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Fabrication of Hybrid Materials from Titanium Dioxide and Natural Phenols for Efficient Radical Scavenging against Oxidative Stress

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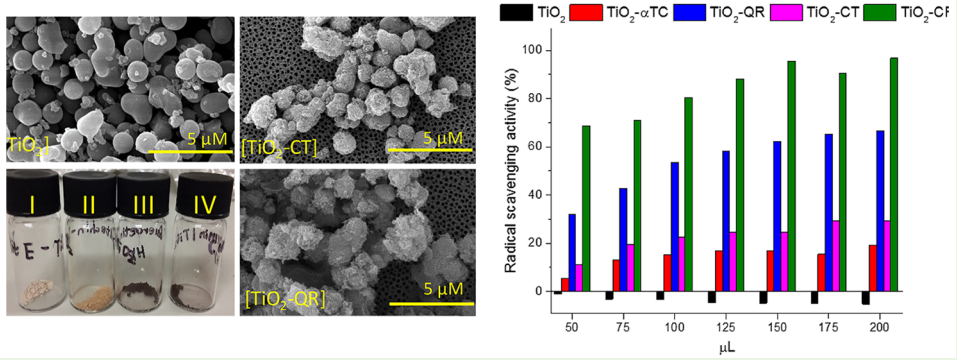

ABSTRACT: Oxidative stress caused by free radicals is one of the great threats to inflict intracellular damage. Here, we report a convenient approach to the synthesis, characterization, and evaluation of the radical activity of titanium-based composites. We have investigated the potential of natural antioxidants (curcumin, quercetin, catechin, and vitamin E) as radical scavengers and stabilizers. The titanium oxide composites were prepared via three steps including sol−gel synthesis, carboxylation, and esterification. The characterization of the titanium−phenol composites was carried out by FTIR, PXRD, UV−vis and SEM methods. The radical scavenging ability of the novel materials was evaluated using DPPH and an in vitro LPO assay using isolated rat liver mitochondria. The novel materials exhibit both a higher stability and an antioxidant activity in comparison to bare TiO_2. It was found that curcumin and quercetin based composites show the highest antioxidant efficiency among the composites under study followed by catechin and vitamin E based materials. The results from an MTT assay carried out on the Caco-2 cell line indicate that the composites do not contribute to the cytotoxicity in vitro. This study demonstrates that a combination of powerful antioxidants with titanium dioxide can change its functional properties and provide a convenient strategy against oxidative stress.

KEYWORDS: *oxidative stress, titanium dioxide, polyphenols, surface modification, antioxidants, LPO and MTT assay, cytotoxicity*

INTRODUCTION

Reactive species (ROS/RNS) can be considered as a double-edged sword: they play an important role in regulating vital cellular functions, but at the same time, they can also trigger oxidative stress, therefore contributing to the acceleration of aging, diabetes, and development of neurodegenerative and cardiovascular diseases as well as initiation and progression of cancer.[1−9] Oxidative stress has a detrimental effect on cell life cycle by inducing extensive damage to DNA, lipids, and proteins.[1,10−14] Antioxidative defense mechanisms involve both enzymatic and nonenzymatic pathways.[15,16] Common antioxidants include the vitamins A, C, and E and the superoxide and glutathione enzymes; other examples cover mixed carotenoids, flavonoids, antioxidants, minerals, and cofactors.[16,17] Polyphenolic compounds such as flavonoids show scavenging activity for various reactive species, including superoxide,[18,19] peroxyl radicals,[20,21] and peroxynitrite.[22] Their structure and ability to interact and to penetrate the membrane of a phospholipid bilayer are thought to be the major factors of flavonoid antioxidant activity.[23,24] Flavonoids such as quercetin and catechin are common and are found in many edible plants consumed daily in a variety of products: e.g., chocolate, red wine, and tea.[25]

Titanium dioxide (TiO_2) is a naturally occurring mineral and is used as a white pigment. The global market of TiO_2 was estimated to be USD 15.76 billion in 2018 and is expected to increase until at least 2025. Its production accounts for ca. 70% of the total volume of pigments manufactured worldwide with a large proportion used in the food−drug−cosmetics (FDC) sector. Many applications of titania are related to human health

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and include personal care products, pharmaceuticals, and food products (E171 in the EU) to name a few.[26] However, recent studies suggest that nanoparticulate TiO_2 can pose a risk to human health, as it can initiate the production of strong oxidants, e.g. free radicals leading to the formation of hydrogen peroxide, on exposure to TiO_2-containing products.[26−36] However, even food grade TiO_2 (E171) contains at least 36% of nano-TiO_2, with a particle size of less than 100 nm.[28] While some sectors cannot avoid using nano-TiO_2, some such as the FDC sector could benefit from alternative approaches. TiO_2 is often associated with its surface morphology.[37] Electronic properties of TiO_2 vary significantly depending on physical properties such as the particle size and crystallinity and the photochemical activity. Therefore, it is possible to alter these properties through surface functionalization. Many natural phenols and polyphenols are biocompatible and economically viable, and some have been demonstrated to provide protection from oxidative stress.[38−42] Their use to manipulate the surface properties of substrates by coating, precipitation, and encapsulation strategies has become very attractive.[43−49]

In this study, we demonstrate that plant-derived natural phenols such as quercetin (capers), curcumin (curry), α-tocopherol (vitamin E), and catechin (green tea) can be used to covalently modify the surface of titanium dioxide, leading to a significant increase in the radical scavenging capability of the hybrid material. A consequence of intracellular ROS accumulation is the lipid peroxidation (LPO) of mitochondrial membrane and/or an increase in the permeability of the latter. Thus, this study aims to assess the effects of the materials on iron-induced LPO and explores the effect of these composites on hydrogen peroxide production in isolated mitochondria. The final objective is to investigate the effect of polyphenol−TiO_2 materials on mitochondrial oxidative stress production and cytotoxicity.

MATERIALS AND METHODS

The materials were characterized by attenuated total reflectance (ATR-PLATINUM) and Fourier transform infrared spectroscopy (FTIR, BRUKER ALPHA). The zeta potential of the products was measured by dynamic light scattering (DLS, Malvern ZETASIZER NANO ZSP). X-ray diffraction (XRD, D2 PHASER BRUKER and Diffrac Commander Software) was used to identify the amorphous or crystalline properties of the products. Scanning electron microscopy (SEM, Quorum Q150RS, coating unit 13 nm of gold) was used to analyze the topography of the particle surface.

Synthesis of Titanium Dioxide Microspheres via Sol−Gel Assisted Method. 1-Hexadecylamine (98%, 5 g) was dissolved in 825 mL of absolute EtOH. An aqueous KCl solution (3 mL) was added to the mixture followed by the quick addition of titanium(IV) tetraisopropoxide (17.6 mL) with vigorous stirring at room temperature. The reaction mixture was kept static for 18 h to allow a TiO_2 suspension to be formed. The suspension of TiO_2 was filtered, washed with EtOH, and dried to yield TiO_2 microspheres as a white powder.

Carboxylation of Titanium Dioxide Surface. The TiO_2 microspheres (8 g) were dispersed in 450 mL of doubly distilled water and stirred at room temperature for 30 min. Then, the mixture was ultrasonicated for 30 min, and chloroacetic acid (5 g) was added slowly with vigorous stirring. The reaction mixture was refluxed at 100 °C for 12 h. The reaction mixture was cooled to room temperature, and the supernatant was decanted. The residue was washed with deionized water to reach a neutral pH. Finally, the microspheres were collected by vacuum filtration and dried under vacuum, yielding carboxylated TiO_2 as a white powder.

Synthesis of Titanium Dioxide Composites: [TiO_2-QR], [TiO_2-CT], [TiO_2-αTC], and [TiO_2-CR]. Carboxylated TiO_2 (0.7 g) was dispersed in 95 mL of isopropyl alcohol and stirred under reflux at 80 °C for 30 min. Then 0.05 mL of concentrated H_2SO_4 was added to the reaction mixture. Typically, 0.25 g of an appropriate natural phenol was dissolved in 2.5 mL of isopropyl alcohol and added to the mixture. The resulting reaction mixture was refluxed at 80 °C for 3 h. The reaction mixture was cooled to room temperature, and the suspension was filtered. The esterified TiO_2 composites were washed with absolute ethanol and deionized water and dried under vacuum and then in the oven at 80 °C for 24 h.

Determination of Radical Scavenging Activity by DPPH Assay. Antioxidant ability was measured using a DPPH (2,2-diphenyl-1-picrylhydrazyl hydrate) assay.[50] In brief, a 0.1 mM DPPH stock solution in 60% ethanol and 0.5 mg/mL stock solutions of TiO_2 and its composites in DMSO were prepared. A concentration range from 0.00625 to 10 mg/mL was mixed with DPPH radical stock solutions and incubated for 30 min in the dark. Absorption spectra of the samples were recorded. The A_0 and A_n values were measured as intensities of the absorption peak at 517 nm in UV−vis spectra of each sample after background subtraction. Radical scavenging activity was calculated using eq 1

$$\% \text{ activity} = \frac{A_0 - A_n}{A_0} \times 100\% \quad (1)$$

where A_0 is the absorbance of DPPH solution and A_n is the absorbance of DPPH with the corresponding concentration of TiO_2 material.

Isolation of Mitochondrial Fractions from Rat Liver Tissue. Mitochondrial fractions were isolated from rat liver (obtained from the Animal Unit, University of Leeds) according to the literature.[51] Briefly, fresh liver tissue was minced in ice-cold buffer (0.01 M Tris/MOPS, pH 7.4; 0.2 M sucrose; 0.1 mM EGTA) in a tissue to buffer ratio of 1:5 to 1:10. The homogenate was centrifuged at 600*g* for 10 min at 4 °C to remove cell debris. The resulting supernatant was centrifuged at 5000*g* for 20 min at 4 °C and the supernatant discarded. The pellet was washed with isolation buffer and centrifuged again (5000*g*, 20 min, 4 °C). The pellet containing mitochondria was carefully resuspended and frozen in aliquots at −80 °C. Mitochondrial protein was quantified using a BCA assay (Pierce, Fisher Scientific, Loughborough, U.K.) with bovine serum albumin as standard.

Inhibition of Mitochondria Oxidative Stress. The test compound dependent inhibition of iron sulfate induced lipid peroxidation was assayed as thiobarbituric acid reactive substances in mitochondria as recently described.[52] Briefly, mitochondrial samples (1 mg of protein/mL) were preincubated with test compounds (0.025, 0.05, 0.1, 0.2, 0.4 mg/mL) for 30 min at 37 °C, centrifuged to remove nonassociated compounds, and subsequently challenged with 5 mM iron(II) sulfate. Samples were incubated for 20 min at 90 °C after protein precipitation and addition of TBA. The resulting dye was extracted with butanol, and absorbance was measured at 540 nm using a plate reader.

Cytotoxicity Assessment by MTT Assay. Human intestinal Caco-2 cells (obtained from the ECACC) were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal bovine serum, 1% (v/v) nonessential amino acids, and 1% (v/v) penicillin/streptomycin mix. Cells were seeded at 8.2×10^4 cells/cm^2 in 24-well plates and after 48 h (≥90% confluence) incubated with TiO_2−composites at concentrations ranging from 0 to 0.25−2.5 μg/mL for 24 h. Then, the medium was replaced by DMEM containing MTT (0.5 mg/mL) and the formazan dye solubilized with DMSO following incubation. Absorbance was measured at 570 nm using a plate reader, and cell viability was calculated as percent change in comparison to control cells.

Statistical Analysis. The data are expressed as means ± SD; results were considered statistically significant when the p value was <0.05. All experiments were independent and were conducted in triplicate or more.

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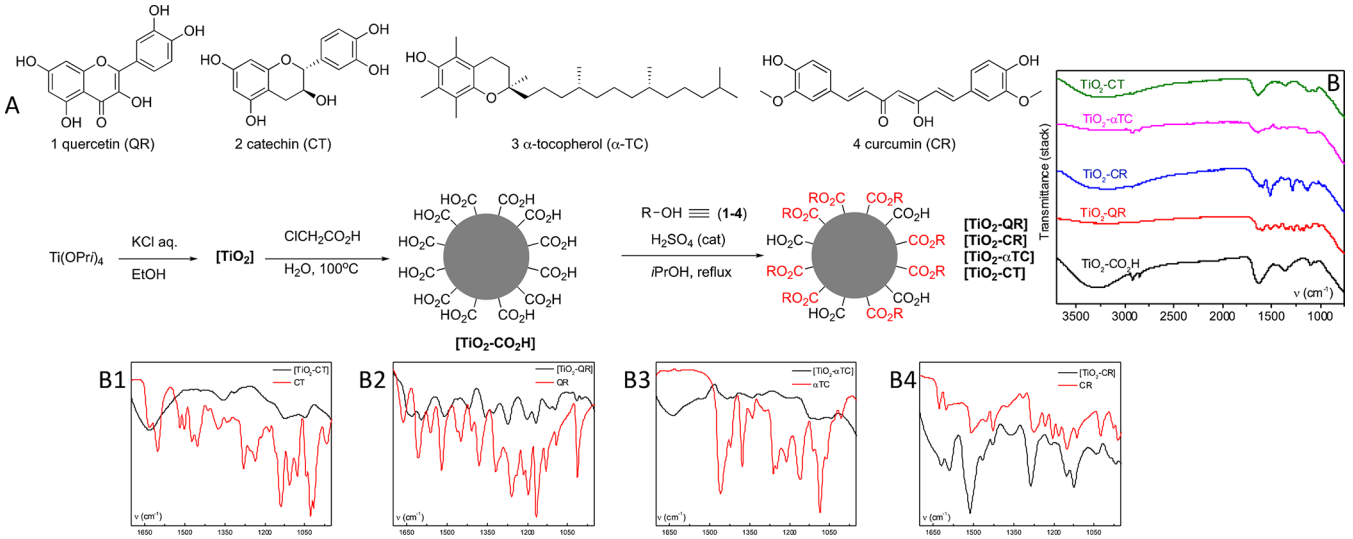

Figure 1. (A) Preparation of [TiO_2], [TiO_2-CO_2H], [TiO_2-QR], [TiO_2-CT], [TiO_2-αTC], and [TiO_2-CR]. (B) FTIR spectra of the modified TiO_2 composites (stack) and (B1−B4) the detailed IR region (1700−950 cm^{-1}) showing the spectra of the pure polyphenol and the respective TiO_2-modified material.

RESULTS AND DISCUSSION

Surface Functionalization of Titanium Dioxide with Natural Phenols. Figure 1A summarizes a modification of titanium dioxide (TiO_2) with natural phenols such as quercetin (QR), catechin (CT), curcumin (CR), and α-tocopherol (TC) as the most biologically active form of vitamin E. The hybrid materials have been prepared in three steps: (1) sol−gel synthesis of TiO_2, (2) carboxylation of the TiO_2 surface, and (3) surface esterification with polyphenols. The sol−gel approach allows control of the structure, size, and shape of TiO_2 particles and thus has been used in this study. The titanium dioxide microspheres [TiO_2] were produced from $Ti(OPr^i)_4$ via a surfactant-assisted sol−gel method.[53−55] This is an efficient synthetic strategy based on the hydrolysis of titanium(IV) alkoxides. The rate of hydrolysis and consequently the desired particle size were controlled by the amount of aqueous KCl used in the reaction. It has been shown that low calcination temperatures (<300 °C) lead to a relatively low photoactivity due to a low crystallinity of the TiO_2 sample.[37] To avoid an increase in the photoactivity, the synthesized amorphous TiO_2 was used without hydrothermal treatment. Functionalization of the TiO_2 surface with carboxylic groups was achieved using chloroacetic acid in water. A surface of carboxylated TiO_2 microspheres [TiO_2-CO_2H] was esterified with the natural phenols to produce covalently modified titanium dioxide composites: from quercetin [TiO_2-QR], catechin [TiO_2-CT], α-tocopherol [TiO_2-αTC], and curcumin [TiO_2-CR].

The reaction was carried out in isopropyl alcohol in the presence of sulfuric acid as a catalyst. The progress of the esterification was followed by FTIR; the strong peak at 3273 cm^{-1} associated with the O−H stretching band of [TiO_2-CO_2H] decreased over time with typical reaction times of 90 min (Figure 1B). FTIR was also used as a primary tool to confirm the success of the functionalization of the carboxylated titanium dioxide with the natural phenols (Figure 1B). The FTIR spectrum of the starting [TiO_2-CO_2H] features strong bands at 1630 cm^{-1} (C═O) and 1356 cm^{-1} (C−O) and a large band starting from 900 cm^{-1} associated with Ti−O−Ti stretching. All esterified TiO_2-based composites (Figure 1B1−B4) show a strong change in C═O signal accompanied by the characteristic bands of the attached natural phenol moieties, confirming the successful esterification. For instance, the flavonoid-based material [TiO_2-QR] shows strong peaks at 1635 (C═O), 1592 (C═C), 1510 (B-ring) and 1270 cm^{-1} (C−O), which are common features of quercetin. Similarly, [TiO_2-CT] displays strong bands at 1629 (C═O), 1463−1440 (C−H), 1179−1127 (C−H), and 1049 cm^{-1} (C−O−C). FTIR spectra of the lipophilic polyphenol-based materials feature the following bands: [TiO_2-CR], 1617−1588 (C═O) and 1289−1123 cm^{-1} (C−C, C−O, and C−O−C); [TiO_2-αTC], 1630 (C═O) and 1289−1123 cm^{-1} (C−C, C−O, C−O−C).

Optical Properties, Crystallinity, Morphology, and Stability. Optical properties of the TiO_2 materials were analyzed using UV−vis absorption spectroscopy. It is evident from Figure 2 that the light absorption ability of the synthesized composites has been efficiently enhanced in comparison to that of the bare [TiO_2] (λ_{max} < 260 nm).

The covalent functionalization with polyphenols leads to drastic changes in blocking of UVA (320−400 nm) and visible light radiation as the synthesized composites absorb between 320 and 600 nm. The UV−vis spectrum of the carboxylated

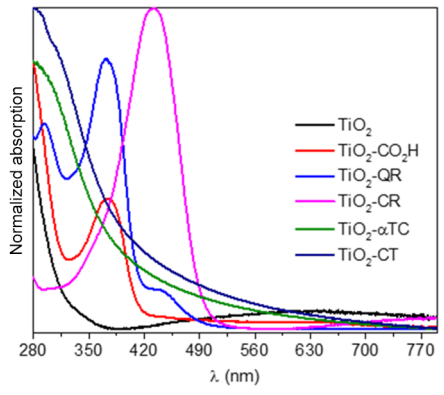

Figure 2. Normalized UV−vis absorption spectra of the TiO_2 materials.

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[TiO_2] exhibits a bathochromic shift of the TiO_2 band (λ_{max} 265 nm) and a new signal at 345−422 nm (λ_{max} 375 nm). Significant broadening in the absorption spectra is observed for [TiO_2-αTC] and [TiO_2-CT] composites, suggesting that the spectra are represented by a few overlapping absorption signals with an increased contribution of long-wavelength bands with maxima of absorption at 277 and 264 nm, respectively. Since the absorbance of both wavelengths increased, the ability of UV blocking also has improved. The TiO_2 materials modified with quercetin and curcumin, which have large $\pi-\pi$ systems, show a distinct color change from the original TiO_2 and carboxylated TiO_2 samples. [TiO_2-QR] sample exhibits a near-blue absorption (λ_{max} 372 nm) with a shoulder (λ_{max} 440 nm) extending into the visible region of up to 500 nm. The absorption spectrum of [TiO_2-CR] indicates a significant contribution from the curcumin fragment, as the material has a large band (λ_{max} 433 nm) in the visible region with a cutoff at around 500 nm.

Powder XRD (PXRD) was used to identify the amorphous or crystalline nature of the titanium dioxide based materials. PXRD analysis shown in Figure 3 confirms the structure of the

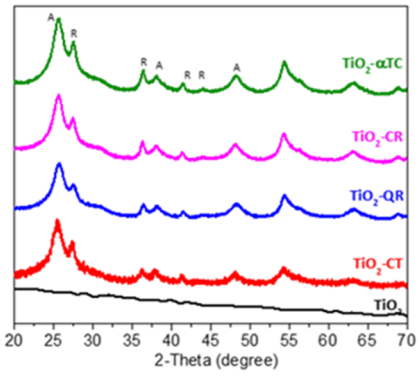

Figure 3. Powder XRD patterns of TiO_2 and modified TiO_2 composites: [TiO_2-CT], [TiO_2-QR], [TiO_2-CR], and [TiO_2-αTC].

TiO_2 microspheres to be amorphous. While an annealing step of synthesis of bare TiO_2 was omitted, PXRD of [TiO_2-CO_2H] shows the mixed-phase pattern of two titania polymorphs. The surface-modified composites show a similar arrangement. The coexistence of anatase (A) and rutile (R) can be clearly identified from characteristic diffraction peaks: anatase (101) peak at 2θ = 25.6° and rutile (110) at 2θ = 27.5°.

The surface morphologies of TiO_2 composites have been analyzed using scanning electron microscopy (SEM). SEM images (Figure 4) show a smooth surface of bare TiO_2 particles with a particle size of around 1.7 μm. A carboxylated TiO_2 sample shows particles of a similar size with etched surfaces. A similar effect on the surface morphology is observed in the titania−polyphenol materials, with crystal clusters clearly visible on the TiO_2 surface. In the series, the particle sizes are comparable with an average diameters of 1.5, 1.3, 1.6, and 1.3 μm for [TiO_2-QR], [TiO_2-CT], [TiO_2-CR], and [TiO_2-αTC], respectively.

Generally, polyphenols show a low stability; thus, ζ potential analysis has been carried out to assess the stability of the TiO_2 composites and the effect of esterification. Water was used as a dispersion medium, and while the surface charge remains negative for all materials, the analysis reveals that the surface modification with polyphenols results in an improved stability of the materials. In comparison to other samples, [TiO_2-QR] and [TiO_2-αTC] composites are the most stable with ζ potentials of −31.1 mV and −30.4 mV, respectively. Lower ζ values have been recorded for other samples: [TiO_2-CO_2H], −27.9 mV; [TiO_2-CT], −27.5 mV; [TiO_2-CR], −28.6 mV. Bare TiO_2 (−24.9 mV) is the least stable. Interestingly, the overall stability has been improved with the attachment of antioxidants.

Antioxidant Properties and in Vitro Activity. The initial radical scavenging efficacy of the TiO_2 composites has been evaluated using a DPPH radical assay. DPPH (1,1-diphenyl-2-picrylhydrazyl) is a stable free radical, which can be neutralized by antioxidants.[50] It produces a deep violet solution with an absorption maximum at 517 nm. In this study, a final DPPH concentration of 50 μM was used, which falls within the range of accuracy for spectrophotometric

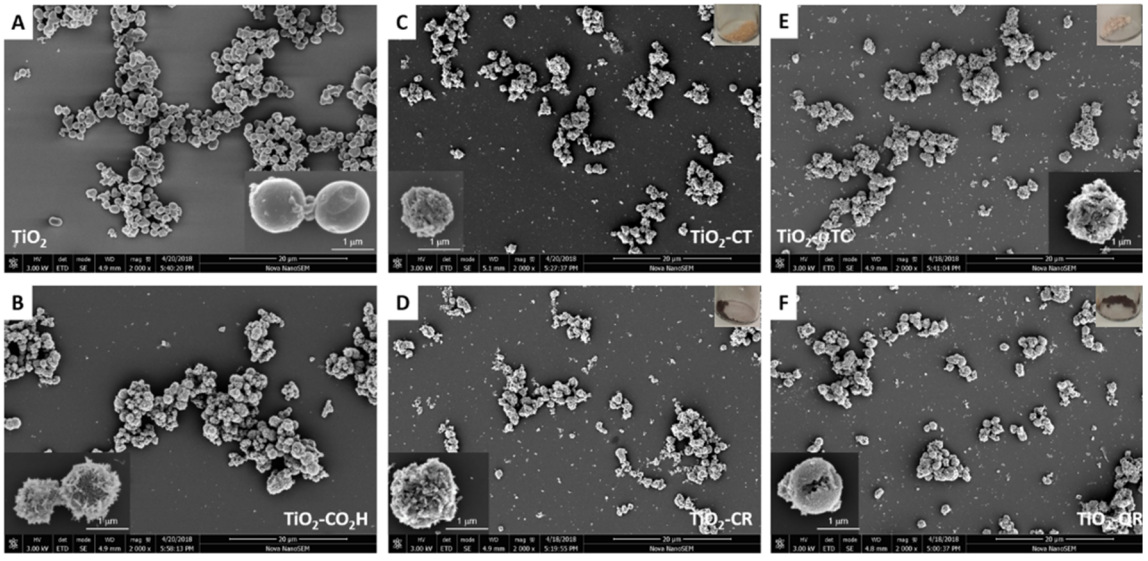

Figure 4. Surface morphology of titanium dioxide and the composites acquired by SEM imaging: (A) [TiO_2]; (B) [TiO_2-CO_2H]; (C) [TiO_2-CT]; (D) [TiO_2-CR]; (E) [TiO_2-αTC]; (F) [TiO_2-QR].

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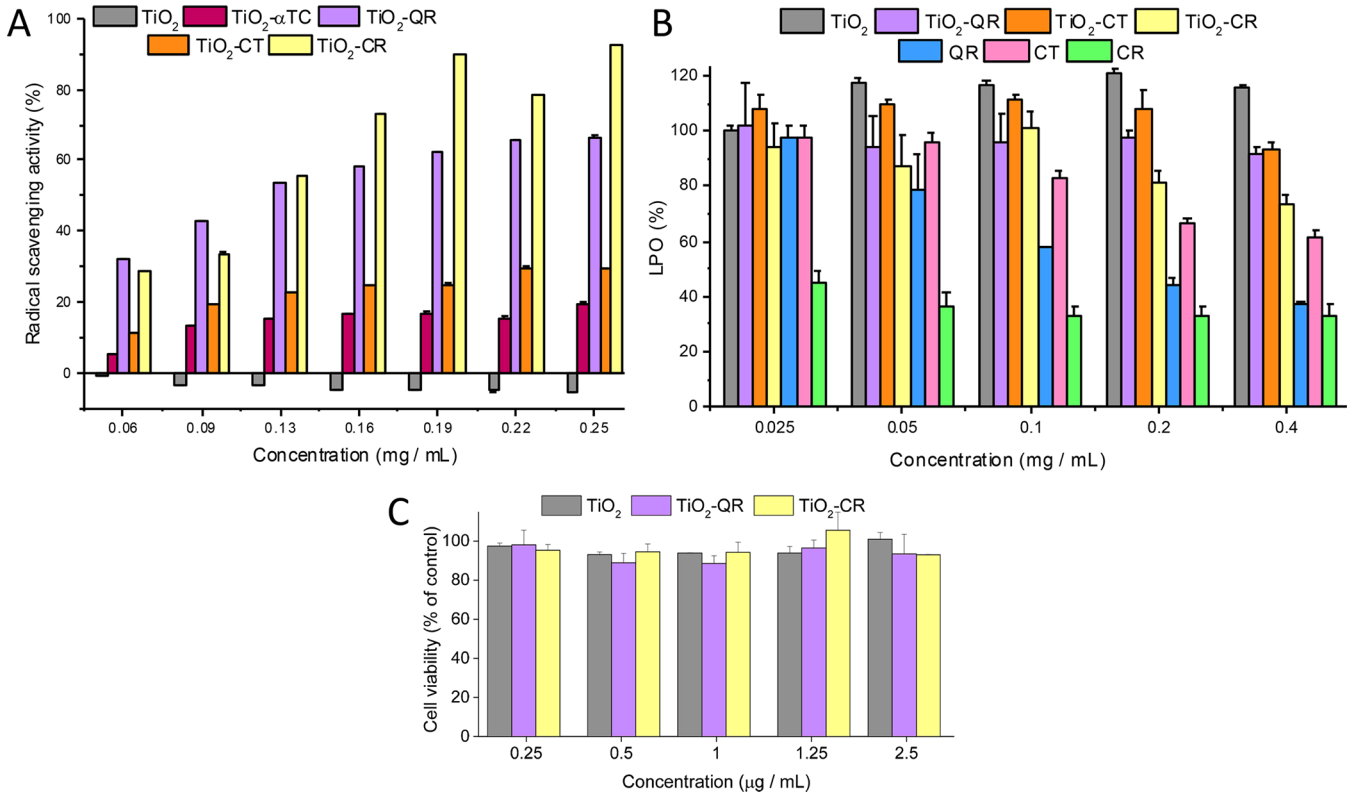

Figure 5. (A) Antioxidant activity of bare TiO_2 and the composites [TiO_2-QR], [TiO_2-CT], [TiO_2-αTC], and [TiO_2-CR] assessed by a DPPH radical scavenging assay. The A_0 and A_n values have been measured as intensities of the absorption peak at 517 nm from the control (final DPPH concentration 0.05 mM) and the samples (the final concentrations of the composites: 0.06, 0.09, 0.13, 0.16, 0.19, 0.22, and 0.25 mg/mL) incubated in the dark at 25 °C for 30 min. (B) Effects of polyphenols: QR, CT and CR and polyphenol−TiO_2 composites: [TiO_2-QR], [TiO_2-CT] and [TiO_2-CR] on iron(II) sulfate induced lipid peroxidation (LPO) in rat liver mitochondria. (C) Effect of polyphenol−TiO_2 composites on Caco-2 cells as tested by an MTT assay. Cells were cultured with [TiO_2-QR] and [TiO_2-CR] at various concentrations (0, 0.25, 0.5, 1.0, 1.25, and 2.5 μg/mL) for 24 h. Untreated control was considered as 100%, and data are expressed as the percentage of untreated control ($p < 0.05$).

measurements corresponding to a transmittance of between 20% and 60%.[56] A stock solution (0.5 mg per mL) of the corresponding TiO_2 material was prepared in DMSO, and a concentration of TiO_2 and its composites in the range of 62.5−250 μg/mL was used. Aliquots (50−200 μL) of the corresponding materials were taken, and the volume was made to 200 μL with 60% ethanol. The radical scavenging reaction was started by the addition of 200 μL of 0.1 mM DPPH stock solution. Figure 5A summarizes the antioxidant activity of TiO_2 and its composites. Bare TiO_2 is inefficient in radical scavenging, showing a low activity even at high concentrations. Moreover, at a higher concentration of TiO_2, an increase in DPPH signal is observed. In contrast, the radical scavenging activity of the polyphenol−TiO_2 hybrids has increased, showing that the antioxidant properties of free polyphenols have been successfully transferred to TiO_2 hybrids upon covalent functionalization. All composites enhance the radical scavenging even at a very low concentration. [TiO_2-CR] shows the highest efficiency of 93% in comparison to other polyphenol−TiO_2 conjugates, followed by [TiO_2-QR] (67%), while [TiO_2-CT] and [TiO_2-αTC] exhibit much lower activity, reaching only 30% and 20%, respectively.

To study the effects of the composites on antioxidant activity in vitro, mitochondrial lipid oxidation (LPO) and MTT cytotoxicity tests have been carried out. The effects of polyphenols and their conjugates on inhibition of lipid peroxidation have been studied in isolated mitochondria, a model that has been employed in previous research[25,57] and is linked to the properties of polyphenols to associate with cellular membranes.[25,41] Mitochondria membrane association of quercetin has been demonstrated recently[58] and affects mitochondria function.[23,42] The LPO assay determines the activity of a membrane-associated compound as an excess of the material is removed and therefore only test compounds integrated or associated with the membrane fraction remain to react in the assay. To understand a trend in antioxidant activity, LPO assay has also been carried out on free polyphenols used for modifications. As shown in Figure 5B, among free polyphenols, curcumin and quercetin are far more effective than catechin. They show similar effects on LPO, reducing it to 33% and 37%, respectively, at the highest concentration used, while the presence of catechin reduces it only to 62%. At the lowest concentration (0.05 mg/mL), only curcumin inhibits LPO. Due to its low DPPH activity, [TiO_2-αTC] was not tested. A similar trend was observed among TiO_2−polyphenol conjugates: again, the highest potent inhibition in mitochondrial LPO was achieved by [TiO_2-CR] followed by [TiO_2-QR]. Both hybrids show an increase in LPO inhibition with higher concentrations. The curcumin conjugate [TiO_2-CT], which slightly increases the mitochondrial LPO at lower concentrations, demonstrates inhibition in comparison to the bare TiO_2, which displays an approximately 20% increase in LPO at all concentrations investigated. The photocatalytic properties of titanium dioxide are likely to have contributed to the increase in lipid peroxidation.[59,60]

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We have carried out a toxicology assessment of TiO_2 and the most efficient [TiO_2-CR] and [TiO_2-QR] composites using an MTT assay. In this experiment, the Caco-2 intestinal cell line has been used, a model commonly employed for in vitro toxicology studies to assess the cell toxicity effects of micro- and macronutrients.[61−63] To choose an appropriate concentration range, we have considered the cytotoxicity of food grade TiO_2 (E171, which typically contains 30% of nanoparticles). Since a daily intake of TiO_2 from food is in the range of 15−37.5 mg per day for an average body weight of 75 kg, an adult would ingest between 6 and 15 ng of TiO_2 per cm^2 of intestine (250 m^2) or 1.8−4.5 ng/cm^2 if TiO_2 is nanoscaled.[64] In addition, the concentration should be below 50 μg/mL to avoid interactions between MTT-dye and TiO_2, which can affect the assay results.[65] Moreover, a high concentration of the pigments can interfere with MTT reading due to a residual absorption of the pigments even after washing. DMSO was used to prepare stock solutions of the materials; the final concentration of DMSO was kept in the range of 0−0.5% to minimize its cytotoxic effect.[66] Thus, Caco-2 cells were cultured with various concentrations (2.5, 1.25, 1, 0.5, 0.25, and 0 μg/mL) of TiO_2, [TiO_2-QR], and [TiO_2-CR] in the dark for 24 h before carrying out the MTT assay as described in Materials and Methods. The results of the MTT assay are summarized in Figure 5C. The data indicate that the cell viability in the presence of bare TiO_2 does not drop below 5% even at the highest concentration of 2.5 μg/mL, which is 400-fold (1.35 μg/cm^2) as high as the daily intake of an adult. MTT results for the [TiO_2-QR] and [TiO_2-CR] materials show a small decrease in the cell viability similar to that observed in the TiO_2 sample. The results indicate that the composites do not contribute to the cytotoxicity in vitro. Moreover, as the nanosized TiO_2 content has been decreased through our preparation method, this is in accordance with the published data on the cytotoxicity driven by particle size.

CONCLUSIONS

In this study, we have shown a versatile method to fabricate polyphenol-titanium dioxide materials using a combination of sol−gel synthesis and surface modification approach. Further analysis such as PXRD and SEM reveals that the surface modification introduces a degree of crystallinity into amorphous TiO_2, while the particle size stays almost unaffected at about 1.4 μm. Importantly, the covalent functionalization leads to a desirable tuning of the optical properties, resulting in a bathochromic shift and an increased efficiency to absorb UV−vis light. The antioxidant activity of the composites has been evaluated using DPPH radical scavenging and in vitro LPO assays. DPPH results indicate that TiO_2 hybrid materials possess an antioxidant activity in contrast to bare TiO_2. It was found that curcumin and quercetin based composites show the highest antioxidant efficiency, followed by catechin and vitamin E based materials. A similar trend has been observed in vitro, with the studied antioxidants exhibiting inhibitory potency on mitochondrial lipid peroxidation. An MTT assay has been carried on the Caco-2 cell line for [TiO_2-QR], [TiO_2-CR], and bare TiO_2 samples. The materials did not induce any significant cytotoxicity even at concentrations as high as 400-fold of daily intake. This study shows that the combination of powerful antioxidants with titanium dioxide can improve its optical properties and antioxidant activity.

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

Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Finkel, T.; Holbrook, N. J. Oxidants, oxidative stress and the biology of ageing. *Nature* **2000**, *408* (6809), 239−247.
(2) Lin, M. T.; Beal, M. F. Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. *Nature* **2006**, *443* (7113), 787−795.
(3) Gorrini, C.; Harris, I. S.; Mak, T. W. Modulation of oxidative stress as an anticancer strategy. *Nat. Rev. Drug Discovery* **2013**, *12* (12), 931−947.
(4) Andersen, J. K. Oxidative stress in neurodegeneration: cause or consequence? *Nat. Med.* **2004**, *10* (S7), S18−S25.
(5) Lavie, L. Oxidative stress in obstructive sleep apnea and intermittent hypoxia - Revisited - The bad ugly and good: Implications to the heart and brain. *Sleep Med. Rev.* **2015**, *20*, 27−45.
(6) Hybertson, B. M.; Gao, B.; Bose, S. K.; McCord, J. M. Oxidative stress in health and disease: the therapeutic potential of Nrf2 activation. *Mol. Aspects Med.* **2011**, *32*, 234−246.
(7) Uttara, B.; Singh, A. V.; Zamboni, P.; Mahajan, R. T. Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options. *Curr. Neuropharmacol.* **2009**, *7*, 65−74.
(8) Rinnerthaler, M.; Bischof, J.; Streubel, M. K.; Trost, A.; Richter, K. Oxidative stress in aging human skin. *Biomolecules* **2015**, *5*, 545−589.
(9) Markesbery, W. R. Oxidative stress hypothesis in Alzheimer's disease. *Free Radical Biol. Med.* **1997**, *23*, 134−147.
(10) Avery, S. V. Molecular targets of oxidative stress. *Biochem. J.* **2011**, *434*, 201−210.
(11) Davies, M. J. Protein oxidation and peroxidation. *Biochem. J.* **2016**, *473*, 805−825.
(12) Stadtman, E. R.; Levine, R. L. Protein oxidation. *Ann. N. Y. Acad. Sci.* **2000**, *899*, 191−208.
(13) Berlett, B. S.; Stadtman, E. R. Protein oxidation in aging, disease, and oxidative stress. *J. Biol. Chem.* **1997**, *272*, 20313−20316.
(14) Vassallo, N. *Polyphenols and health: new and recent advances*; Nova Biomedical Books: New York. 2008.
(15) Das, K.; Roychoudhury, A. Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. *Front. Environ. Sci.* **2014**, *2*, A53.
(16) Maritim, A. C.; Sanders, R. A.; Watkins, J. B., 3rd Diabetes, oxidative stress, and antioxidants: a review. *J. Biochem. Mol. Toxicol.* **2003**, *17*, 24−38.
(17) Sheng, Y.; Abreu, I. A.; Cabelli, D. E.; Maroney, M. J.; Miller, A.-F.; Teixeira, M.; Valentine, J. S. Superoxide Dismutases and Superoxide Reductases. *Chem. Rev.* **2014**, *114*, 3854−3918.
(18) Chun, O. K.; Kim, D.-O.; Lee, C. Y. Superoxide Radical Scavenging Activity of the Major Polyphenols in Fresh Plums. *J. Agric. Food Chem.* **2003**, *51*, 8067−8072.

DOI: 10.1021/acsbiomaterials.9b00535
ACS Biomater. Sci. Eng. 2019, 5, 2778−2785

(19) Jovanovic, S. V.; Simic, M. G. Antioxidants in nutrition. *Ann. N. Y. Acad. Sci.* **2000**, *899*, 326−334.
(20) Nakao, M.; Takio, S.; Ono, K. Alkyl peroxyl radical-scavenging activity of catechins. *Phytochemistry* **1998**, *49*, 2379−2382.
(21) Boadi, W. Y.; Iyere, P. A.; Adunyah, S. E. In vitro exposure to quercetin and genistein alters lipid peroxides and prevents the loss of glutathione in human progenitor mononuclear (U937) cells. *J. Appl. Toxicol.* **2005**, *25*, 82−88.
(22) Pollard, S. E.; Kuhnle, G. G.; Vauzour, D.; Vafeiadou, K.; Tzounis, X.; Whiteman, M.; Rice-Evans, C.; Spencer, J. P. The reaction of flavonoid metabolites with peroxynitrite. *Biochem. Biophys. Res. Commun.* **2006**, *350*, 960−968.
(23) Santos, A. C.; Uyemura, S. A.; Lopes, J. L.; Bazon, J. N.; Mingatto, F. E.; Curti, C. Effect of naturally occurring flavonoids on lipid peroxidation and membrane permeability transition in mitochondria. *Free Radical Biol. Med.* **1998**, *24*, 1455−1461.
(24) Oyewole, A. O.; Birch-Machin, M. A. Mitochondria-targeted antioxidants. *FASEB J.* **2015**, *29*, 4766−4771.
(25) Hendrich, A. B. Flavonoid-membrane interactions: possible consequences for biological effects of some polyphenolic compounds. *Acta Pharmacol. Sin.* **2006**, *27*, 27.
(26) Weir, A.; Westerhoff, P.; Fabricius, L.; Hristovski, K.; von Goetz, N. Titanium dioxide nanoparticles in food and personal care products. *Environ. Sci. Technol.* **2012**, *46*, 2242−2250.
(27) Grande, F.; Tucci, P. Titanium Dioxide Nanoparticles: a Risk for Human Health? *Mini-Rev. Med. Chem.* **2016**, *16*, 762−769.
(28) Shi, H.; Magaye, R.; Castranova, V.; Zhao, J. Titanium dioxide nanoparticles: a review of current toxicological data. *Part. Fibre Toxicol.* **2013**, *10*, 15.
(29) Warheit, D. B.; Donner, E. M. Risk assessment strategies for nanoscale and fine-sized titanium dioxide particles: Recognizing hazard and exposure issues. *Food Chem. Toxicol.* **2015**, *85*, 138−147.
(30) Jayaram, D. T.; Runa, S.; Kemp, M. L.; Payne, C. K. Nanoparticle-induced oxidation of corona proteins initiates an oxidative stress response in cells. *Nanoscale* **2017**, *9*, 7595−7601.
(31) De Angelis, I.; Barone, F.; Zijno, A.; Bizzarri, L.; Russo, M. T.; Pozzi, R.; Franchini, F.; Giudetti, G.; Uboldi, C.; Ponti, J.; Rossi, F.; De Berardis, B. Comparative study of ZnO and TiO_2 nanoparticles: physicochemical characterisation and toxicological effects on human colon carcinoma cells. *Nanotoxicology* **2013**, *7*, 1361−1372.
(32) Runa, S.; Lakadamyali, M.; Kemp, M. L.; Payne, C. K. TiO_2 Nanoparticle-induced oxidation of the plasma membrane: importance of the protein corona. *J. Phys. Chem. B* **2017**, *121*, 8619−8625.
(33) Gurr, J. R.; Wang, A. S.; Chen, C. H.; Jan, K. Y. Ultrafine titanium dioxide particles in the absence of photoactivation can induce oxidative damage to human bronchial epithelial cells. *Toxicology* **2005**, *213*, 66−73.
(34) Hussain, S. M.; Hess, K. L.; Gearhart, J. M.; Geiss, K. T.; Schlager, J. J. In vitro toxicity of nanoparticles in BRL 3A rat liver cells. *Toxicol. In Vitro* **2005**, *19*, 975−983.
(35) Maynard, A. D.; Warheit, D. B.; Philbert, M. A. The new toxicology of sophisticated materials: nanotoxicology and beyond. *Toxicol. Sci.* **2011**, *120*, S109−S129.
(36) Jugan, M. L.; Barillet, S.; Simon-Deckers, A.; Herlin-Boime, N.; Sauvaigo, S.; Douki, T.; Carriere, M. Titanium dioxide nanoparticles exhibit genotoxicity and impair DNA repair activity in A549 cells. *Nanotoxicology* **2012**, *6*, 501−513.
(37) Schneider, J.; Matsuoka, M.; Takeuchi, M.; Zhang, J.; Horiuchi, Y.; Anpo, M.; Bahnemann, D. W. Understanding TiO_2 photocatalysis: mechanisms and materials. *Chem. Rev.* **2014**, *114*, 9919−9986.
(38) Akhlaghi, M.; Bandy, B. Mechanisms of flavonoid protection against myocardial ischemia−reperfusion injury. *J. Mol. Cell. Cardiol.* **2009**, *46*, 309−317.
(39) Bjorklund, G.; Dadar, M.; Chirumbolo, S.; Lysiuk, R. Flavonoids as detoxifying and pro-survival agents: What's new? *Food Chem. Toxicol.* **2017**, *110*, 240−250.
(40) Lagoa, R.; Graziani, I.; Lopez-Sanchez, C.; Garcia-Martinez, V.; Gutierrez-Merino, C. Complex I and cytochrome c are molecular targets of flavonoids that inhibit hydrogen peroxide production by mitochondria. *Biochim. Biophys. Acta, Bioenerg.* **2011**, *1807*, 1562−1572.
(41) Sandoval-Acuña, C.; Ferreira, J.; Speisky, H. Polyphenols and mitochondria: an update on their increasingly emerging ROS-scavenging independent actions. *Arch. Biochem. Biophys.* **2014**, *559*, 75−90.
(42) Steffen, Y.; Gruber, C.; Schewe, T.; Sies, H. Mono-O-methylated flavanols and other flavonoids as inhibitors of endothelial NADPH oxidase. *Arch. Biochem. Biophys.* **2008**, *469* (2), 209−219.
(43) Lee, H.; Dellatore, S. M.; Miller, W. M.; Messersmith, P. B. Mussel-inspired surface chemistry for multifunctional coatings. *Science* **2007**, *318*, 426−430.
(44) Kang, S. M.; Rho, J.; Choi, I. S.; Messersmith, P. B.; Lee, H. Norepinephrine: material-independent, multifunctional surface modification reagent. *J. Am. Chem. Soc.* **2009**, *131*, 13224−13225.
(45) Ye, Q.; Zhou, F.; Liu, W. Bioinspired catecholic chemistry for surface modification. *Chem. Soc. Rev.* **2011**, *40*, 4244−4258.
(46) Hong, S.; Kim, J.; Na, Y. S.; Park, J.; Kim, S.; Singha, K.; Im, G. I.; Han, D. K.; Kim, W. J.; Lee, H. Poly(norepinephrine): ultrasmooth material-independent surface chemistry and nanodepot for nitric oxide. *Angew. Chem., Int. Ed.* **2013**, *52*, 9187−9191.
(47) Cordoba, A.; Monjo, M.; Hierro-Oliva, M.; Gonzalez-Martin, M. L.; Ramis, J. M. Bioinspired quercitrin nanocoatings: a fluorescence-based method for their surface quantification, and their effect on stem cell adhesion and differentiation to the osteoblastic lineage. *ACS Appl. Mater. Interfaces* **2015**, *7*, 16857−16864.
(48) Cordoba, A.; Satue, M.; Gomez-Florit, M.; Hierro-Oliva, M.; Petzold, C.; Lyngstadaas, S. P.; Gonzalez-Martin, M. L.; Monjo, M.; Ramis, J. M. Flavonoid-modified surfaces: multifunctional bioactive biomaterials with osteopromotive, anti-inflammatory, and anti-fibrotic potential. *Adv. Healthcare Mater.* **2015**, *4*, 540−549.
(49) Lee, J. S.; Lee, J. S.; Lee, M. S.; An, S.; Yang, K.; Lee, K.; Yang, H. S.; Lee, H.; Cho, S. W. Plant flavonoid-mediated multifunctional surface modification chemistry: catechin coating for enhanced osteogenesis of human stem cells. *Chem. Mater.* **2017**, *29*, 4375−4384.
(50) Sharma, O. P.; Bhat, T. K. DPPH antioxidant assay revisited. *Food Chem.* **2009**, *113* (4), 1202−1205.
(51) Frezza, C.; Cipolat, S.; Scorrano, L. Organelle isolation: functional mitochondria from mouse liver, muscle and cultured fibroblasts. *Nat. Protoc.* **2007**, *2*, 287−295.
(52) Boesch-Saadatmandi, C.; Wagner, A. E.; Wolffram, S.; Rimbach, G. Effect of quercetin on inflammatory gene expression in mice liver in vivo - role of redox factor 1, miRNA-122 and miRNA-125b. *Pharmacol. Res.* **2012**, *65*, 523−530.
(53) Brinker, C. J.; Scherer, G. W. *Solgel Science: The Physics and Chemistry of Solgel Processing*; Academic Press: San Diego, 1990.
(54) Wang, C.-C.; Ying, J. Y. Sol−gel synthesis and hydrothermal processing of anatase and rutile titania nanocrystals. *Chem. Mater.* **1999**, *11*, 3113−3120.
(55) Liu, H.; Yang, W.; Ma, Y.; Cao, Y.; Yao, J.; Zhang, J.; Hu, T. Synthesis and characterization of titania prepared by using a photoassisted sol−gel method. *Langmuir* **2003**, *19*, 3001−3005.
(56) Ayres, G. H. Evaluation of accuracy in photometric analysis. *Anal. Chem.* **1949**, *21*, 652−657.
(57) Dorta, D. J.; Pigoso, A. A.; Mingatto, F. E.; Rodrigues, T.; Pestana, C. R.; Uyemura, S. A.; Santos, A. C.; Curti, C. Antioxidant activity of flavonoids in isolated mitochondria. *Phytother. Res.* **2008**, *22*, 1213−1218.
(58) Fiorani, M.; Guidarelli, A.; Blasa, M.; Azzolini, C.; Candiracci, M.; Piatti, E.; Cantoni, O. Mitochondria accumulate large amounts of quercetin: prevention of mitochondrial damage and release upon oxidation of the extramitochondrial fraction of the flavonoid. *J. Nutr. Biochem.* **2010**, *21*, 397−404.
(59) Brezová, V.; Gabcová, S.; Dvoranová, D.; Stasko, A. Reactive oxygen species produced upon photoexcitation of sunscreens containing titanium dioxide (an EPR study). *J. Photochem. Photobiol., B* **2005**, *79*, 121−134.

DOI: 10.1021/acsbiomaterials.9b00535
ACS Biomater. Sci. Eng. 2019, 5, 2778−2785

(60) Kiwi, J.; Nadtochenko, V. New Evidence for TiO_2 photocatalysis during bilayer lipid peroxidation. *J. Phys. Chem. B* **2004**, *108*, 17675−17684.
(61) Alvarez-Hernandez, X.; Nichols, G. M.; Glass, J. Caco-2 cell line: a system for studying intestinal iron transport across epithelial cell monolayers. *Biochim. Biophys. Acta* **1991**, *1070*, 205−208.
(62) Artursson, P.; Palm, K.; Luthman, K. Caco-2 monolayers in experimental and theoretical predictions of drug transport. *Adv. Drug Delivery Rev.* **2001**, *46*, 27−43.
(63) Sambuy, Y.; de Angelis, Y.; Ranaldi, G.; Scarino, M. L.; Stammati, A.; Zucco, F. The Caco-2 cell line as a model of the intestinal barrier: influence of cell and culture-related factors on Caco-2 cell functional characteristics. *Cell Biol. Toxicol.* **2005**, *21*, 1−26.
(64) Brun, E.; Barreau, F.; Veronesi, G.; Fayard, B.; Sorieul, S.; Chanéac, C.; Carapito, C.; Rabilloud, T.; Mabondzo, A.; Herlin-Boime, N.; Carriére, M. Titanium dioxide nanoparticle impact and translocation through ex vivo, in vivo and in vitro gut epithelia. *Part. Fibre Toxicol.* **2014**, *11* (1), 13.
(65) Lupu, A.R.; Popescu, T. The noncellular reduction of MTT tetrazolium salt by TiO_2 nanoparticles and its implications for cytotoxicity assays. *Toxicol. In Vitro* **2013**, *27*, 1445−1450.
(66) Timm, M.; Saaby, L.; Moesby, L.; Hansen, E. W. Considerations regarding use of solvents in in vitro cell based assays. *Cytotechnology* **2013**, *65*, 887−894.

DOI: 10.1021/acsbiomaterials.9b00535
ACS Biomater. Sci. Eng. 2019, 5, 2778−2785