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Bioinspired Artificial Melanosomes As Colorimetric Indicators of Oxygen Exposure

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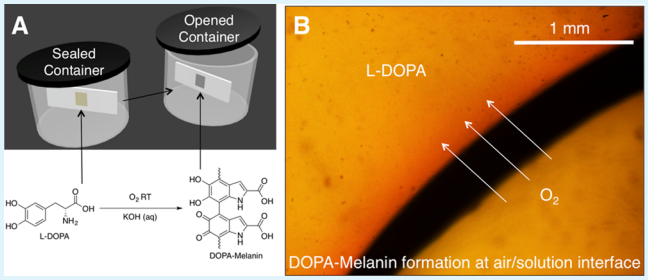
Supporting Information

ABSTRACT: Many industries require irreversibly responsive materials for use as sensors or detectors of environmental exposure. We describe the synthesis and fabrication of a nontoxic surface coating that reports oxygen exposure of the substrate material through irreversible formation of colored spots. The coating consists of a selectively permeable rubber film that contains the colorless organic precursors to darkly pigmented synthetic melanin. Melanin synthesis within the film is triggered by exposure to molecular oxygen. The selectively permeable rubber film regulates the rate of oxygen diffusion, enabling independent control of the sensitivity and response time of the artificial melanosome, while preventing leaching of melanin or its precursors.



KEYWORDS: *L-DOPA, oxygen exposure, tampering, stimuli responsive materials, melanin, melanosome*

There is increasing interest in materials whose chemical and physical properties change when exposed to specific environmental stimuli. These materials are finding use in sensors,[1−5] controlled-release devices,[6,7] self-healing technology,[8] photoresponsive materials,[3,9,10] and homeostatic materials,[11−13] to name a few applications. For many of these applications the response must be reversible and reproducible against many response cycles.[9,13−15] As a result, there has been a large body of recent research aimed at the synthesis of materials with reversible responses to a large number of stimuli.[1,4,16,17]

There are, however, a few applications where irreversible responses are preferred. One such example is that of tamper-indicating materials, whose incorporation into consumer or military products has become an increasingly popular strategy to counteract the widespread threats of forgery and tampering, or to detect the exposure to the environment that may have caused product degradation.

An important indicator of compromised integrity for many consumer products is prior opening of a sealed container (e.g., opening the packaging, uncapping a bottle, exposing a sensitive pharmaceutical, etc.). Materials designed to indicate product opening can do so through response to either mechanical forces associated with the act of opening a container or to exposure to elements of the outside environment (e.g., light, oxygen, humidity). Chemicals that are acutely sensitive to oxygen or humidity have promise for very high tamper-sensitivity, but their generally extreme reactivity in ambient conditions is frequently accompanied by significant toxicity concerns, limiting their practicality in consumer applications (e.g., food, liquor, pharmaceuticals).[1,9] Toxicity is also a challenge faced when developing stimuli-responsive materials for medical applications. Partly due to this challenge, there has recently been a trend to explore biologically inspired synthetic approaches, focusing on actuation mechanisms and formulations that mimic the wide range of responsive systems in our bodies, which can perform sophisticated functions without collateral damage.[1,9,14,15]

Here, we apply a bioinspired approach to design a tamper-indicating oxygen sensor using only nontoxic materials. We draw inspiration from the chemical processes that take place in our skin, which provide a highly visible account of skin's exposure history through the environmentally triggered production of melanin in melanosomes.[18] Because of their interesting photoprotective, optical, electronic, adhesive, and biochemical properties, a large number of synthetic routes for producing melanin-like polymers have been developed.[19−29] While the synthesis of melanin in our bodies occurs through more complex biochemical pathways, melanins can be synthesized in the lab from amino-acid derivatives through much simpler reactions,[19−21,25] and occurs spontaneously when alkaline solutions of L-DOPA are oxidized upon exposure to oxygen.[19−22]

These "artificial melanosomes" consist of deoxygenated pockets of basic L-DOPA solution contained within oxygen-permeable polymer films (see the Supporting Information for

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fabrication methods). Although the underlying chemical processes in artificial melanosomes are much simpler than the complex enzymatic synthesis of melanin in the actual organelle, it performs a very analogous function by producing melanin and the associated color changes in response to an external environmental stimulus. Polymer encapsulation allows the precursors to be exposed to oxygen, but prevents their leaching into the outside environment. We chose water as a medium not only because both L-DOPA and synthetic melanin are sparingly soluble in organic solvents, but also because we sought to design a completely nontoxic response system that can be patently incorporated into consumer goods without risk of chemical leaching. When the film is exposed to oxygen, the gas diffuses through the polymer layer and is reduced in water, leading to the synthesis of the melanin pigment via spontaneous oxidative polymerization of L-DOPA under weakly alkaline (pH 8), aqueous conditions (Figure 1A), and

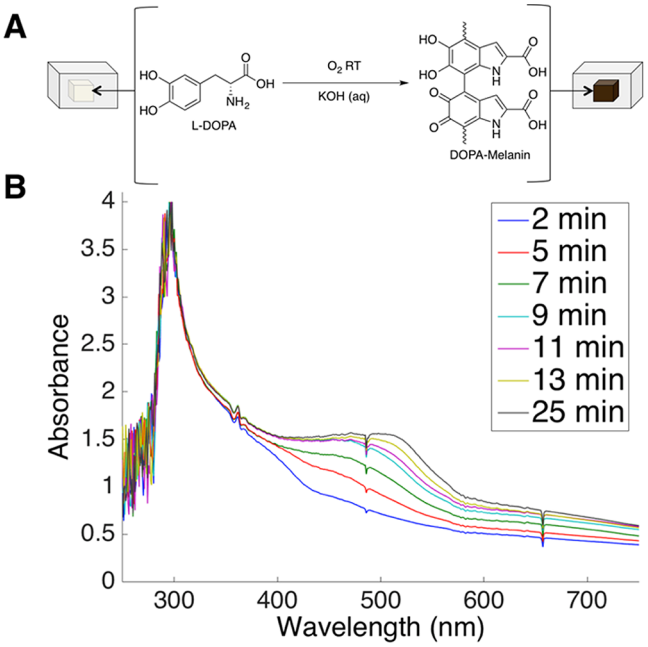

Figure 1. (A) Schematic showing the oxygen-triggered synthesis of a portion of the DOPA-melanin polymer. (B) Time-resolved UV−vis spectra of the L-DOPA polymerization process.

yielding the appearance of a dark brown color. The oxidative polymerization of L-DOPA has a characteristic absorption profile, which permits quantitative determination of the oxygen diffusion rate through the rubber casing and the response time of melanosomes of different thicknesses. The dihydroquinone moiety on L-DOPA absorbs strongly at 297 nm; this peak is retained in the DOPA-melanin spectrum since there are reduced hydroquinones within the polymer structure. Oxidation of L-DOPA to generate the quinone produces an increase in absorbance intensity in the visible range (400−650 nm) and a small peak around 340 nm (Figure 1B).[27,30]

A demonstration of how artificial melanosomes may function as tamper-indicating materials is shown in Figure 2. Glass slides containing 250 μL depressions with a circular (d ∼ 1 cm) exposed surface filled with a 0.3 M L-DOPA solution, capped with 2 mm thick layers of polydimethylsiloxane (PDMS, Figure 2A), were placed in two sealed glass jars (250 mL total volume) under nitrogen (see schematic in Figure 2B). After both jars

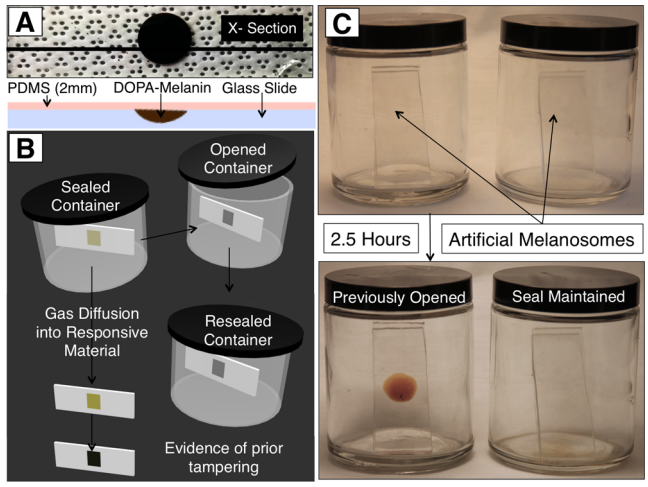

Figure 2. (A) Image (top) and schematic cross-section of the artificial melanosome (bottom). (B) Schematic and (C) images of the melanosome functioning as an indicator in a jar that has been previously opened and purged with argon gas. The thickness of the polymer film controls the delay of the response.

were removed from the inert atmosphere, one jar was opened for 30 s, purged with argon gas for 1 min, and resealed (Figure 2C). After a delay of 2.5 h the artificial melanosome in the jar that was previously opened changed from a pale, nearly colorless solution to a darker brown solution (Figure 2C) and after 5 h the sample contains a completely opaque, black pigment (Figure 2A, Figure S1). No color change occurred in the unopened jar. There is a time delay of approximately 30 min before color change is noticeable to the naked eye. Because our system is highly sensitive to oxygen exposure, residual oxygen that has already dissolved into the encapsulating polymer and that cannot be removed by purging will initiate melanin formation.

The absolute sensitivity of the artificial melanosome to oxygen is tied stoichiometrically to the amount of L-DOPA it contains and is therefore controlled by the melanosome size, given fixed L-DOPA concentration. The L-DOPA concentration of 0.3 M used in Figure 2 provides an oxygen uptake capacity of 6 μmol per 1 mm^3 of melanosome volume, the equivalent of 67 mm^3 of air. The time delay of the response is tuned independently from this absolute sensitivity by adjusting the thickness of the encasing polymer layer, as illustrated in Figure 3. Figure 3A shows the time-evolution of the color change. Figure 3B shows the time response of the absorbance at 550 nm as a function of the membrane thickness. Figure 3C shows the response time (time to 50% reduction in transmission) as a function of the membrane thickness. This time delay is tuned over a broad spectrum ranging from a few seconds (as the thickness approaches 0) to several hours (for thicknesses of ∼2−5 mm). The linearity of the absorbance curves (Figure 3B) and their slopes' dependence on membrane thickness suggests that reaction speed is determined by the rate of oxygen diffusion through the membrane and not by the rate of conversion; this rate is constant for a given thickness. It is important to note that the color changed associated with L-DOPA polymerization is nearly instantaneous in the presence of oxygen and is only limited by the rate of oxygen diffusion through the encapsulating polymer membrane. Figure 3D shows how this diffusion rate varies with membrane thickness.

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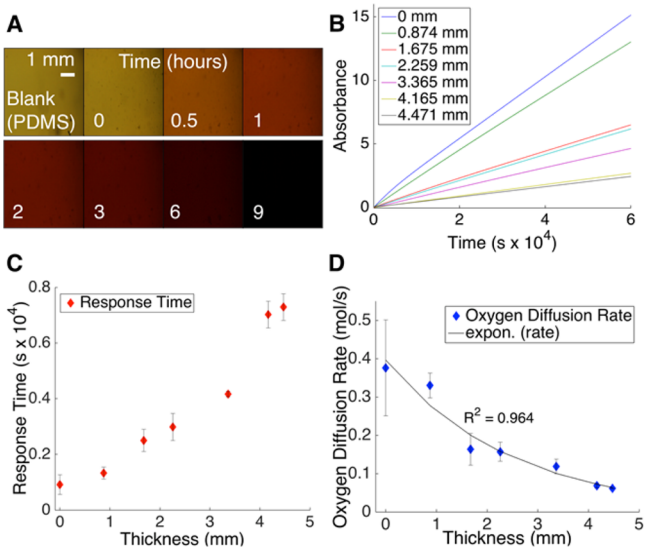

Figure 3. (A) Time evolution of color in a melanosome with a 0.874 mm thick polymer membrane. (B) Time evolution of absorbance (550−552 nm) from artificial melanosomes with different polymer membrane thicknesses. (C) Response time (to 50% transmittance reduction) as a function of membrane thickness. (D) Calculated oxygen diffusion rate into the melanosome as a function of membrane thickness.

Separately adjusting the size of the melanosome reagent pocket and the gas-permeable membrane thickness allows determination of the response time and sensitivity to be decoupled. The ability to engineer long delay times for highly sensitive responses provides an added level of covertness for artificial melanosomes used as tamper-indicating materials. Delaying response until long after the bottle has been opened, tampered with, and resealed would keep the response hidden from the perpetrator.

We used UV−vis spectroscopy to confirm the long-term stability of artificial melanosomes in an inert atmosphere. In our synthetic procedure, all stock solutions were repeatedly degassed under high vacuum and backfilled with dry nitrogen before melanosome preparation in the glovebox. Any residual dissolved oxygen is rapidly consumed once the precursor solution has been fully prepared, which renders the mixture a pale, translucent, yellow in lieu of the preferred clear solution (Figure 2C). After this slight initial change, however, we found that the pale yellow is conserved as long as the solution remains under nitrogen. Figure S2A shows the long-term stability of artificial melanosomes stored in sealed containers for up to 40 days. Hydroquinone absorbance intensity at 297 nm shows an initial increase between 1 and 4 days but remains stagnant over time. Quinone absorbance in the UV range (450−490 nm) also increases initially but plateaus after 13 days. Most importantly, there is no discernible color change after 40 days. There is likely a finite and nearly negligible amount of molecular oxygen that drives the slow oxidation of L-DOPA and is consumed after about 2 weeks, after which the reaction terminates.

Safe integration of artificial melanosomes into consumer products also requires confirmation that the melanin precursors do not leach through the silicone membrane and into the outside environment. Figure S2B describes the leaching test setup. Sealed artificial melanosomes containing DOPA-KOH (deoxygenated) and DOPA-melanin solutions (after oxygen exposure) were fabricated by curing the silicone polymer around a suspended droplet of the oxygen-responsive solution, so as to avoid leaving behind injection holes through which the liquid might diffuse. Artificial melanosomes with surface thicknesses of 3.5 mm and 0.2 mm were submerged in DI water for 2 weeks (under an inert atmosphere when testing leaching of deoxygenated precursors). The surrounding media were extracted and analyzed for traces of DOPA-melanin and L-DOPA using HPLC. In both cases, signal intensity was far below the limit of detection for the surrounding media of all samples after 14 days of incubation (Figure S3), suggesting that leaching through the encapsulating polymer was negligible. Fluorescence intensity of the media surrounding encapsulated DOPA-melanin was also below the limit of detection after 21 days (Figure S2C).

Inspired by nature's own stimuli-responsive systems, we created a surface coating out of biocompatible materials that reports oxygen exposure through irreversible color change. The color change derives from the oxygen-dependent synthesis of dark melanin pigment from a colorless, catecholamine precursor (L-DOPA) encapsulated in an oxygen-permeable polymer film. The thickness of the encapsulating layer regulates the rate of oxygen diffusion, and enables the response time to be tuned separately from the sensitivity, the latter being limited by the melanosome size. Artificial melanosomes could find use as low-cost indicators for validating the integrity of sealed consumer packaging and detecting tampering. The detection requires no specialized equipment or electrical input, in contrast to many other types of oxygen sensors (e.g., Clark type electrode sensors, paramagnetic gas sensors, optical sensors, etc.).[1] Nontoxic and remaining stable and nonreactive for at least 40 days, artificial melanosomes have the potential to endure the functional lifetime of many packaged goods that might benefit from this type of integrity monitoring, including food, beverages, pharmaceuticals, or chemical products.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsami.5b11933.

Experimental methods and additional figures relating to chemical leaching experiments (PDF)

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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Notes

The authors declare no competing financial interest.

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