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Oxygen Sensing Difluoroboron β -Diketonate Polylactide Materials with Tunable Dynamic Ranges for Wound Imaging

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Abstract

Difluoroboron β -diketonate poly(lactic acid) materials exhibit both fluorescence (F) and oxygen sensitive room-temperature phosphorescence (RTP). Introduction of halide heavy atoms (Br and I) is an effective strategy to control the oxygen sensitivity in these materials. A series of naphthyl-phenyl (nbm) dye derivatives with hydrogen, bromide and iodide substituents were prepared for comparison. As nanoparticles, the hydrogen derivative was hypersensitive to oxygen (0–0.3%), while the bromide analogue was suited for hypoxia detection (0–3% O₂). The iodo derivative, BF₂nbm(I)PLA, showed excellent F to RTP peak separation and an 0–100% oxygen sensitivity range unprecedented for metal-free RTP emitting materials. Due to the dual emission and unconventionally long RTP lifetimes of these O₂ sensing materials, a portable, cost-effective camera was used to quantify oxygen levels via lifetime and red/green/blue (RGB) ratiometry. The hypersensitive H dye was well matched to lifetime detection, simultaneous lifetime and ratiometric imaging was possible for the bromide analogue, whereas the iodide material, with intense RTP emission and a shorter lifetime, was suited for RGB ratiometry. To demonstrate the prospects of this camera/material design combination for bioimaging, iodide boron dye-PLA nanoparticles were applied to a murine wound model to detect oxygen levels. Surprisingly, wound oxygen imaging was achieved without covering (i.e. without isolating from ambient conditions, air). Additionally, wound healing was monitored via wound size reduction and associated oxygen recovery, from hypoxic to normoxic. These single-component materials provide a simple tunable platform for biological oxygen sensing that can be deployed to spatially resolve oxygen in a variety of environments.

Graphical Abstract

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Supplementary contents include the materials and methods, the optical properties in CH₂Cl₂ solution, details of oxygen sensitivity, photostability measurements, oxygen calibrations, instrumentation used for imaging oxygen levels, detailed schematics of daily imaging, examples of mouse wounds, and wound healing quantification. Supplementary videos illustrate the response of **I-NP** to hypoxia within the wound bed. These figures and videos are available free of charge at pubs.acs.org.

Keywords

Difluoroboron β -diketonate complexes; poly(lactic acid); fluorescence; room-temperature phosphorescence; ratiometric oxygen sensing; RGB camera imaging; wound healing

Oxygenation is powerful indicator of health and healing.^{1,2} Deficits and excesses in oxygen levels are associated with medical conditions such as cancer, cardiac ischemia, and chronic wounds, however oxygen levels are rarely used for diagnosis in the clinical setting. For example, although it is well known that the oxygen level within wounds is directly related to healing potential, clinical monitoring of oxygen levels is not commonly practiced largely because the current tools are invasive, expensive, rely on indirect quantification of oxygenation, and do not provide oxygenation levels within the entire wound bed. Instead, clinical assessment of wound healing is largely qualitative and can be highly subjective.² Therefore, new and effective methods for imaging and diagnosis would be valuable.³

A promising new approach for spatiotemporal wound mapping combines luminescent nanomaterials and camera imaging.⁴⁻⁷ Phosphorescence imaging agents, with emission susceptible to oxygen quenching, have been used to quantify oxygen via lifetime or intensity. Typically, nanoprobe are incorporated into a polymer matrix to monitor local wound oxygenation.^{8,9} Wolfbeis and coworkers prepared a multi-functional foil comprised of oxygen sensing Pt porphyrin polystyrene microparticles, pH sensitive FITC polyacrylonitrile microparticles, with a diphenylanthracene standard on a poly(vinylidene chloride) support.⁸ With a conventional digital camera, wound pH and O_2 levels were monitored in a clinical setting.⁹ Evans and coworkers have developed Pt and Pd porphyrin “clickaphors” with bright and tunable red phosphorescence conjugated to biocompatible poly(l-lysine) dendrimer scaffolds.^{10,11} These materials were applied as rapid drying bandages¹² or as probes for NIR oxygen mapping. With a high-speed color camera, lifetime or coumarin referenced red/green/blue (RGB) imaging, can be used to monitor wound oxygen levels. These approaches rely on barriers (e.g. Saran,⁹ Tegaderm¹²) to block or slow ambient O_2 diffusion into the sensor and tissue, and a delay time before imaging.

Boron dyes are another class of responsive luminescent materials,¹³⁻¹⁷ that includes mechanofluorochromic powders,¹⁸⁻²¹ photo-switchable devices,²²⁻²⁴ ion sensors,^{25,26} and dual emissive oxygen reporters.²⁷⁻²⁹ Dual emissive difluoroboron β -diketonate poly(lactic acid) (BF_2bdkPLA) (PLA) materials are alternative main group oxygen sensing systems that combine oxygen sensitive phosphorescence, a fluorescence standard, and a material support all in one.^{27,30} Boron dye-PLA biomaterials may be fabricated in myriad ways, as nanoparticles,^{31,32} nanofibers,³³ and films.²⁷ The value of these bright, photostable, nontoxic materials for biomedicine has been demonstrated. When applied to HeLa and CHO cells, boron nanoparticles (BNPs) were taken up and accumulated in perinuclear regions, as

verified by fluorescence and 2-photon confocal microscopies.³² Intratumoral administration of boron dye-PLA nanoparticles in conjunction with hyperspectral imaging enabled tumor hypoxia imaging in a murine dorsal window chamber model.²⁷ When injected intravenously, PLA nanoparticles illuminated vasculature, whereas, PEG-PLA systems accumulate in tumor tissue.^{34,35} Electrospun nanofibers of boron biomaterials have found application in tissue engineering, specifically in tracking ischemia in cell transplant models.³³

Single component BF₂bdkPLA materials, that combine phosphor, standard and matrix in one, pose unique challenges for sensing optimization.^{36–39} First, it was necessary to increase the phosphorescence intensity by the heavy atom effect via introduction of bromide or iodide.^{27,36–38} Secondly, for ratiometric sensing, ideally the fluorescence and phosphorescence peaks should be distinct, and the F/P intensity ratio must be optimized.^{36,37} This was achieved through a combination of ligand modification and molecular weight (i.e. dye loading) effects. Third, for ratiometric camera imaging, it is best if dye singlet and triplet emissions are well aligned with RGB channels of the camera.³⁶ Emission tuning is possible through dye loading and ligand modification. Fourthly, photostability is an important consideration.³⁸ This was addressed via careful consideration of the propensity of dyes to aggregate (i.e. solubility in different media) and dye loading. For selected dyes, lower dye loadings (i.e. higher MW BF₂bdkPLA) resulted in greater photostability.

In this study, we draw upon all of these considerations to advance materials design, imaging, and application. Dye sets shown in Figure 1 were evaluated based on luminescence energies (color) and spectral overlap of F and P, and photostability, and the naphthyl-phenyl dye fit the key requirements. Based on this selection, we describe here a simple method to tune oxygen sensitivity in naphthyl-phenyl BF₂bdkPLA materials^{36,40} by varying a single dye arene substituent (i.e. H, Br or I). These materials, in conjunction with a portable cost effective camera, are well tuned to anoxia, hypoxia (<3% O₂), normoxia (0–21% O₂) and even full range (0–100% O₂) imaging in real time via lifetime (τ), ratiometric (RGB), or dual lifetime/ratiometric modes. Finally, we show how the nanoprobe/camera imaging system enabled non-invasive mapping of oxygen gradients in wound beds.

RESULTS and DISCUSSION

Materials Synthesis

Three materials were prepared for comparison; the unsubstituted BF₂nbmPLA (**HP**),⁴⁰ and two heavy atom analogues, BF₂nbm(Br)PLA (**BrP**)³⁶ and newly synthesized BF₂nbm(I)PLA (**IP**). Polymers were generated from boron dye initiators (BF₂nbm(X)OH, where X = H, Br, and I) by solvent-free ring-opening polymerization (ROP) of DL-lactide (200 equivalents) at 130 °C with stannous octoate (Sn(oct)₂) as the catalyst.³⁰ Reactions were stopped once the mixture became slightly viscous (3–5 hours; ~70% conversion) to yield polymers with comparable molecular weights (M_n = 16 – 20 kDa) and narrow polydispersities (M_w/M_n = 1.12 – 1.17). Polymers were prepared with similar MWs to ensure that differences in optical properties stem from the halide substituent and not dye concentration effects.⁴¹ Furthermore, in a previous report we discovered higher MW polymers (>16 kDa) have larger fluorescence to room temperature phosphorescence (RTP) wavelength gaps,

important for ratiometric oxygen quantification techniques, and they are more photostable (Figures S1–S5).^{37,38}

Optical Properties

The polymer luminescence was analyzed in CH₂Cl₂ solution (Table S1) and as thin films in glass vials (Table 1 and Figure 2). Dye initiators and dye-PLA conjugates had very similar properties in dilute CH₂Cl₂ solutions, indicating that the integrity of the dye is maintained during the polymerization process (i.e. negligible boron decomplexation).^{41,42}

For solid state films, all polymers have indistinguishable blue fluorescence at ~460 nm and lifetimes, $\tau_F < 2$ ns. Phosphorescence red-shifted and the RTP intensity increased relative to the fluorescence, while lifetimes decreased more dramatically, as is expected for the **HP** to **BrP** and **IP** series given the heavy atom effect.⁴³ A weak phosphorescence shoulder and long lifetime ($\tau_P = 453$ ms) were observed for **HP**. The bromide polymer, **BrP**, showed two distinguishable peaks for fluorescence and phosphorescence, and a decreased lifetime (14.5 ms). Whereas, phosphorescence dominated for the iodide polymer (**IP**), and the lifetime further shortened (1.9 ms). These results show that halide substitution primarily influenced the RTP, while features of the fluorescence (e.g. color) are well maintained. Because changes in color are negligible, detection methods can be broadly applied without changing settings (e.g. filters), and the three materials can be easily interchanged to screen and identify the optimal material for a given sensing application.

Oxygen Sensing

The halide substituted dye-PLA conjugates are distinguished by their RTP intensities and unquenched lifetimes, which relate to two ways to quantify oxygen quenching (I_0/I or τ_0/τ).^{7,44} As shown in equations 1 and 2, the unquenched RTP lifetime (τ_0) is directly correlated to the Stern-Volmer quenching constant (K_{SV}).

$$\frac{I_0}{I} = \frac{\tau_0}{\tau} = 1 + K_{SV}[Q] \quad (1)$$

$$K_{SV} = k_q \tau_0 \quad (2)$$

As a result, materials with long RTP lifetimes are more sensitive to oxygen (Q) quenching (large K_{SV}), and will operate within a narrower O₂ sensing range. Furthermore, materials with longer lifetimes can be detected with less costly instrumentation (e.g. lower frame rate).

Here, we show that halide activated RTP influences the oxygen sensitivity and mode of detection via lifetime (τ/τ_0) or intensity (I/I_0) techniques. The hydrogen substituted dye-polymer (**HP**), with weak RTP and a long lifetime (~400 ms) serves as an ultrasensitive lifetime oxygen sensor, whereas the iodide derivative (**IP**), with a short lifetime (~2 ms) but intense RTP, functions as a full range ratiometric sensor. The bromide derivative showed

balanced F and RTP intensities, and a relatively long RTP lifetime (~14 ms). Therefore, with **BrP**, oxygen can be sensed via both detection modes.

To elucidate the oxygen sensing ranges and generate materials suitable for wound application, the polymers were fabricated as nanoparticles³² (**X-NPs**, where X = H, Br, and I) and subjected to oxygen calibration²⁷ (Table 2, Figures S6–11). The nanoparticles showed similar optical properties as the dye-polymer films and, as expected given varying phosphorescence lifetimes, each derivative showed a different dynamic range (Table S2). For **H-NP**, phosphorescence lifetime vs [O₂] showed an oxygen sensing range of ~0–0.3% (Figure S6). Also, because RTP is very weak compared to F, only RTP lifetime is practical for detection, not ratiometry.

For **Br-NP**, the phosphorescence intensity is strong enough for ratiometric imaging. Ratiometric referenced intensity calibration (i.e. comparing intensities at λ_F and λ_{RTP} maxima; I_F/I_P) revealed a linear correlation from 0–1% O₂ with reliable detection to 3% (Figure S7). Although it is possible to sense oxygen to 21%, changes in the F/P ratio are minimal from 3–21%, making it difficult to distinguish the O₂ level in these ranges.

This may be attributed to the tail of the broad fluorescence emission spanning into the red region of the spectrum (~430–600 nm). This causes a “jack-knife” shape in the ratiometric calibration curve (Figure S9).^{7,45} At higher oxygen concentrations, oxygen insensitive fluorescence interferes with the oxygen sensitive RTP observed at the same wavelength. Lifetime techniques can circumvent this problem by eliminating fluorescence effects.⁴⁶ Because the fluorescence emission is on the order of nanoseconds, and RTP, milliseconds, an appropriately timed gate can be applied to eliminate the singlet emission. When a lifetime calibration is performed on **Br-NP**, the Stern-Volmer quenching constant (τ_P vs %O₂) had a linear correlation from 0–21% O₂ (Figure S7). Thus, both ratiometry and lifetime methods are suitable for **Br-NPs** within appropriate [O₂] ranges.

The iodide derivative **I-NP**, in contrast, showed oxygen-sensing capability unprecedented for boron bdk materials. From 0–100% O₂, the F/P ratio is linearly correlated ($R^2 = 0.993$; Figures 2, S10–S11). Furthermore, because the RTP is much stronger than F and is never fully quenched even in pure O₂ (Figure 3; red line spectrum), interference from fluorescence is negligible. Ratiometry works well for this material, while the unquenched lifetime (1.7 ms) is on the edge of the detection limits with our current camera instrumentation (2.0 ms). While **H-NP** and **Br-NP** are best matched to anoxia and hypoxia (e.g. in tumors) respectively (Table S2), the reduction in oxygen sensitivity for **I-NP** dramatically increases potential uses in normoxic tissue (e.g. veins vs arteries: 10 vs 15% O₂) while still illuminating low O₂ and deoxygenated regions.^{47,48} The increased RTP intensity and decreased oxygen sensitivity of **I-NP** expands bio-imaging capabilities of BF₂bdkPLA materials.

Camera Imaging

With these properties and diverse oxygen sensing applications in mind, we developed a portable cost-effective camera imaging system (Figures S12–S13).⁴⁹ Previously, lifetime imaging with long-lived BF₂nbmPLA (**HP**) films was demonstrated. Here, we extend

camera lifetime imaging to **H-NP** nanoparticle suspensions, as shown in Figure 4. As expected, the long-lived RTP of **H-NP** was quenched very quickly (i.e. in ~6 s) when 1% O₂ is bubbled into a nitrogen purged sample. Because the RTP is ultrasensitive to oxygen, this type of probe could be deployed for “turn-on” sensing to detect the most oxygen-starved regions (e.g. anoxic, ischemic, necrotic tissue).^{50–52}

The camera, in conjunction with **Br-NPs**, on the other hand, enables simultaneous, multimodal lifetime and ratiometric detection (Figure 5, S7–S9). Because the phosphorescence is strong for **Br-NPs**, the red/green/blue (RGB) color channels of the camera can be used to independently monitor changes in F and P for referenced (F/P) oxygen sensing. RGB camera calibration of **Br-NP** revealed that using the blue channel for the reference (F) and the red channel as the sensor (P) generated the best calibration curve for this material. The green channel was excluded from the measurements to provide the most spectrally isolated features of the material. It is common for the camera RGB calibration to have reduced precision compared to spectral referenced intensity (RI) as shown in Figure S11. However, these are goals for further optimization, namely, either to push the material F and P signals farther apart or into better alignment with camera channels, or to better tailor the instrumentation to the material via camera selection, filter selection and customization.

As illustrated in Figure 5, **Br-NPs** were purged with N₂ (i.e. 0% O₂) then exposed to a stream of air under steady UV illumination, with a pulse of excitation every second.⁴⁷ Regions between the excitation pulse and decay were used to quantify O₂ via RGB, while regions of decay monitored the O₂ via phosphorescence lifetime. Oxygen levels acquired by the two methods are in good agreement with each other. At higher oxygen concentrations, the lifetime method provided higher resolution images than the RGB images. This may be due to interfering fluorescence intensity, as discussed above for F/P calibration and previously, for RGB curves.⁴⁵ This limitation is addressed with **I-NPs**, with very strong RTP versus F. Like hydrogen and bromide analogues, iodo nanoparticles show different total emission colors when purged with also gases ranging from 0–100% oxygen (Figure 3, left). And we will show below for wound imaging, **I-NPs** are ideal for ratiometric camera imaging.

Wound Imaging

Tissue healing requires energy to facilitate increased extracellular matrix synthesis and cell proliferation.² The production of adenosine triphosphate (ATP) and other activated carrier molecules requires oxygen, so tissues are dependent on proper oxygenation to provide the energy to enable healing. Wounds that do not heal properly, given incomplete repair, are known as chronic wounds and they can be hypoxic. In fact, tissue oxygenation (e.g. hyperbaric chambers) is a chronic wound treatment.⁵⁰

To demonstrate the utility of nanoparticle/camera imaging for biological oxygen sensing, nanoparticles were applied to a murine full thickness skin wound (Figures S14–26). Upon **I-NP** application, the wound bed displayed bright yellow emission (strong P + weak F) even without covering the wound (Figure 6). When **I-NP** treated wounds were later covered, the

phosphorescence signal persisted and intensified over the course of image acquisition (Supplemental Videos 1 and 2). Finer wound bed detail is also evident in certain UV and processed RGB oxygenation images. For example, some **I-NP** ratiometric images reveal microvasculature (Figures 6F, S25–S26).

Because **Br-NPs** possess a narrower sensing range, they too, were tested, in attempts to detect details and microenvironmental changes at higher resolution. For example, on day 0, the periphery of the **I-NP** treated wound bed appeared to be highly deprived of oxygen (Figure 6F). However, this effect was less evident when **Br-NPs** were applied to the wound, as ratio changes were barely above the baseline fluorescence. When attempting lifetime mode imaging with **Br-NP**, no delayed emission was observed, or it was too short-lived for detection (<2 ms). This could be a result of the increased O₂ sensitivity of the material, or because wounds can be situational and diverse, and the same features did not arise between trials. When isolated from air (covered) however, RTP did activate. However, the response of **Br-NPs** was less dynamic than **I-NP**, indicating that **Br-NPs** are not as well suited for wound imaging as **I-NP**, which gave consistent measurements of the oxygen levels day to day for covered and uncovered measurements. Biological mechanisms of nanoparticle cell uptake, differences in oxygen diffusion, and tissue accumulation may all influence hypoxia responses, and are under investigation.

Wound Healing

The nanoparticle camera imaging system was also used to correlate wound oxygenation with recovery. In a prior study with early generation BF₂bdkPLA nanoparticles²⁷ (BF₂dbm(I)PLA; Figure S27), nanoparticle solutions were applied to murine skin wounds and monitored for 9 days with a digital single-lens reflex (DSLR) camera equipped with a light-emitting diode camera attachment (LED-ring). The bright fluorescence from a single application on day 1 was still observable in the wound bed for 9 days. However, RTP within the wound bed was weak, and F/P ratios generated from the camera were unclear and difficult to interpret. The oxygen sensing material and imaging techniques required further optimization for wound imaging, and thus, served as the motivation for this study.

To test for stability and retention of nanoparticles with the current improved camera imaging system, we applied a single treatment of **H-NPs** (10 μ L of 1 mg/mL NP solution), the brightest of the set, to the 3 mm wound bed to monitor the fluorescence over time. Fluorescence from this sample could be observed for ~2 days after initial application to these small wounds (3 mm). But for the dimmer iodo NPs, luminescence from previous applications diminished. Thus, **I-NPs** were applied fresh daily. Residual fluorescence from the previous day was removed from the analysis with a background subtraction before fresh NPs were applied. Reasons for differences in emission signal persistence from the preliminary study could stem from differences in fluorescence brightness, wound size (10 mm vs 3 mm diameter), and NP dose (100 vs 10 μ L of 1 mg/mL solution). The more rapid attenuation in luminescence for these **X-NP** samples could also be due to lower initial dye loadings (i.e. dyePLA MWs) affecting BF₂ hydrolysis⁴¹ or PLA degradation. And because phosphorescence is typically only present for BF₂bdk dyes in ordered media (e.g. below the

glass transition temperature, T_g of PLA), it is also possible that hydration, swelling or other factors affecting the T_g may be playing a role.⁵³

Serial application of **I-NPs** and imaging of the wounds were performed each consecutive day for five days, and images were analyzed as described above. As expected, individual wound areas decreased over time (Figure 6) and oxygenation levels within the wound bed increased up to Day 4 (Figure 7). In the first days, the periphery of the wound bed showed distinguishable hypoxia, possibly from high cell activity in tissue granulation for the healing processes (Figure S26).⁵⁴ Wound contracture and re-epithelialization began to occur at Day 4, which is consistent with the well-documented wound-healing cascade. Furthermore, application of the **I-NPs** did not significantly slow or delay the wound healing process, proving this to be a minimally invasive tool for monitoring wound oxygenation levels (Figure S28).

CONCLUSION

To conclude, we describe a simple method to generate materials with varying and useful oxygen sensitivity. The new iodide substituted BF_2nbmPLA material is capable of distinguishing oxygen levels from 0–100% O_2 . The distinct fluorescence and strong phosphorescence peaks facilitated red/green/blue camera imaging. Nanoparticle suspensions applied to wound beds tracked spatially resolved oxygen gradients and daily monitoring of wound healing with repeated application. Table 3 summarizes camera based methods for luminescence imaging of oxygen in skin and wounds. To our knowledge, this is the first technique that does not rely on covering the wound bed to differentiate features.

As shown in Table 3, an oxygen impermeable SaranTM support layer, or TegadermTM diffusion barrier, is normally used to slow or block the wound from ambient oxygen in air. Covering can cause healthy tissue to become hypoxic overtime through consumption of available oxygen, while damaged tissue remains at the same oxygen level, or slightly lower based on the degree of damage. Other sensing systems typically reported percent O_2 consumed, rather than the percent oxygen present and available for consumption. Therefore, where past methods used oxygen consumption to distinguish normal and damaged tissue, this cover-free method enabled imaging of the native oxygen environment in the wound bed and surrounding skin, with minimal perturbation and relatively rapid readout. Both approaches are useful in different ways. For example, stable sensor foils reported by Wolfbeis et al. that track oxygen consumption as the measurement of health can be cleaned, sterilized and reused repeatedly for 72 hours of excitation without re-calibration.⁸ A drawback to this photostable foil method is that repeated application and removal of the foil to the wound to measure oxygen can perturb the wound. Furthermore, leaving the oxygen impermeable sensor foil for prolonged periods would starve the wound of needed oxygen for proper wound healing.

The nanoparticle-camera method that we describe here for monitoring wound oxygenation addresses some of these issues. The PLA nanoparticle suspensions are applied, wounds are imaged, and then particles degrade away with minimal wound perturbation using this rapidly equilibrating diagnostic method. While a synthetic process is required to generate the O_2

sensitive dye-polymers, these main group boron dyes are less expensive and much easier to make than heavy atom Pt and Pd porphyrin dyes. Readily processed boron dye/polymer blends may even be suitable for certain applications and could one day be commercialized. Other benefits of these “all in one” materials are facile nanoparticle fabrication, and very little nanoparticle suspension is required for imaging. Though “cocktail” oxygen sensing foils can sometimes utilize commercially available dyes and polymers, they have involved fabrication to avoid problems with energy transfer and dye leaching. The covalent linkage of a dual emissive dye (BF₂bdk) to a biocompatible matrix (PLA) eliminates problems with dye leaching and energy transfer seen in the conventional two dye, sensor/standard approach.

Evans and coworkers also have an excellent solution for wound healing diagnosis.^{10,12} A cocktail mixture of fluorophore/phosphor is applied as a rapid-drying bandage, followed by covering with oxygen permeable Tegaderm. This barrier protects the wound and slows the oxygen diffusion, but doesn't completely block it, allowing for both minimal perturbation without starving the wound of oxygen. Furthermore, newly designed dyes are excitable by ambient light, removing the need for a supplementary excitation (i.e. UV lamp). Other promising new materials include a thermogelating spray that binds to skin.⁵⁵

Given that boron dye-PLA nanoparticles, films and nanofibers are likely compatible with many of these state-of-the-art techniques (i.e. cocktail foils, paint on bandages and spray on thermogelation and others in Table 3) BF₂bdkPLA materials provide a metal-free alternative with tunable oxygen sensitivity. As demonstrated by these examples, phosphorescence wound healing indicators are increasingly utilized in research and clinical settings to track healing, drug efficacy, and general health.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Comparison of single component BF₂bdk(X)PLA dual-emissive, oxygen sensitive dye-polymers.

Figure 2.

Optical properties of polymer films. Photographs of dye-polymers under air, N_2 , and under N_2 with the lamp turned off (delay) and corresponding total emission spectra in air and N_2 ($\lambda_{\text{ex}} = 385 \text{ nm}$).

Figure 3.

I-NP Oxygen Calibration. Images of an aqueous nanoparticle suspension under oxygen, air and nitrogen (left). Total emission spectra at 0–100% O_2 ($\lambda_{ex} = 385$ nm; black line = 0% O_2) (right).

Figure 4.

Lifetime Imaging with **H-NPs**. (A) Photographs of **H-NP** nanoparticles in air (F) (UV lamp on) and under N₂ (P) (UV lamp turned off). (B) Selected video frames of oxygen quenching from 0% (N₂; start) to 1% oxygen, as a steady stream of 1% O₂ (Praxair) is blown into the nanoparticle suspension.

Figure 5. Dual-Mode **Br-NP** Oxygen Sensing. (A) Images of **Br-NP** in N₂ and air (B) Selected video frames of oxygen quenching from 0% (N₂) to 21% oxygen (Praxair) as a steady stream of 21% O₂ (Praxair) is blown into the nanoparticle suspension.

Figure 6.

In Vivo Camera Imaging with I-NPs. (A) Illustration of mouse dorsal wounds (gray circles = control wounds, blue circles = NP treated wounds; details in Figures S20 and S21). (B) Bright field image. (C) Raw image under UV excitation (D) Blue channel (fluorescence). (E) Red channel (phosphorescence). (F) RGB image (blue/red channel).

Figure 7. Wound oxygenation and healing time course monitored by **I-NPs**. Top row: Brightfield images of the uncovered wound. Bottom row: Ratiometric images of the uncovered wound taken 5 min after nanoparticle application.

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Table 1

Optical Properties of Polymer Films

Sample	λ_F^a (nm)	τ_F^b (ns)	λ_P^c (nm)	τ_P^d (ms)
BF ₂ nbmPLA	HP	459	545	453
BF ₂ nbm(Br)PLA	BrP	462	561	14.5
BF ₂ nbm(I)PLA	IP	461	569	1.90

^aSteady-state fluorescence emission maximum (λ_{ex} = 385 nm)
^bFluorescence lifetime (λ_{Ex} = 369 nm LED)
^cDelayed emission spectra maxima under N₂ (λ_{ex} = 385 nm)
^dPre-exponential weighted RTP lifetime.

Table 2

Optical Properties of Nanoparticles

NP	DLS ^a		Fluorescence			Phosphorescence		
	R_H (nm)	PD	λ_f (nm)	τ_f^b (ns)	λ_p^d (nm)	τ_p^e (ms)	$\tau_0/\tau_{10\%}^f$	
H	38.5	0.09	459	1.76	543	127	32.0	
Br	37.0	0.11	462	0.86	559	12.3	3.3	
I	41.4	0.18	461	0.47	565	1.7	0.8	

^aNP hydrodynamic radius (R_H) and polydispersity (PD) determined by dynamic light scattering (DLS).

^bSteady-state fluorescence spectra emission maximum under air. Excitation source: monochromator set to 385 nm with xenon lamp.

^cFluorescence lifetime excited with a 369 nm light-emitting diode (LED) monitored at the emission maximum.

^dDe-layed emission spectra maxima under N₂. Excitation source: monochromator set to 385 nm with xenon lamp.

^ePre-exponential weighted RTP lifetime.

^fSensitivity measurement of NPs.⁷

Table 3
An Overview of Camera Based Methods for Luminescence Imaging of Oxygen in Skin and Wounds.

Sensor ^a	Reference ^b	Matrix ^c	λ_F/λ_P ^d (nm)	τ_F ^e (μ s)	Range (%)	Application ^f	Cover ^g	Imaging Methods	Ref
Oxyphor R2	Coumarin 500	New Skin [®] liquid bandage ^h	510/ 690	700	0–100	Rapid drying paint	Tegaderm [™]	Lifetime/ RGB	12
Clickaphor Red G2	Coumarin 500	New Skin [®] liquid bandage ^h	510/ 660	N.D.	0–100	Rapid drying paint	Tegaderm [™]	RGB	10
PTFPP in PS ⁱ microparticles	DPA in PAN ^j microparticles	Poloxamer 407 ^k	445/ 650	N.D.	0–100	Sprayable; Thermogelation occurs on skin	N.A.	RGB	55
PTFPP in PS ⁱ microparticles	DPA in PAN ^j microparticles	Polyurethane hydrogel	445/ 650	N.D.	0–100	Sensor foil	PVdC ^l support/ barrier	RGB	8
Pd-PTPBP in PSAN ^m microparticles	Ru(dpp) ₃ in PAN ^j microparticle	Polyurethane hydrogel	600/ 797	300	0–100	Sensor foil	PVdC ^l support/ barrier	Dual Life- time/ RGB referencing	9
PTFPP	N.A.	Polyurethane hydrogel	N.A./ 650	350	0–100	Sensor foil	Polyester support/ barrier	Lifetime	56
PTFPP	N-(5-carboxypentyl)-4-piperidino-1,8-naphthalimide	Polyurethane hydrogel	510/ 650		0–100	Sensor foil	Polyester support/ barrier	RGB	56
Pt-OEP	N.A.	PS	N.A./ 645	70	0–100	Sensor film	Polyester support/ barrier	Lifetime	57
Pd-PTPBP in PSAN ^m microparticles	N.A.	Polyurethane hydrogel	N.A./ 797	300	0–100	Sensor foil	PVdC ^l support/ barrier	Lifetime	58
BF ₂ dbm(1)PLA	N.A.	N.A.	445/ 526	4370	0–21	Nanofibers	Glass coverslip	Microscope + Custom Filters	33
BF ₂ nbm(1)PLA	N.A.	N.A.	461/ 569	1900	0–100	Nanoparticles; dropwise	N.A.	RGB	This work

^aOxygen sensitive dye (phosphor); Note: Sometimes phosphors are embedded into a separate matrix before mixing with the reference fluorophores to prevent energy transfer processes or to control oxygen diffusion for multiplexing applications where other indicators are added.

^bOxygen insensitive dye (fluorophore) used as the reference signal; for ratiometric methods; similarly, sometimes the references are embedded into a separate microparticle matrix before being “cocktailed” with the sensor dye.

^cPolymer matrix used for film, foil, or gel on the surface.

^dFluorescence maximum/phosphorescence maximum.

^ePhosphorescence lifetime (linked to oxygen sensitivity).

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f Description of sensor material applied to the region of interest oxygen.

g Oxygen impermeable support (or diffusion barrier in the case of TegadermTM usage).

h New Skin[®] liquid bandage is comprised of nitrocellulose plus some rapid drying organic solvents.

i PS = polystyrene.

j PAN = polyacrylonitrile.

k Polaxamer 407 is a tri-block copolymer of poly(ethylene glycol)—poly(propylene glycol)—poly(ethylene glycol).

l PVdC = polyvinylidenechloride (SaranTM)

m PSAN = poly (styrene-co-acrylonitrile).