to the data on the bare glass, indicating no signs of cytotoxicity of our films (Figure 4c). This further confirms the biocompatibility of these materials.

Figure 4. Dye leakage studies in 10% fetal bovine serum in water. (a) Absorption spectra of the serum samples after 2h incubation at 37°C with **R18/CIO₄** and **R18/F5-TPB** (5 wt% each) thin films. (b) Emission response of serum samples after 2h and 24 h incubation at 37°C with **R18/CIO₄** and **R18/F5-TPB** thin films. Error bars are standard deviation of the mean (n = 3). (c) WST-1 cytotoxicity evaluation after 24 h cell seeding on the substrates containing PMMA polymer films and **R18/CIO₄** and **R18/F5-TPB** dye loaded films. Error bars are standard deviation of the mean (n = 6).

Fabrication of dye loaded electrospun fibers and photophysical studies. Next, we transformed these dyes into fluorescent nanofibrous materials by electrospinning technique. Biocompatible PMMA was used as the polymer matrix. Spinning parameters were optimized (see SI) to obtain smooth, continuous fibers. To this end, we prepared the nanofibers either with PMMA polymer alone or with the polymer loaded with **R18/F5-TPB** and **R18/CIO₄**. Representative scanning electron microscope (SEM) images of the electrospun fibers showed randomly oriented nanofibers

with smooth surfaces (Figure 5a-c and Figure S6). Nanofibers prepared with only PMMA polymer showed an average diameter of 364 ± 20 nm, whereas fibers loaded with 5 wt% of **R18/F5-TPB** and **R18/CIO₄** showed a slightly larger diameter of 429 ± 18 nm and 439 ± 20 nm respectively (Figure 5d-f).

Figure 5. SEM micrographs of electrospun (a) PMMA polymer fibers alone. Inset in (a) shows an enlarged portion of the fibers. (b) PMMA fibers loaded with 5 wt% **R18/CIO₄**. (c) PMMA fibers loaded with 5 wt% **R18/F5-TPB**. (d-f) Represents corresponding size distribution of fibers of a-c respectively.

Emission spectra of the electrospun fibers at different loading of **R18/F5-TPB** and **R18/CIO₄** were characterized by a single band centered around 600 nm. Already at 0.5 wt% dye loading, fluorescence intensity was significantly higher for the **R18/F5-TPB** loaded fibers compared **R18/CIO₄** fibers (Figure 6a). At 5 wt% loading of **R18/CIO₄**, the emission band almost vanished indicating severe ACQ, whereas in the case of **R18/F5-TPB** loaded fibers, a strong emission was observed (Figure 6b). Remarkably, the emission intensity of **R18/F5-TPB** fibers was >290 fold

higher than the **R18/C1O₄** fibers with this dye loading. Using home-built imager set up (see SI), we confirmed a bright emission from **R18/F5-TPB** loaded nanofiber mats, almost negligible emission of **R18/C1O₄** fiber mats, especially at higher (5 wt%) loading (Figure 6c). Optical microscopy images of electrospun nanofibers prepared with 5 wt% **R18/F5-TPB** dye loading showed they are indeed fluorescent and the staining is homogenously distributed without apparent dots or irregularities (Figure 6d). Moreover, the absolute quantum yield values for the **R18/F5-TPB** loaded electrospun fibers were significantly higher compared to the **R18/C1O₄** loaded fibers (Figure 6e and Table S3): $57 \pm 2\%$ vs $2.5 \pm 0.5\%$ at 5 wt% dye loading. Interestingly, **R18/F5-TPB** loaded electrospun fiber mats showed strong fluorescence emission even at 30 wt% dye loading (Figure S6), with QY values of $31 \pm 0.5\%$ (Figure 6e and Table S3). Previous reports on dye-doped nanofibers used rhodamine 6G dye, which allowed only 0.3 wt% (6 mM)⁵⁵ and 0.1 wt% (2 mM)⁵⁶ dye doping, probably because of a significant aggregation of this dye starting from 6 mM loading.⁵⁷ Moreover, a recent study using rhodamine, fluorescein and acridine dyes showed significant quenching beyond 0.5 wt% loading in the polymeric nanofibers.⁵⁸ Owing to the bulky counterion approach, we achieved nearly 100-fold stronger loading of a rhodamine derivative, while preserving minimal ACQ and high QY values, which corresponds to brightness improvement by nearly two orders of magnitude.

Figure 6. Characterization of fluorescent electrospun nanofibers. (a) Emission spectra of electrospun nanofibers prepared with 0.5 wt% dye loading. (b) Emission spectra of electrospun

nanofibers prepared with 5 wt% dye loading. (c) Upper panels: represents the white light (i, ii) and fluorescence (iii, iv) images of electrospun fiber mats loaded at 0.5 wt% with **R18/C1O₄** (i,iii) and **R18/F5-TPB** (ii,iv). Bottom panels: represents the white light (v, vi) and fluorescence (vii, viii) images of electrospun nanofiber mats loaded at 5 wt% with **R18/C1O₄** (v,vii) and **R18/F5-TPB** (vi,viii). Scale bar, 1 cm. (d) Fluorescence microscopy image of **R18/F5-TPB** (5 wt%) loaded fibers. Scale bar, 10 μ m. Fluorescence images were captured with a home-built imager setup. (e) Absolute quantum yield values of electrospun nanofibers at different dye loading (Note: Electrospun nanofibers loaded with **R18/C1O₄** at 30 wt% were not prepared). Error bars are standard deviation of the mean (n = 2).

Coatings on steel wires and 3D printed objects. Finally, we explored the capacity of our polymer blend with the rhodamine dye salt to be used as a coating for different surfaces and shapes. According to our previous studies, microns-thick coatings can be obtained by dip-coating of materials with a PMMA solution in acetonitrile.¹²

Figure 7. Application of PMMA blends with rhodamine dye salts as coatings of different objects of medical relevance. (a) Upper panels: represents the white light (i, ii) and fluorescence (iii, iv) images of the steel wires dip-coated in a PMMA solution containing 0.1 wt% of **R18/C1O₄** (i, iii) and **R18/F5-TPB** (ii, iv). Bottom panels: the same as top panels but at 5 wt% dye loading of **R18/C1O₄** (v, vii) and **R18/F5-TPB** (vi, viii). Scale bar is 1 cm. (b) Fluorescence intensity analysis

of the coated steel wires. (c) 3D-printed polymer medical stent mimics coated with **R18/F5-TPB** (5 wt%) doped PMMA. (d) Fluorescence images of coated stents observed under 365 nm UV lamp.

Using this method, we first coated stainless steel wires with PMMA containing different concentration of **R18/CIO₄** and **R18/F5-TPB** dyes (0.1 and 5 wt% each). According to fluorescence images, at low dye loading (0.1 wt%), both **R18/CIO₄** and **R18/F5-TPB** showed detectable but rather heterogeneous fluorescence along the wire (Figure 7a top panels). Interestingly, at higher dye loading (5 wt%), complete fluorescence quenching was observed for the steel wires coated with **R18/CIO₄**/PMMA, whereas a strong emission was observed for those containing **R18/F5-TPB** (Figure 7a bottom panels). This observation was further supported by the fluorescence intensity analysis, wherein a clear superiority of the **R18/F5-TPB** coating was observed (Figure 7b).

Optical coatings on medical devices are valuable tools in the image-guided surgery¹². Here, we asked a question whether we can apply this coating on medical devices having complex geometries. As a proof of concept, we designed a cylindrical object that mimics the cardiovascular medical stents⁵⁹ and 3D-printed it with the help of a resin-based stereolithography 3D-printer (see SI for details). The resulting transparent polymer objects (Figure 7c), which we call here as polymer medical stents, were further dip-coated (2 times) with a solution of PMMA (30 mg/mL) containing **R18/F5-TPB** (5 wt%) (Figure 7c). Due to high dye concentration used, this simple coating protocol was sufficient to obtain strong surface staining, which can be seen as pink color on RGB images. These coated polymer stents showed bright fluorescence when observed under UV-light (Figure 7d). This result show that 3D printed polymeric object of a desired complex shape can be easily made fluorescent by a simple dip-coating with our dye salt/polymer blend.

CONCLUSIONS

In this work, based on a concept of ionic dye insulation with bulky hydrophobic counterions, we have developed ultrabright fluorescent polymeric materials in form of films, nanofibers and coatings. Bulky counterion acts as a spacer between the dye molecules and thus helps to overcome the fundamental problem of ACQ of dyes in polymeric matrices. With the help of a bulky hydrophobic counterion fluorinated tetraphenylborate (**F5-TPB**) and PMMA polymer, we transformed a series of ionic dyes into ultrabright fluorescent materials such as spin-coated thin films, electrospun nanofibers and coatings on fiducials and 3D printed medical stents.

Polymeric thin films containing dyes with small counterions were poorly emissive because of ACQ, whereas the dye films with **F5-TPB** systematically showed strong fluorescence and quantum yields reached as high as 130-fold in case of **R18/F5-TPB**. The counterion concept worked well for both rhodamine and cyanine dyes, which enabled preparation of bright polymeric materials with varied emission color from blue to near-infrared. We also found that the bulky counterion ensures much higher stability of the fluorescent films against dye leakage in the presence of cells and serum-containing medium. Indeed, **R18/CIO₄** films exhibited significant dye leakage in these conditions, in contrast to **R18/F5-TPB** showing excellent stability. Moreover, these fluorescent films were not cytotoxic, allowing normal cell culture.

Fluorescent nanofibers prepared by electrospinning revealed even stronger ACQ effects for **R18/CIO₄** dye, where their emission was completely quenched at 5 wt% loading. On the contrary, **R18/F5-TPB** loaded nanofibers showed strong emission and their fluorescence intensity was >290-fold higher compared to **R18/CIO₄** at 5 wt % loading. **R18/F5-TPB** loaded fibers displayed a strong fluorescence and high QY (31 %) even at a very high dye loading of 30 wt%. Thus, we obtained highly emissive nanofibers at nearly 100-fold higher loading than currently reported in literature,⁵⁵⁻⁵⁷ which implies that we improved two orders of magnitude the brightness of this type

of materials. These studies highlight the dramatic influence of bulky counterions on the emission properties of polymeric nanofibers.

Finally, we explored our fluorescent polymeric materials as coatings. First, we showed that the formulation with the bulky counterion is essential to produce bright coating on the top of model stainless steel fiducials. Then, we have demonstrated a proof-of-concept for coating 3D-printed medical devices having complex geometries, which will open-up new possibilities for several medical applications. For example, such bright coatings on medical stents/implants or catheters can guide the surgeons to precisely place the medical devices inside the body or such coatings can be used as a fluorescent barcode on medical implants, that will help to quickly track/locate the implants inside the body as and when there is an infection or the replacement is necessary.⁶⁰ Another interesting application would be to use the coating as an optical waveguide to deliver the light precisely into the deep tissues for phototherapeutic applications and this ultrabright coating material could be a handy tool in photomedicine and biosensing. We have successfully demonstrated the universality of the counterion approach by taking different ionic dyes and produced bright fluorescent materials of different colors covering the entire visible spectrum. The developed approach opens the route to a great variety of functional fluorescent polymeric materials in form of films, coating and nanofibers.

ASSOCIATED CONTENT

Supporting Information.

The supporting information is available free of charge.

Analytical and photo physical characterization, absorption spectra of the films, absolute quantum yields, SEM and fluorescence images.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENT

This work and AHA are supported by SATT Conectus grants NICE and Biosensing and European Research Council ERC Consolidator grant BrightSens 648528. We acknowledge Jean-Luc Weickert, IGBMC for SEM measurements.

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