Coffee Bean Polyphenols Can Form Biocompatible Template-free Antioxidant Nanoparticles with Various Sizes and Distinct Colors

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ABSTRACT: Plant polyphenols have attracted attention in recent years due to their ability to undergo oxidative coupling reactions enabled by the presence of multiple phenolic hydroxyl groups, forming chemically versatile coatings and biocompatible nanoparticles (NPs) for various applications. The aim of this study was to investigate whether coffee bean aqueous extracts, which are known to be rich in polyphenols, could serve as a natural source of NP building blocks. Extracts were prepared by heating ground Arabica beans of varying roasting degrees in water with or without the addition of sodium metaperiodate or copper sulfate as an oxidizing agent, followed by filtration. NP formation was verified by dynamic light scattering and transmission electron microscopy, which revealed the presence of nano-sized particles with varying sizes and polydispersities as a function of the coffee type and oxidizing agent used. NP colors ranged from light to medium to dark brown, and particle sizes were between 44 and 250 nm with relatively low polydispersity indices. In vitro antioxidant assays showed that oxidizing agent-treated coffee NPs had lower antioxidant potency compared to air-oxidized NPs, but the free-radical scavenging activity was still retained. Coffee NPs exhibited no antimicrobial activity against common bacterial and fungal strains. Cell viability assays demonstrated that the NPs were biocompatible in human dermal fibroblasts, while exhibiting antiproliferative activity against MCF7 breast cancer cells, particularly copper sulfate-oxidized NPs. This study presents a facile and economical method to produce template-free antioxidant NPs that may be explored for various applications such as drug delivery and cosmetics.

INTRODUCTION

Natural products are an abundant source of bioactive compounds that have been extensively investigated to alleviate various ailments. In addition to their well-established therapeutic benefits, plant polyphenols have recently attracted attention as a source of precursors for nanomaterials synthesis. In particular, green tea polyphenols and the flavonoid quercetin (QCT) have been reported to form untemplated nanoparticles (NPs) under a variety of conditions.2−6 The most common denominator for the synthesis conditions for these materials is oxidation-triggered polymerization of the polyphenol precursors. Oxidative coupling of polyphenols has been reported by several groups and leveraged their chemical versatility to produce functional surface coatings and nanomaterials for many biomedical applications.7−13 The mechanism is inspired by the naturally occurring self-defense phenomenon in the plant kingdom which involves browning of freshly cut fruits and vegetables upon air exposure.14

Conventional NP synthesis from metal salts or polymers typically involves harsh reaction conditions, toxic organic solvents, and multi-step reactions, resulting in significant adverse environmental impacts.15,16 Thus, there is an emergent need for the sustainable synthesis of eco-friendly nanomaterials for various practical applications. Biomedical applications in particular require the materials to be nontoxic, biocompatible, and/or biodegradable. The various types of NPs that are commonly applied in drug delivery can trigger certain biological effects that largely depend on their unique physicochemical properties. Naahidi et al. considered materials to be biocompatible when they interact with the body without inducing adverse effects such as immunogenic, thrombogenic, and carcinogenic responses.17 In addition to their safety profile, the availability and cost of raw materials for nanomaterials synthesis can impede their wide-scale application.18 In this context, plant polyphenols provide an attractive toolbox of precursors for nanomaterials synthesis. They are readily available from a variety of renewable resources, relatively cheap and biocompatible, and can impart additional therapeutic benefits by retaining some of their antioxidant activity.19,20 So far, only green tea polyphenols, tannic acid, and QCT have been investigated in this regard. In particular, our group has synthesized biocompatible NPs from QCT via a variety of approaches and leveraged their chemical versatility to load anticancer drugs and surface ligands.21−23 Other than these
precursors, NP synthesis from plant polyphenols via oxidative coupling has not yet been adequately explored.

Coffee is one of the most popular beverages in the world. The most abundant species are *Coffee arabica* (Arabica) and *Coffee canephora* (Robusta), both of which are considered a rich source of biologically active compounds, especially polyphenols.\(^{21}\) The composition of these polyphenols varies as a function of the source and degree of roasting, with hydroxycinnamic acids such as chlorogenic and caffeic acid being considered key components that contribute to coffee’s health benefits.\(^{22}\) Processing of green coffee beans by roasting is commonly performed to produce roasted coffee beans with distinct aromas, taste, and color. These organoleptic changes take place upon degradation and transformation of coffee constituents depending on the roasting conditions. For example, the carbohydrate, protein, and chlorogenic acid content of green beans is reduced upon roasting, whereas melanoidins are formed upon condensation of carbohydrates with amino acids at high roasting temperatures.\(^{23}\) Despite changes in the chemical constituents, studies have shown that unroasted and roasted coffee beans still maintain considerable antioxidant activity, which is mainly attributed to the persistence of polyphenolic compounds.\(^{24,25}\)

In this work, we hypothesized that the polyphenol-rich aqueous extracts of Arabica coffee beans represented by chlorogenic acids and melanoidins may produce nanostructures through oxidative coupling, similar to previous reports involving QCT and green tea catechins. To test our hypothesis, aqueous extracts of green (unroasted), medium-roasted, and dark-roasted ground beans were prepared either by air oxidation or by using oxidizing agents previously employed by our group and others to synthesize polyphenol NPs. The ability of the extracts to form NPs was verified by dynamic light scattering (DLS) and transmission electron microscopy (TEM). The NPs were further investigated through antioxidant, antimicrobial, and cell viability assays to confirm their biocompatibility and potential bioactivity, thus providing the first evidence on the ability of coffee polyphenols to serve as NP building blocks.

**RESULTS AND DISCUSSION**

**Synthesis and Characterization of NPs from Polyphenol-Rich Coffee Bean Aqueous Extracts.** In this study, we investigated the ability of coffee bean aqueous extracts to form NPs via oxidative coupling of polyphenols. As depicted in Figure 1, extracts were prepared from ground coffee beans with different roasting degrees (green/unroasted, medium-roasted, and dark-roasted) simply by mixing the grounds in boiling hot water, followed by filtration. Oxidation was triggered by air or upon the addition of one of two oxidizing agents, NaIO\(_4\) or CuSO\(_4\), which led to the formation of distinctly colored extracts (Figure 1). In the case of air-oxidized extracts, the different colors were attributed to the difference in roasting degrees between green, medium-roasted, and dark-roasted coffee beans. The characteristic green color of Cu(II) complexes was more evident in CuSO\(_4\)-oxidized green coffee extracts, while NaIO\(_4\)-oxidized extracts were associated with dark-brown tones, indicating the persistence of iodine complexes after purification.

The simplest way to detect NP formation was through DLS measurements. The hydrodynamic diameters and the corresponding polydispersity indices (PDIs) for the various extracts are depicted in Figure 2 and Table S1 of the Supporting Information. In general, extracts from all coffee types were able to produce colloidal particles spontaneously, even without the addition of oxidizing agents, most likely through oxidative self-polymerization of the polyphenol constituents. Under similar reaction conditions, green tea polyphenols reportedly formed insoluble aggregates upon air oxidation and microspheres with different morphologies with the addition of CuSO\(_4\).\(^{2}\) On the other hand, QCT NPs previously reported by our group typically required the presence of an oxidizing agent (NaIO\(_4\)) or an alkaline medium for oxidative coupling reactions to occur under ambient conditions, producing NPs between 30 and 200 nm in diameter.\(^{4,5}\)

As shown in Figure 2A, the addition of oxidizing agents altered the particle size of coffee NPs compared to air oxidation. Among all the groups tested, CuSO\(_4\)-oxidized green coffee extracts produced the smallest NPs with an average diameter of 44 nm, and the largest NPs were obtained from both NaIO\(_4\) and CuSO\(_4\)-oxidized dark coffee extracts (249 and 250 nm, respectively). Notably, the particle size for air-oxidized green coffee extracts was significantly reduced from 121 to 73 nm (\(p < 0.05\)) and 44 nm (\(p < 0.001\)) upon oxidation with NaIO\(_4\) and CuSO\(_4\), respectively. A similar trend was observed in medium-roasted coffee, where the particle size was significantly reduced from 175 to 85 nm (\(p < 0.001\)) and 134 nm (\(p < 0.05\)) in NaIO\(_4\)- and CuSO\(_4\)-oxidized extracts, respectively. These findings may be attributed to the accelerated oxidative coupling reactions in the presence of the oxidizing agents, which enabled more efficient oxidation of the polyphenol moieties (e.g., the catechol groups of chlorogenic acids) into reactive quinones. These reactive intermediates have been shown to mediate the formation of oligomeric and/or polymeric structures capable of self-assembly into NPs.\(^{3,5}\) In the absence of oxidizing agents as catalysts, auto-oxidation of coffee extracts’ polyphenol constituents most likely proceeded at a much slower rate, resulting in the formation of larger NPs.

Conversely, the size of dark-roasted coffee extracts upon air oxidation was 89 nm, which was significantly increased to 249 and 250 nm after adding NaIO\(_4\) and CuSO\(_4\), respectively (\(p < 0.0001\)). This discrepancy may have resulted from the changes in coffee constituents upon roasting. Compared to green and medium-roasted coffee, dark-roasted coffee is known to be rich in melanoidins, which are high-molecular weight (MW) Maillard reaction products formed during the roasting process.\(^{25}\) Although their exact composition is not well elaborated, coffee melanoidins are generally condensation...
products of carbohydrates, amino acids, and chlorogenic acids. Upon the addition of the oxidizing agents, oxidative coupling reactions mediated by the catechol moieties of chlorogenic acid pendant groups in melanoidins likely led to further growth of these oligomeric structures and a significant increase in NP size compared to air oxidation. When comparing NPs produced under the same oxidation conditions, there was no significant difference in size in air-oxidized NPs; only green coffee extracts were associated with a much larger PDI (see below). In the case of NaIO₄-treated extracts, there was no difference between green and medium-roasted coffee, but dark-roasted coffee produced significantly larger NPs. In the case of CuSO₄-treated extracts, there was a trend of size increase going from green to medium to dark, which may be attributed to the difference in the oxidation mechanism of CuSO₄ compared to NaIO₄ and the difference in coffee constituents as a function of the roasting degree.

As shown in Figure 2B, all NPs exhibited relatively low polydispersity except for air-oxidized green coffee extracts, which were associated with a PDI of 0.48. However, upon adding NaIO₄ and CuSO₄, PDI values were markedly reduced to 0.19 and 0.25, respectively, which were not significantly different from all other NPs. Surface charge represented by the zeta potential is another important feature of colloidal particles as it can greatly affect their stability under various conditions. As shown in Table S1, all the NPs exhibited partially negative zeta potential values, which may be attributed to the abundance of electron-rich aromatic structures. Although the magnitude of the surface charge may not be high enough to provide colloidal stability by electrostatic repulsion, the hydrophilic nature of the NPs should make them sufficiently stable in aqueous solutions. Alternatively, the reactivity of oxidized polyphenols toward nucleophiles may be exploited to immobilize hydrophilic polymers such as polyethylene glycol on the NP surface to impart steric stability.

Next, the particle size and shape of the coffee NPs prepared under various conditions were visualized by TEM. As depicted in Figure 3, all NPs appeared as dark spheres without the need for staining, confirming their electron-rich aromatic structures. Although the magnitude of the surface charge may not be high enough to provide colloidal stability by electrostatic repulsion, the hydrophilic nature of the NPs should make them sufficiently stable in aqueous solutions. Alternatively, the reactivity of oxidized polyphenols toward nucleophiles may be exploited to immobilize hydrophilic polymers such as polyethylene glycol on the NP surface to impart steric stability.

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which were associated with the highest PDI, revealed the presence of both small and large particles indicative of their broad size distribution. On the other hand, all other NPs appeared uniform in size. In particular, NPs produced from NaIO₄- and CuSO₄-oxidized dark-roasted coffee extracts appeared significantly larger than air-oxidized extracts in line with DLS results. Taken together, these findings provided a definitive proof that the aqueous extracts of various types of coffee are able to form NPs upon oxidation, most likely enabled by oxidative coupling reactions of their polyphenolic constituents.

The NPs were further characterized by differential scanning calorimetry (DSC) to obtain better insights into their solid-state characteristics. As shown in Figure 4, NPs produced from green coffee extracts as well as air-oxidized medium- and dark-roasted coffee extracts exhibited no characteristic thermal transitions. NPs produced from CuSO₄-treated medium- and dark-roasted coffee extracts showed a broad endothermic peak between 80 and 100 °C, which was attributed to the evaporation of moisture from the samples. Broad exothermic transitions were observed at temperatures > 200 °C in NaIO₄- and CuSO₄-treated extracts of medium- and dark-roasted coffees, consistent with thermal degradation at elevated temperatures. Overall, the absence of crystal melting peaks in all the samples provides evidence on the amorphous nature of the coffee NPs. Similar results were reported for polyphenol NPs which were synthesized by oxidative coupling of QCT, where the characteristic crystal melting peak of unreacted QCT was completely diminished upon polymerization and NP formation.⁶

**Spectroscopic Characterization of Coffee NPs.** Fourier transform infrared (FT-IR) analysis of coffee NPs (Figure S1 of the Supporting Information) revealed several peaks that were consistent with polyphenolic species such as O−H(̈)C−H (3690−2990 cm⁻¹), C−H (2980−2780 cm⁻¹), and C=C/C=O (1820−1490 cm⁻¹) stretching bands. These peaks correspond to the heterogeneous constituents of coffee beans including chlorogenic acids, carbohydrates, proteins, lipids, and caffeine as previously reported.²⁸,²⁹ UV-visible (UV-vis) spectra of the NPs were scanned alongside air-oxidized extracts which were obtained after removal of insoluble coffee grounds before ultrafiltration (UF) (Figure S2 of the Supporting Information). Before UF, all extracts exhibited two peaks at around 290 and 330 nm, which correspond to the characteristic bands of plant polyphenols such as chlorogenic acid, the most common constituent of coffee polyphenols. The peaks became less prominent and underwent significant broadening after oxidation and further purification by UF, particularly in NPs prepared from dark-roasted coffee extracts. These spectral changes are likely attributed to the conversion of phenolic −OH groups to quinones, coupled with the light scattering effect of colloidal aggregates, both of which have been shown to alter the UV absorbance pattern of polyphenols undergoing oxidative coupling reactions.¹−⁶

**Polyphenol Content and Antioxidant Capacity of Coffee NPs.** Coffee NPs prepared under the various conditions were analyzed by the Folin–Ciocalteu method in order to examine the possible alteration in polyphenol content both as a function of coffee roasting degree and as a function of the oxidizing agent used. Extracts prepared by air oxidation after removal of insoluble coffee grounds and before UF served as the control. As shown in Figure SA, extracts from green and medium-roasted coffee beans before UF were associated with a similar polyphenol content ranging between 38.3 and 44.2 mg gallic acid equivalents (GAE)/g ground beans. The total phenol content of dark-roasted coffee extracts before UF was significantly lower compared to that of green coffee (p < 0.05), with a value of 35.2 mg GAE/g ground beans, indicating that the roasting degree can affect the polyphenol content of coffee extracts. Although the roasting process is known to cause alterations in the relative composition of coffee beans, polyphenolic species can still be present after roasting.²² After UF, the total phenol content of the NPs prepared by air oxidation was significantly reduced across all coffee types owing to the removal of small-molecule phenolic compounds that were not incorporated in the NPs. Interestingly, NPs prepared by NaIO₄ oxidation were characterized by an even more significant reduction in total polyphenol content compared to those prepared by CuSO₄ oxidation, which is similar to QCT NPs synthesized by NaIO₄ oxidation.⁹ This may be attributed to differences in the oxidation mechanism of the two reagents. NaIO₄ is known to mediate the oxidation of catechol moieties such as those found in coffee polyphenols into quinones, which either remain as such or undergo intermolecular addition reactions.³⁰ Consequently, these moieties will not be available for complexation with the Folin–Ciocalteu reagent, resulting in an apparent reduction in the polyphenol content. On the other hand, although it also causes the conversion of catechols into quinones, oxidation of polyphenols by CuSO₄ has been shown to increase their reactivity toward the Folin–Ciocalteu reagent, with Cu(II) acting as a redox carrier.³¹,³²

The antioxidant activity of coffee NPs prepared from air-, NaIO₄-, or CuSO₄-oxidized extracts was compared using a 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) assay. The colored reagent was incubated with increasing concentrations of each material, and the antioxidant activity was calculated by recording the decrease in absorbance of the ABTS radical which is proportional to the concentration of free-radical scavengers, that is, antioxidants. The results were expressed as % antioxidant activity relative to the ABTS blank solution and are presented in Figure S3–D. Antioxidant potency of the different NPs was calculated by fitting the data into dose–response curves to obtain the concentration equivalent to 50% antioxidant activity or EC₅₀.
As the antioxidant capacity of coffee extracts is closely related to their polyphenol content, it was expected that all air-oxidized extracts would result in similar ABTS radical scavenging activities. This was indeed the case with green and medium-roasted extracts, which exhibited comparable antioxidant potency (EC50 = 75.5 and 68.5 μg·mL−1, respectively). However, dark-roasted coffee extracts had significantly lower antioxidant activity with an EC50 of 149.7 μg·mL−1 (p < 0.05). This may be attributed to the roasting process, which has been shown to cause degradation of chlorogenic acid, the main constituent responsible for the antioxidant activity.33 Generally, there was a decrease in antioxidant activity for the NPs prepared by adding the oxidizing agents compared to those prepared by air oxidation. For example, NPs prepared from NaIO4-oxidized green coffee extracts exhibited ≈2-fold increase in their EC50 value compared to air oxidation (p < 0.01), but the value was not significantly different from that of NPs prepared through CuSO4 oxidation. In the case of medium-roasted coffee, oxidation by NaIO4 resulted in NPs with a slightly lower antioxidant potency compared to CuSO4 oxidation, and both types of NPs were less potent as antioxidants compared to NPs formed by air oxidation (p < 0.01). In the case of dark-roasted coffee, the antioxidant potency of NaIO4-oxidized NPs was comparable to those prepared by air oxidation.

Figure 5. (A) Polyphenol content of coffee extracts before UF and coffee NPs prepared by air, NaIO4, or CuSO4-mediated oxidation. The results (mean ± SD; n = 3) are expressed as milligram GAE per gram ground beans. ***p < 0.0001; **p < 0.001 based on two-way ANOVA, followed by Sidak’s post-hoc test; antioxidant activity measured by an ABTS assay for coffee NPs prepared from (B) green/unroasted, (C) medium-roasted, and (D) dark-roasted beans. The results are expressed as % antioxidant activity versus concentration of each material.

Table 1. Antioxidant Activity of Coffee NPs Expressed as the Mean ± SD (n = 3) of the Concentration Equivalent to 50% Antioxidant Activity (EC50)

| sample | coffee type | oxidant | EC50 (μg·mL−1) | p-valuea |
|--------|-------------|---------|----------------|----------|
| 1      | green       | air     | 75.5 ± 23.3    |          |
| 2      | NaIO4       | air     | 155.2 ± 29.0   | 0.0068 (relative to air-oxidized green coffee) |
| 3      | CuSO4       | air     | 101.1 ± 30.4   | 0.4218 (relative to air-oxidized green coffee) |
| 4      | medium      | air     | 68.5 ± 36.6    | ns (relative to air-oxidized green coffee) |
| 5      | NaIO4       | medium  | 219.0 ± 24.5   | 0.0001 (relative to air-oxidized medium coffee) |
| 6      | CuSO4       | medium  | 159.6 ± 23.2   | 0.0041 (relative to air-oxidized medium coffee) |
| 7      | dark        | air     | 149.7 ± 30.4   | 0.0184 (relative to air-oxidized dark coffee) |
| 8      | NaIO4       | dark    | 205.4 ± 23.1   | 0.0517 (relative to air-oxidized dark coffee) |
| 9      | CuSO4       | dark    | 276.6 ± 29.6   | 0.0157 (relative to air-oxidized dark coffee) |

aData based on one-way ANOVA, followed by Tukey’s post-hoc test.
obtained from air-oxidized extracts, whereas CuSO₄-oxidized NPs were associated with a significantly higher EC₅₀ (p < 0.05).

Overall, NPs prepared from green coffee bean extracts exhibited the greatest antioxidant activity after adding the oxidizing agents compared to those prepared from medium- and dark-roasted beans. Conversely, NPs prepared from dark-roasted coffee through CuSO₄ oxidation were associated with the lowest antioxidant activity at an EC₅₀ of 276.6 μg·mL⁻¹. Note that NaIO₄-oxidized NPs showed significant antioxidant activity despite the seemingly lower polyphenol content as revealed by the Folin–Ciocalteu assay. This finding indicates that the polyaromatic-rich nature of coffee polyphenols can still mediate free-radical scavenging activity even after oxidation of catechols into quinones, consistent with previous reports involving polyphenol NPs.³⁵⁻⁶

**Biocompatibility and Potential Bioactivity of Coffee NPs.** The antimicrobial activity of the prepared coffee NPs was assessed against bacterial (Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa) as well as fungal (Candida albicans) strains using the standard well diffusion method. None of the NPs displayed any inhibitory activity at the tested concentration (20 mg·mL⁻¹) as indicated by the lack of the zone of inhibition. Previous studies have reported the antimicrobial activity of coffee extracts against various microorganisms.³⁴,³⁵ However, these activities were imparted by small-molecule constituents such as chlorogenic acid, caffeic acid, trigonelline, and caffeine.³⁶ which were likely removed during UF or transformed during NP formation.

Biocompatibility assessment of NPs is usually performed in vitro using normal cell lines such as fibroblasts, macrophages, and keratinocytes by conducting viability assays. Alternatively, hemolysis assays may be applied to evaluate the potential adverse effects of the NPs on red blood cells.³⁷⁻⁴⁰ In this study, biocompatibility of coffee NPs was assessed in human dermal fibroblasts as a model normal cell line. The effect of coffee NPs on the viability of MCF7 cells as a representative human cancer cell line was also investigated. Cells were incubated with various concentrations of the NPs, and cell viability was expressed relative to untreated controls (Figure 6). In fibroblasts (Figure 6A–C), the results showed that the NPs were well tolerated up to 1 mg·mL⁻¹. NPs prepared from CuSO₄-oxidized green and medium-roasted coffee extracts started to exhibit moderate cytotoxicity (≈50% viability) at the highest concentration tested of 1 mg·mL⁻¹, but no half-maximal inhibitory concentration (IC₅₀) could be determined from the dose–response curves. The observed reduction in cell viability may be ascribed to the pro-oxidant properties of chlorogenic acid-Cu(II) complexes, which have been shown to induce DNA damage.⁴¹⁻⁴³ This effect was more pronounced in MCF7 cancer cells, where NPs prepared by CuSO₄ oxidation exhibited IC₅₀ values of 42, 40, and 57 μg·mL⁻¹ for green, medium-roasted, and dark-roasted coffee NPs. Air-oxidized green and medium-roasted coffee NPs exhibited lower potencies with IC₅₀ values of 212 and 154 μg·mL⁻¹, respectively, whereas no IC₅₀ could be determined for air-oxidized dark coffee NPs or all NPs prepared by NaIO₄ oxidation. These differences likely resulted from the changes in coffee’s chemical constituents, particularly the chlorogenic acid content, as a function of roasting. For example, a recent study by Mojica et al. found that lighter coffee roasts were associated with more potent anticancer activity than dark roasts, which was correlated with the total phenol content.³⁴ This also explains the limited antiproliferative activity of coffee NPs prepared by NaIO₄ oxidation, which were associated with the lowest total phenol content (Figure 5A). Similar results were reported for QCT NPs synthesized by NaIO₄-mediated oxidative coupling.⁵ The fact that coffee NPs were able to maintain some of the anticancer activity but not the antimicrobial activity of the crude extracts may be attributed to differences in molecular mechanisms of action at the cellular level, which warrants further investigation.
CONCLUSIONS

In this work, we report the facile formation of untemplated NPs from coffee bean aqueous extracts via oxidative coupling of soluble polyphenols. NPs were synthesized from green, medium-roasted, and dark-roasted ground Arabica beans by air-, NaIO\textsubscript{4}, or CuSO\textsubscript{4}-mediated oxidation, resulting in the formation of spherical particles with unique colors and variable sizes depending on the reaction conditions. While auto-oxidation by air may be sufficient to produce NPs from coffee extracts, adding oxidizing agents such as NaIO\textsubscript{4} and CuSO\textsubscript{4} is recommended in order to maintain batch-to-batch consistency. NPs with skin tone-matching colors (e.g., NPs from medium- and dark-roasted coffee and NPs from air- and NaIO\textsubscript{4}-oxidized green coffee) are good candidates for cosmetic applications. NPs with sizes <100 nm may be suited for targeted drug delivery applications. All NPs exhibited significant antioxidant activity, highlighting their potential utility in biomedicine by delivery applications. All NPs exhibited significant antioxidant activity, highlighting their potential utility in biomedicine by delivery applications.

Verification of NP Formation. The ability of coffee bean extracts to form NPs was verified by DLS and TEM. For DLS, freshly prepared extracts were diluted appropriately with ultrapure water and both the particle size and zeta potential were measured using a Nicomp Nano Z3000 instrument (Particle Sizing Systems, Santa Barbara, CA, USA). Measurements were reported as the mean ± SD from at least three trials. For TEM imaging, 10 μL of each freshly prepared extract was placed on 300-mesh Formvar-coated copper grids (Electron Microscopy Sciences, Hatfield, PA, USA) for 1 min. The excess liquid was blotted with a filter paper, and the grids were air-dried. Images were captured using a Morgagni 268 TEM (FEI, Netherlands) at an accelerating voltage of 60 kV.

Spectroscopic Characterization of Coffee NPs. Coffee NPs were characterized by FT-IR and UV–vis spectroscopy. For FT-IR, lyophilized coffee NPs were prepared as KBr discs and FT-IR spectra were recorded between 4500 and 650 cm\textsuperscript{-1} using an IR Affinity-1 spectrometer (Shimadzu, Kyoto, Japan). For UV–vis, freshly prepared NP dispersions were diluted 100× in ultrapure water and the absorbance spectra were scanned between 220 and 500 nm using a Shimadzu UV-1800 spectrophotometer (Kyoto, Japan).

Differential Scanning Calorimetry Analysis. DSC analysis was performed on lyophilized samples of each NP. Thermograms were recorded using a DSC 1 STARE System (Mettler Toledo, Columbus, OH, USA). Approximately 1 mg of each sample was heated in an aluminum pan from 25 to 350 °C, and the scanning rate was conducted at 10 °C/min.

Determination of the Total Phenol Content of Coffee NPs. The total phenol content of the NPs was determined by the Folin–Ciocalteu method using gallic acid standards as previously described with some modification.\textsuperscript{45} Briefly, 1 mL of Folin–Ciocalteu’s phenol reagent (diluted 10 times with ultrapure water) was added to 15 mL conical tubes containing 250 μL of each NP dispersion or gallic acid standards (15, 25, 50, 75, and 100 μg·mL\textsuperscript{-1} in ultrapure water). After 5 min, 1 mL of 10% NaHCO\textsubscript{3} was added to each tube, the samples were incubated at room temperature in the dark for 30 min, and then the absorbance was measured at 765 nm (Shimadzu UV-1800 spectrophotometer). Gallic acid standards were used to construct a calibration curve of absorbance at 765 nm versus concentration, from which the total phenol content in coffee NPs was determined. The results were expressed as milligram GAE per gram ground coffee beans (mean ± SD) from three independent experiments.

Antioxidant Activity of Coffee NPs. Antioxidant activity of the NPs was determined using the ABTS method.\textsuperscript{46} For the assay, 30 mg of the ABTS salt was dissolved in 15 mL of ultrapure water. The ABTS radical was generated by incubating the solution with an equal volume of KMnO\textsubscript{4} (2.4 mM in ultrapure water) under vigorous stirring in the dark for 16 h. The ABTS working solution was diluted with ultrapure water until the absorbance at 743 nm reached 0.7. Then, 1 mL of the working solution was added to 200 μL of ultrapure water containing 0–5000 μg·mL\textsuperscript{-1} coffee NPs. The mixture was incubated in the dark for 30 min before reading the absorbance at 743 nm. Antioxidant activity was expressed according to eq 1.

EXPERIMENTAL SECTION

Samples and Reagents. Ground Arabica coffee beans (green, medium-roasted, and dark-roasted), of Brazilian origin, were obtained from a local store (Amman, Jordan). Sodium metaperiodate (NaIO\textsubscript{4}), copper(II) sulfate (CuSO\textsubscript{4}), Folin–Ciocalteu’s phenol reagent, gallic acid, and sodium bicarbonate (NaHCO\textsubscript{3}) were obtained from Sigma-Aldrich (St Louis, MO, USA). ABTS was obtained from Abcam (Cambridge, UK). Water (ultrapure grade) was prepared using a Direct-Q 5UV system (EMD Millipore, Thermo Fisher Scientific, Waltham, MA, USA). ABTS was obtained from Abcam (Cambridge, UK). ABTS was obtained from Abcam (Cambridge, UK). ABTS was obtained from Abcam (Cambridge, UK).

Preparation of Coffee Bean Aqueous Extracts. For the preparation of air-oxidized extracts, ground coffee beans (2 g) were weighed in a 100 mL Erlenmeyer flask, followed by adding boiling water (25 mL), and the mixture was stirred vigorously for 15 min. Afterward, the mixture was filtered using a filter paper to remove insoluble coffee grounds and cooled to room temperature. The mixture was further purified by UF (Pierce Protein Concentrator, 10 kD MW cutoff, Thermo Scientific, Waltham, MA, USA) at 4000 g and 4 °C for 15 min (Hermle Z326K centrifuge, Wehingen, Germany), with repeated washing with ultrapure water twice. For the preparation of NaIO\textsubscript{4}- and CuSO\textsubscript{4}-oxidized extracts, 200 mg of NaIO\textsubscript{4} or CuSO\textsubscript{4} was added to the ground beans (2 g) before adding boiling water (25 mL), and the mixture was further processed as described above. The obtained extracts were stored at 4 °C or lyophilized using a FreeZone 4.5 L Benchtop Freeze Dryer (Labconco Corporation, Kansas City, MO, USA).

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antioxidant activity (%) = \[1 - (A_{\text{sample}}/A_{\text{ABTS}})\] \times 100\%

(1)

where \(A_{\text{sample}}\) is the absorbance of each sample after incubation with the ABTS working solution and \(A_{\text{ABTS}}\) is the absorbance of the ABTS working solution diluted with 200 \(\mu\)L of ultrapure water. The results were expressed as the mean antioxidant activity ± SD from three independent experiments.

**Antimicrobial Activity of Coffee NPs.** To assess the antimicrobial activity of the coffee NPs, lyophilized samples were dissolved in ultrapure water at a concentration of 20 mg mL\(^{-1}\). Stock solutions were sterilized for 30 min under a UV sterilizer. Sterility was tested by inoculating the post-sterilized material into a nutrient broth (Oxoid, Basingstoke, UK). No growth under aerobic incubation at 37 °C indicated sterility. The antimicrobial activity of the prepared coffee NPs was assessed using the standard well diffusion method according to the Clinical and Laboratory Standards Institute (CLSI). The experiment was performed using the following standard bacterial and fungal strains: S. aureus ATCC 43300, S. aureus ATCC 29213, E. coli ATCC 8739, P. aeruginosa PAO1 ATCC 47085, and C. albicans ATCC 10231. Briefly, microorganisms were evenly spread onto the surface of Muller Hinton agar media (Oxoid, Basingstoke, UK) using a sterile cotton swab immersed into 0.5 McFarland standard of microbial suspension. Wells of 8 mm were punched aseptically with a sterile cork borer. Each well was filled with 50 \(\mu\)L of NP stock solutions. The plate was then incubated overnight aerobically at 37 °C. The zone of inhibition resulting after incubation was assessed using the standard well diffusion method according to the CLSI.

**Effect of Coffee NPs on In Vitro Cell Viability.** Coffee NPs were evaluated in human dermal fibroblasts as a representative normal cell line and MCF7 breast cancer cells as a model cancer cell line. Cells were obtained from American Type Culture Collection (ATCC, Manassas, VA, USA) and cultured as previously reported. One day before the experiment, cells were trypsinized from a confluence flask and seeded in 96-well plates at 1.0 \(\times\) 10\(^4\) cells per well (\(n = 4\)). After allowing the cells to adhere for 24 h, each well was treated with 0–1000 \(\mu\)g mL\(^{-1}\) NPs in the complete medium for 24 h. At the end of the incubation period, the MTT assay was carried out as previously described. Cell viability was expressed as % viability relative to untreated controls.

**Statistical Analysis.** Data analysis was performed in Graphpad Prism 6.0e. All values were reported as the mean ± SD from at least three independent experiments. Differences in sample means were evaluated by one- or two-way analysis of variance (ANOVA), followed by Tukey’s or Sidak’s post-hoc test, respectively, where \(p < 0.05\) was considered statistically significant.

**ASSOCIATED CONTENT**

1. *Supporting Information*

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c05061.

**FT-IR and UV−vis spectra and characterization of coffee NPs by DLS (PDF)**

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**Notes**

The authors declare no competing financial interest.

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**REFERENCES**

(1) Panche, A. N.; Diwan, A. D.; Chandra, S. R. Flavonoids: An overview. *J. Nutr. Sci.* 2016, *S*, No. E47.

(2) Chen, Z.; Wang, C.; Chen, J.; Li, X. Biocompatible, functional spheres based on oxidative coupling assembly of green tea polyphenols. *J. Am. Chem. Soc.* 2013, 135, 4179–4182.

(3) Xiang, S.; Yang, F.; Guo, H.; Zhang, S.; Zhang, X.; Zhu, F.; Li, Y. Green tea makes polyphenol nanoparticles with radical-scavenging activities. *Macromol. Rapid Commun.* 2017, 38, 1700446.

(4) Sunoqrot, S.; Al-Debsi, T.; Al-Shalabi, E.; Hasan Ibrahim, L.; Faruq, F. N.; Walters, A.; Palgrave, R.; Al-Jamal, K. T. Bioinspired polymerization of quercetin to produce a curcumin-loaded nanomedicine with potent cytotoxicity and cancer-targeting potential in vivo. *ACS Biomater. Sci. Eng.* 2019, *S*, 6036–6045.

(5) Sunoqrot, S.; Al-Shalabi, E.; Hasan Ibrahim, L.; Zalloum, H. Nature-inspired polymerization of quercetin to produce antioxidant nanoparticles with controlled size and skin tone-matching colors. *Molecules* 2019, *24*, 3815.

(6) Sunoqrot, S.; Al-Shalabi, E.; Messersmith, P. B. Facile synthesis and surface modification of bioinspired nanoparticles from quercetin for drug delivery. *Biomater. Sci.* 2018, *6*, 2656–2666.

(7) Sileka, T. S.; Barrett, D. G.; Zhang, R.; Lau, K. H. A.; Messersmith, P. B. Colorless multifunctional coatings inspired by polyphenols found in tea, chocolate, and wine. *Angew. Chem., Int. Ed.* 2013, *52*, 10766–10770.

(8) Zhang, S.; Jiang, Z.; Wang, X.; Yang, C.; Shi, J. Facile method to prepare microcapsules inspired by polyphenol chemistry for efficient enzyme immobilization. *ACS Appl. Mater. Interfaces* 2015, *7*, 19570–19578.
varieties affected by the degree of roasting. Comparative study of polyphenols and caffeine in different coffee samples. ACS Appl. Mater. Interfaces 2018, 10, 39353–39362.

(12) Shin, M.; Park, E.; Lee, H. Plant-inspired pyrogallol-containing functional materials. Adv. Funct. Mater. 2019, 29, 1903022.

(13) Fernandes, L.; Messias, B.; Pereira-Neves, A.; Azevedo, E. P.; Araújo, J.; Foguel, D.; Palhano, F. L. Green tea polyphenol microparticles based on the oxidative coupling of EGCG inhibit amyloid aggregation/cytotoxicity and serve as a platform for drug delivery. ACS Biomater. Sci. Eng. 2020, 6, 4414–4423.

(14) Quideau, S.; Deffieux, D.; Douat-Casassus, C.; Pouységuy, L. Plant polyphenols: Chemical properties, biological activities, and synthesis. Angew. Chem., Int. Ed. 2011, 50, 586–621.

(15) Gómez-López, P.; Puente-Santiago, A.; Castro-Beltrán, A.; Santos do Nascimento, L. A.; Balu, A. M.; Luque, R.; Alvarado-Beltrán, C. G. Nanomaterials and catalysis for green chemistry. Curr. Opin. Green Sustain. Chem. 2020, 24, 48–55.

(16) Kobayashi, S. Green polymer chemistry: new methods of polymer synthesis using renewable starting materials. Struct. Chem. 2017, 28, 461–474.

(17) Naahidi, S.; Jafari, M.; Edalat, F.; Raymond, K.; Khademhosseini, A.; Chen, P. Biocompatibility of engineered nanoparticles for drug delivery. J. Controlled Release 2013, 166, 182–194.

(18) Duan, H.; Wang, D.; Li, Y. Green chemistry for nanoparticle synthesis. Chem. Soc. Rev. 2015, 44, 5778–5792.

(19) Guo, J.; Suma, T.; Richardson, J. J.; Ejima, H. Modular assembly of biomaterials using polyphenols as building blocks. ACS Biomater. Sci. Eng. 2019, 5, 5578–5596.

(20) Zhou, J.; Lin, Z.; Ju, Y.; Rahim, M. A.; Richardson, J. J.; Caruso, F. Polyphenol-mediated assembly for particle engineering. Acc. Chem. Res. 2020, 53, 1269–1278.

(21) Hečamolinov, I.; Beličák-Cvitanović, A.; Horžić, D.; Komes, D. Comparative study of polyphenols and caffeine in different coffee varieties affected by the degree of roasting. Food Chem. 2011, 129, 991–1000.

(22) Król, K.; Gantner, M.; Tatarak, A.; Hallmann, E. The content of polyphenols in coffee beans as roasting, origin and storage effect. Eur. Food Res. Technol. 2020, 246, 33–39.

(23) Moreira, A. S. P.; Nunes, F. M.; Domingues, M. R.; Coimbra, M. A. Coffee melanoidins: Structures, mechanisms of formation and potential health impacts. Food Funct. 2012, 3, 903–915.

(24) Marcucci, C.; Dias, R.; Almeida, M.; Benassi, M. Antioxidant activity of commercial soluble coffees. Beverages 2017, 3, 27.

(25) Rao, N. Z.; Fuller, M. Acidity and antioxidant activity of cold brew coffee. Sci. Rep. 2018, 8, 1–9.

(26) Herawati, D.; Giriwono, P. E.; Dewi, F. N. A.; Kashiwagi, T.; Andarwulan, N. Critical roasting level determines bioactive content and antioxidant activity of Robusta coffee beans. Food Sci. Biotechnol. 2019, 28, 7–14.

(27) Kim, J. Y.; Kim, S.; Han, S.; Han, S. Y.; Passos, C. P.; Seo, J.; Lee, H.; Kang, E. K.; Mano, J. F.; Coimbra, M. A.; Park, J. H.; Choi, I. S. Coffee melanoidin-based multipurpose film formulation: Application to single-cell nanoencapsulation. ChemNanoMat 2020, 6, 379–385.

(28) Craig, A. P.; Franca, A. S.; Oliveira, L. S. Evaluation of the potential of FTIR and chemometrics for separation between defective and non-defective coffees. Food Chem. 2012, 132, 1368–1374.

(29) Correia, R. M.; Loureiro, L. B.; Rodrigues, R. R. T.; Costa, H. B.; Oliveira, B. G.; Filgueiras, P. R.; Thompson, C. J.; Lacerda, V.; Romão, W. Chemical profiles of Robusta and Arabica coffee by ESI(−)-FT-ICR MS and ATR-FTIR: A quantitative approach. Anal. Methods 2016, 8, 7678–7688.

(30) Muñoz, J.; García-Molina, F.; Varon, R.; Rodríguez-Lopez, J. N.; García-Ruiz, P. A.; García-Cañovas, F.; Tudela, J. Kinetic characterization of the oxidation of chlorogenic acid by polyphenol oxidase and peroxidase. Characteristics of the ß-quinone. J. Agric. Food Chem. 2007, 55, 920–928.

(31) Evrett, J. D.; Bryant, Q. M.; Green, A. M.; Abbey, Y. A.; Wang, G. W.; Walker, R. B. Thorough study of reactivity of various compound classes toward the Folin-Ciocalteau reagent. J. Agric. Food Chem. 2010, 58, 8139–8144.

(32) Huang, D.; Ou, B.; Prior, R. L. The chemistry behind antioxidant capacity assays. J. Agric. Food Chem. 2005, 53, 1841–1856.

(33) Cho, A. R.; Park, K. W.; Kim, K. M.; Kim, S. Y.; Han, J. Influence of roasting conditions on the antioxidant characteristics of Colombian coffee (Coffea arabica L.) beans. J. Food Biochem. 2014, 38, 271–280.

(34) Bajko, E.; Kalinowska, M.; Borowski, P.; Siergiejczyk, L.; Lewandowski, W. 5-O-Caffeoylquinic acid: A spectroscopic study and biological screening for antimicrobial activity. LWT—Food Sci. Technol. 2016, 65, 471–479.

(35) Runti, G.; Pacor, S.; Colomban, S.; Gennaro, N.; Navarini, L.; Scocchi, M. Arabica coffee extract shows antibacterial activity against Staphylococcus epidermidis and Enterococcus faecalis and low toxicity towards a human cell line. LWT—Food Sci. Technol. 2015, 62, 108–114.

(36) Daglia, M.; Papetti, A.; Grisoli, P.; Aceti, C.; Spini, V.; Dacarro, C.; Gazzani, G. Isolation, identification, and quantification of roasted coffee antibacterial compounds. J. Agric. Food Chem. 2007, 55, 10208–10213.

(37) Jadhav, K.; Kr, R.; Deshpande, S.; Jagwani, S.; Dhamecha, D.; Jalapure, S.; Subburayan, K.; Baheti, R. Phytosynthesis of gold nanoparticles: Characterization, biocompatibility, and evaluation of its osteoinductive potential for application in implant dentistry. Mater. Sci. Eng., C 2018, 93, 664–670.

(38) Canfarotta, F.; Waters, A.; Sadler, R.; McGill, P.; Guerreiro, A.; Papkovsky, D.; Haupt, K.; Piletsky, S. Biocompatibility and internalization of molecularly imprinted nanoparticles. Nano Res. 2016, 9, 3463–3477.

(39) Jadhav, K.; Deore, S.; Dhamecha, D.; H R, R.; Jalapure, S.; Bohara, R. Phytosynthesis of Silver Nanoparticles: Characterization, Biocompatibility Studies, and Anticancer Activity. ACS Biomater. Sci. Eng. 2018, 4, 892–899.

(40) Eiras, F.; Amaral, M. H.; Silva, R.; Martins, E.; Lobo, J. M. S.; Silva, A. C. Characterization and biocompatibility evaluation of cutaneous formulations containing lipid nanoparticles. Int. J. Pharm. 2017, 519, 373–380.

(41) Karpinska, J.; Swislocka, R.; Lewandowski, W. A mystery of a cup of coffee; an insight look by chemist. BioFactors 2017, 43, 621–632.

(42) Zheng, L.-F.; Dai, F.; Zhou, B.; Yang, L.; Liu, Z.-L. Prooxidant activity of hydroxycinamic acids on DNA damage in the presence of Cu (II) ions: mechanism and structure—activity relationship. Food Chem. Toxicol. 2008, 46, 149–156.

(43) Iwasaki, Y.; Hiratsawa, T.; Maruyama, Y.; Ishii, Y.; Ito, R.; Saito, K.; Umemura, T.; Nishikawa, A.; Nakazawa, H. Effect of interaction between phenolic compounds and copper ion on antioxidant and pro-oxidant activities. Toxicol. in Vitro 2011, 25, 1320–1327.

(44) Mojica, B. E.; Fong, L. E.; Biju, D.; Muharram, A.; Davis, I. M.; Vela, K. O.; Rios, D.; Osorio-Camacena, E.; Kaur, B.; Rojas, S. M.; Forester, S. C. The impact of the roast levels of coffee extracts on their potential anticancer activities. J. Food Sci. 2018, 83, 1125–1130.

(45) Singleton, V. L.; Orthofer, R.; Lamuela-Raventos, R. M. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods Enzymology; Elsevier, 1999; Vol. 299; pp 152–178.

(46) Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS
radical cation decolorization assay. Free Radical Biol. Med. 1999, 26, 1231–1237.

(47) CLSI. Performance Standards for Antimicrobial Susceptibility Testing, 27th ed.; Clinical and Laboratory Standards Institute: Wayne, PA, 2017.