https://doi.org/10.17221/135/2020-CJFS

Antioxidant potential of tea assessed by optical absorption spectroscopy in DNA-encased carbon nanotubes

Lijun Wang[1], Shusuke Oura[2], Yongbo Wu[3]*

[1]*Department of Materials Science and Engineering, Xihua University, Chengdu, P.R. China*
[2]*Department of Physics, Tokyo University of Science, Tokyo, Japan*
[3]*Department of Mechanical and Energy Engineering, Southern University of Science and Technology, Shenzhen, P.R. China*

**Corresponding author: Wlijun1031@outlook.com*

Citation: Wang L., Oura S., Wu Y. (2021): Antioxidant potential of tea assessed by optical absorption spectroscopy in DNA-encased carbon nanotubes. Czech J. Food Sci., 39: 23–28.

Abstract: It is essential to develop a simple method to assess food quality quantitatively. Available methods primarily rely on nanotechnology and offer high selectivity and sensitivity. In this study, we aimed to develop a sensitive nanoprobe, and, to this end, a double-stranded DNA-encased HiPco carbon nanotube (dsDNA-HiPco) hybrid was prepared and used to evaluate the antioxidant potential of a Chinese tea against hydrogen peroxide (H_2O_2) with a range of irradiation wavelengths. The morphology and dispersion of the hybrids were analysed using atomic force microscopy, which showed that dsDNA wrapped on the SWCNT surface well and homogeneous dispersion of the rod-shaped tubes while the concentration of dsDNA was 1 mg mL^{-1}. The antioxidant effect of Chinese tea was evaluated by using near-infrared absorption and photoluminescence of the hybrid. Experimental results revealed that the tea exerted excellent antioxidant effects when the hybrid was pre-treated with 0.03% wt H_2O_2. Catechin present in the Chinese tea played a pivotal role in exerting the antioxidant effects. Therefore, a simple detection method proposed herein can be successfully applied in various fields, including biology, medicine, and the food industry.

Keywords: antioxidant potency; atomic force microscopy; double-stranded DNA; NIR absorption spectroscopy; single-walled carbon nanotubes

Since its discovery, the single-walled carbon nanotube (SWCNT) has found numerous applications owing to its unique physical and chemical properties (Boghossian et al. 2011; Koh et al. 2011). Despite controversy over its potential toxicity (Fu and Li 2010), SWCNT has been extensively studied in recent years and is considered an ideal material for biological applications ranging from DNA detection (Chen et al. 2008; Boghossian et al. 2011) to drug delivery (Elhissi et al. 2012). Many studies have revealed that the unique structural and chemical properties of SWCNT, which provide a relatively large surface area (i.e. ~300 m^2 g^{-1}), make it a suitable carrier for large molecules such as enzymes and DNA (Xu et al. 2017). Additionally, its near-infrared (NIR) optical properties, which are highly sensitive to environmental changes, led to the development of SWCNT-based optical biosensors for *in vitro* remote detection (Yum et al. 2013). However, the main shortcoming of such applications is related to the lack of its solubility, when SWCNTs tend to form aggregations in water. To address this issue, SWCNT has been coated with DNA oligos, sodium dodecyl sulphate (SDS), and polymers which increase its water solubility (Koh et al. 2011). To date, SDS- and DNA-encased SWCNTs have been commonly used as optical biosensor components for detection in medicine or food (Lei and Ju 2010; Blanch and Shapter 2014).

Additionally, a study reported that DNA-enfolded CNTs could form a stable hybrid material (Zheng et al. 2003) owing to the interaction between negatively charged DNA and the positively charged surface of CNTs. This interaction can be modulated by changing the electronic properties of the CNTs that would thereby reduce aggregation and promote high disper-

https://doi.org/10.17221/135/2020-CJFS

sion of the hybrid materials. Moreover, it was reported that DNA-SWCNT hybrids, prepared by mixing a DNA solution with an aqueous suspension of SWCNT followed by sonication, exhibit different responses to radiation at different wavelengths. When the hybrid is irradiated with an NIR source, the light is absorbed only by the SWCNT because DNA absorbs light at approximately 260 nm (Ishibashi et al. 2018). This selective response of a DNA-CNT hybrid opens up a wide range of possibilities in developing new technologies for gene investigation and food analysis based on near-infrared absorption (NIR-ABS) and photoluminescence (NIR-PL) phenomena. Tu et al. (2007) studied the redox reaction mechanism of single-stranded DNA-encased (ssDNA) high-pressure carbon monoxide (HiPco) nanotubes with H_2O_2 through NIR-ABS. They found that NIR-ABS spectral changes could be reversed by tuning the pH, and the sensitivity of NIR-ABS was enhanced in the pH range of 6–8. A biosensor based on DNA-SWCNT hybrids was developed to measure the antioxidant activity of caffeine, regular coffee, and decaffeinated coffee against reactive oxygen species (ROS), such as H_2O_2 and hydroxyl radicals by NIR-ABS (Zhao et al. 2015). By comparing the PL and NIR properties of SWCNTs dispersed with salmon genomic DNA (SaDNA) and sequence repeats of alternating G and T [d (GT) 20], Kim et al. (2008) showed that genomic DNA specifically enriched (6, 5) SWCNTs. Kurnosov et al. (2017) used PL from semiconducting SWCNTs to detect cysteine and found that the PL intensity was enhanced by 27% at a cysteine concentration of 10^{-3} M. It was hypothesised that the PL intensity increased owing to a passivation effect induced by the reactive thiol groups of cysteine on the nanotube p-defects.

In the present study, dsDNA-encased HiPco SWCNTs were prepared to evaluate the antioxidant activity of Chinese tea. Atomic force microscopy (AFM) was used to confirm the dispersion of HiPco tubes. Additionally, NIR-ABS and PL were both measured to detect the antioxidant activity of the tea in the presence of H_2O_2 with dsDNA-HiPco hybrids using different wavelengths (Zhao et al. 2015; Xu et al. 2017). By conducting measurements using known concentrations of tea, the tea was found to reduce the oxidised dsDNA-HiPco hybrids, and this effect was dependent on the amount of antioxidants. Furthermore, the impact of brewing temperature and the number of brewing cycles on antioxidant potency of the tea was investigated.

This study aimed to provide a simple and effective method for evaluating the antioxidant effect of samples against H_2O_2 using dsDNA-HiPco hybrids as biosensors. The method we provide is expected to satisfy the requirements of biomedical and food applications adequately.

MATERIAL AND METHODS

Material. High purity SWCNTs synthesised by high-pressure co-conversion (HiPco SWCNTs) were purchased from Unidym Inc. (USA). Double-stranded DNA (dsDNA) from salmon testes (D1626 type) was purchased from Sigma-Aldrich (USA). Hydrogen peroxide (H_2O_2, 30% wt) and Tris(hydroxyethyl)aminomethane hydrochloride (Tris-HCl) were obtained from Wako Pure Chemical Industries (Japan). And 3-(2-aminoethyl aminopropyl)-trimethoxysilane and mica were obtained from Life Technologies Japan Ltd. (Japan). Refined Chinese tea (China) was purchased from a local supermarket in China.

Preparation of dsDNA-HiPco SWCNTs hybrids and tea solutions. The stock suspensions of dsDNA-HiPco SWCNTs in Tris-HCl buffer solution (10 mM, pH = 8.0) were prepared as previously described (Wang and Umemura 2019). Briefly, in a typical experiment 0.5 mg of pristine HiPco SWCNT powder was added to 1 mL of dsDNA with different concentrations (i.e. 0.5, 1.0, 2.0 mg mL^{-1}) in a Tris-HCl buffer solution. Next, the mixture was sonicated in an ice-water bath at 3 W (VCX 130; Sonics & Materials, Inc., USA) for 2 h to ensure dispersion of HiPco SWCNTs. The suspension was centrifuged for 3 h (MX-150; Tomy Seiko, Japan) at 17 360 g and 8 °C to remove undispersed HiPco SWCNTs. After centrifugation, a 0.7 mL sample of the supernatant was obtained and stored in a refrigerator for 24 h. The results showed that the supernatant stored in a refrigerator for 24 h retains its properties.

To prepare the tea solution, 5 mg of Chinese tea was brewed in 1 000 mL of purified water at different temperatures (i.e. 20, 30, 40, 50, 60, 70, 80, 90, 100 °C). In addition, the tea was brewed repeatedly to obtain different concentrations. The resulting fresh tea solutions were used for evaluating the antioxidant activity without further treatment.

Atomic force microscopy (AFM). We analysed the hybrids in AC mode using an MFP-3D microscope equipment (Asylum Research, USA). For AFM sample preparation, a hybrid solution with a dilution factor of 1 : 200 was prepared by mixing 1 µL of hybrid with 199 µL of Tris-HCl buffer solution. The surface of newly cleaved mica substrate was pretreated with 0.01% 3-(2-aminoethyl aminopropyl)-trimethoxysilane. Next, 10 µL of the diluted HiPco SWCNT solution was added and incubated for 10 min at room tem-

https://doi.org/10.17221/135/2020-CJFS

perature (23 ± 2 °C). The samples were rinsed with 1 mL of pure water and air-dried overnight. A cross-sectional AFM height analysis was performed for the hybrids prepared with different dsDNA concentrations. Subsequently, we calculated the diameter distributions of the hybrids based on 400 cross-sections of 50 hybrids for each sample.

Antioxidant activity and spectral measurements. To evaluate the tea antioxidant activity using the tea brewed at different temperatures and after several brewing cycles, 10 µL of the tea solution was added to the H_2O_2-reacted dsDNA-HiPco solution, and the absorption spectra were recorded after 10 min. All NIR absorption spectra were measured between 700 and 1 350 nm using 10 mm path length quartz cells and a UV-vis-NIR spectrophotometer (SolidSpec-3700DUV; Shimadzu, Japan) at room temperature. Each measurement was repeated three times for accuracy. According to the report of Koh et al. (2011), the extinction coefficient of HiPco SWCNT is 17.4 mg mL^{-1} cm^{-1} at 808 nm. Hence, the concentration of HiPco SWCNT in the dispersed samples was determined using the IR absorbance at 808 nm.

PL maps were measured using a NIR-PL spectrometer (Shimadzu, Japan) by varying the excitation wavelength from 700 to 850 nm. The sample preparation and experimental procedures performed were similar to those in NIR measurements. The absorbances were also measured in triplicate.

RESULTS AND DISCUSSION

AFM of dsDNA-HiPco hybrids. Representative images for the hybrids at various DNA concentrations are illustrated in Figure 1. The rod-like structures were homogeneously dispersed. Moreover, because the surface of HiPco SWCNT was wrapped with dsDNA to form a stable hybrid, the diameter of the rod-like hybrids is expected to be larger than that of pure SWCNT. To obtain a quantitative measure of rod diameter, 400 cross-sections of 50 hybrids were used. The diameter of the hybrids was found to vary from 0.82 to 1.32 nm. More specifically, the diameter of hybrids containing 0.5 mg mL^{-1} dsDNA as shown in Figure 1A was approximately 0.82 nm. When the concentration was increased to 1.0 mg mL^{-1}, the diameter of the hybrids increased to 1.32 nm (Figure 1B). Further increases in dsDNA concentration (i.e. 2.0 mg mL^{-1}) increased the diameter of the resulting hybrids to approximately 1.92 nm (Figure 1). These results conclusively show that the hybrid diameter

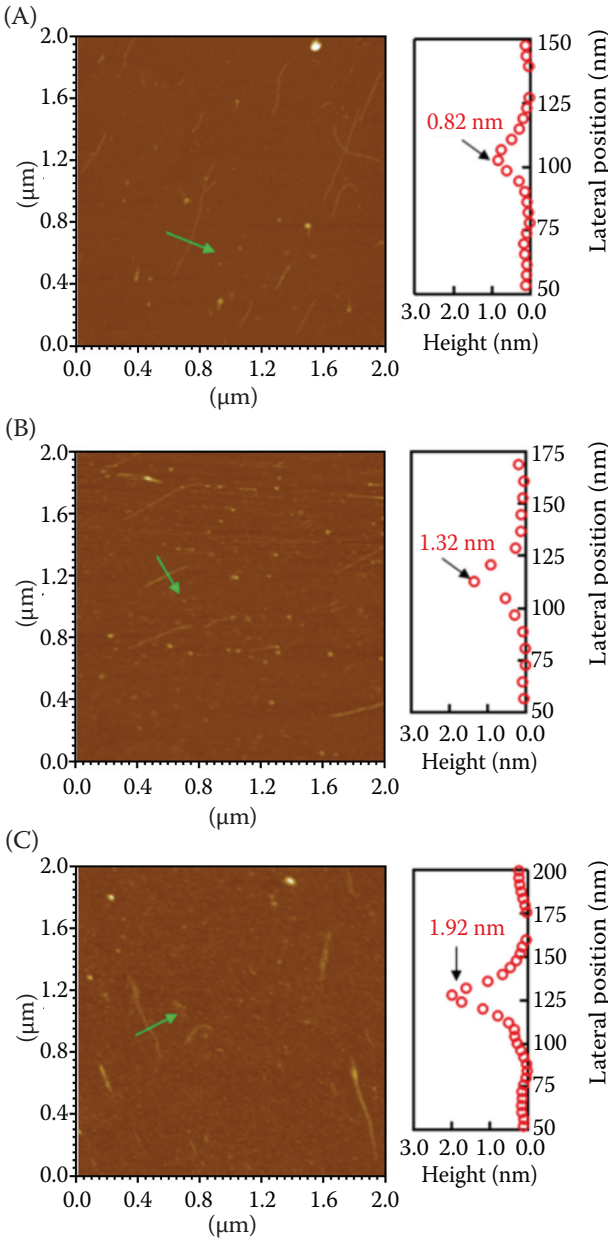

Figure 1. AFM images of dsDNA-HiPco hybrids with different DNA concentrations

increases with the increasing dsDNA concentration. When the concentration of dsDNA was 1 mg mL^{-1}, dsDNA wrapped on the SWCNT surface well, and the resulting SWCNT hybrids were dispersed homogeneously. Therefore, this concentration was selected for subsequent experiments.

Antioxidant activity of Chinese tea. Based on our previous study, the oxidation capacity of H_2O_2 is stable after 60 min (Wang et al. 2019). Therefore, to accurately measure the antioxidant activity of the selected tea, the NIR-ABS and PL properties were measured after reacting the hybrids with H_2O_2 for 60 min. The antioxi-

https://doi.org/10.17221/135/2020-CJFS

dant activity of Chinese tea was evaluated based on NIR-ABS and PL measurements. Two effects were investigated. The first is the effect of tea brewing temperature on the antioxidant activity. The spectra recorded after 10 min for the reaction between 0.03% wt H_2O_2 and the tea solution in the presence of dsDNA-HiPco are illustrated in Figure 2.

As a first observation, it is evident that the addition of the tea solution to the dsDNA-HiPco + 0.03% wt H_2O_2 mixture resulted in a rapid increase in the absorbance intensity of the bands between 1 100 and 1 300 nm, especially the band at 1 264 nm. The most compelling intensity change belongs to the tea solution prepared at 20 °C (i.e. red line) where the absorbance intensity at peak 1 (P1) increased from 0.728 4 to 0.773 5 (6.2%), and the absorbance intensity at peak 3 (P3) increased from 0.555 6 to 0.668 4 (20.3%).

These results clearly reveal the high antioxidant activity of tea against H_2O_2. As stated previously, the band at 1 264 nm is extremely sensitive. The absorbance intensity of this band increases with increasing tea-brewing temperature until 100 °C. Hence, for the reaction performed with the extract prepared at 100 °C (i.e. dark red line), the absorbance intensities at P1 and peak 2 (P2) are approximately 0.801 6 and 0.713 2, respectively, which correspond to a total increase of 10.0% and 28.4%. Regarding the band at P3, its intensity not only increased with the brewing temperature, but also its position was slightly shifted toward higher wavelengths. This is primarily owing to an increase in catechin concentration as the brewing temperature increased, which also resulted in enhanced antioxidant activity. The shift was less than a 1.0% change, which suggests that

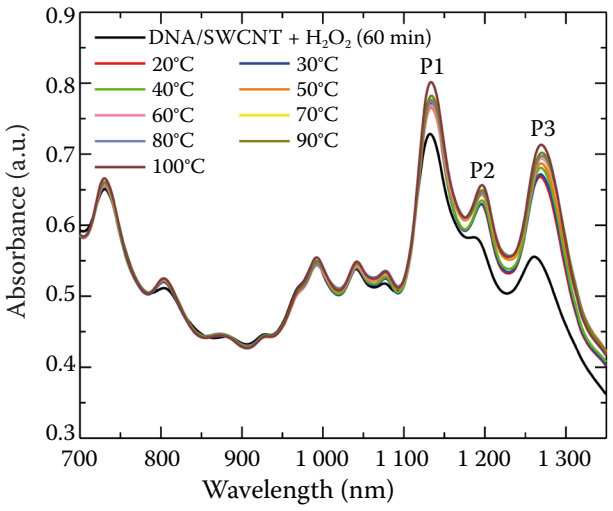

Figure 2. NIR absorption spectra of dsDNA-HiPco + 0.03% wt H_2O_2 reacted with the tea brewed at different temperatures

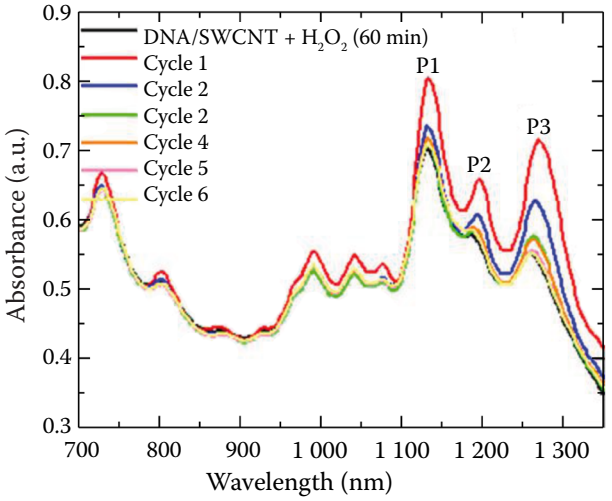

Figure 3. NIR absorption spectra of dsDNA-HiPco + 0.03% wt H_2O_2 in the presence of tea after several cycles (tea brewed at 100 °C)

the wavelength shift was useful for detecting the oxidation-reduction of the samples (Ishibashi et al. 2018).

The effect of brewing cycles (i.e. number of times the same tea sample is brewed) on the antioxidant potency was also evaluated using both NIR-ABS and PL measurements. Since the tea solution obtained at 100 °C exhibited the most pronounced antioxidant properties, this temperature was selected to investigate how repeated brewing influences the antioxidant activity of the resulting solutions. To this end, the tea was subjected to six sequential brewing cycles. Figure 3 illustrates the optical absorption spectrum of dsDNA-HiPco + 0.03% wt H_2O_2 reacted with the tea brewed for six cycles.

As shown in Figure 3, when the tea solution was freshly prepared (i.e. one brewing cycle), the absorbance increased significantly, suggesting that a redox reaction occurred. The absorbance intensities were increased by 10.0% at P1 and 28.4% at P3, indicating potent antioxidant activity of the freshly brewed tea. After the second cycle of brewing, the absorbance intensity decreased to 0.732 4 at P1 and 0.625 9 at P3, owing to a decrease in antioxidant activity of the tea. This can be explained by a decrease in the concentration of catechin in the extract, as it is known that catechins play a major role in tea antioxidant potency (Colon and Nerin 2012). As the number of brewing cycles further increased, the concentration of the antioxidant molecules in the tea solution decreased; thus the antioxidant activity decreased. Thus, the intensity of the bands at P1, P2, and P3 decreased continuously until they reached the level observed in the absence of tea.

Figure 4 shows the photoluminescence (PL) maps of dsDNA-HiPco + 0.03% wt H_2O_2 in the presence

https://doi.org/10.17221/135/2020-CJFS

of tea brewed for an increasing number of cycles. In Figure 4A, the brightest PL spot appeared at 730 nm excitation and 1 127 nm emission, which could have originated from (9, 4) SWCNTs (Weisman and Bachilo 2003). The intensity of these spots (i.e. red circles of Figure 4) decreased in the presence of tea solution. Moreover, a dependence of the spot intensity on the number of brewing cycles was noticed. The spot intensity decreased as the number of cycles increased owing to a decrease in catechin concentration in the tea solution. Accordingly, the antioxidant activity of tea was reduced.

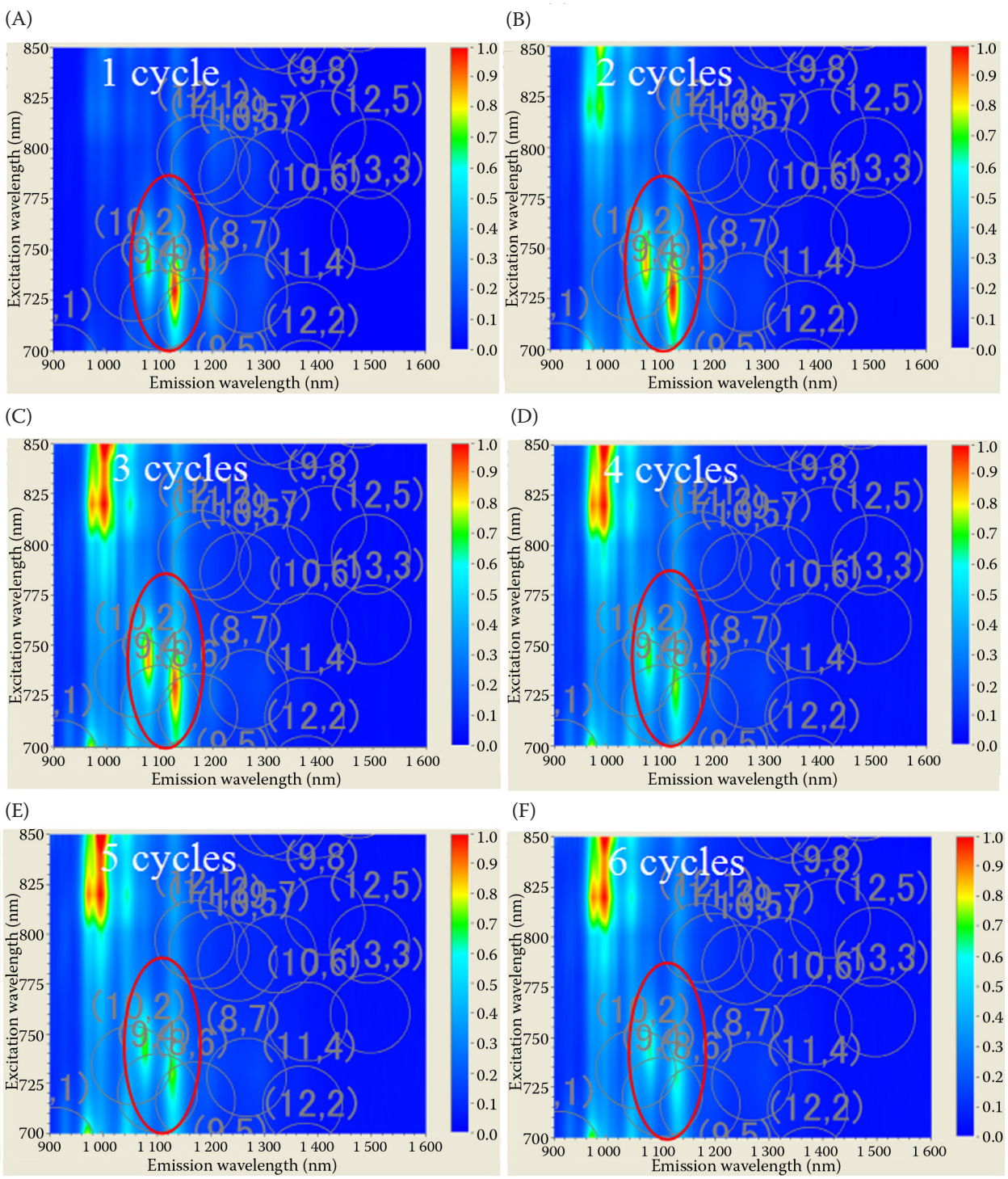

Figure 4. PL maps corresponding to dsDNA-HiPco + 0.03% wt H_2O_2 in the presence of tea after several cycles (tea brewed at 100 °C); (A) 1 cycle, (B) 2 cycles, (C) 3 cycles, (D) 4 cycles, (E) 5 cycles, and (F) 6 cycles

https://doi.org/10.17221/135/2020-CJFS

CONCLUSION

In summary, Chinese tea displayed excellent antioxidant activity towards 0.03% wt H_2O_2 in the presence of the dsDNA-HiPco suspension. The antioxidant activity is mediated by catechins. This study provides a simple dsDNA-SWCNT-based approach to assess the antioxidant potential of catechin from tea against strong oxidants, such as H_2O_2, for various applications. Moreover, it provides fundamental knowledge required to develop novel biosensors for other oxidant systems in living organisms.

Acknowledgement. We would like to thank Editage (www.editage.cn) for English language editing.

REFERENCES

Blanch A.J., Shapter J.G. (2014): Surfactant concentration dependent spectral effects of oxygen and depletion interactions in sodium dodecyl sulfate dispersions of carbon nanotubes. The Journal of Physical Chemistry B, 118: 6288–6296.

Boghossian A., Zhang J., Barone P.W., Reuel N.F., Kim J.H., Heller D.A., Ahn J.H., Hilmer A.J., Rwei A., Arkalgud J.R., Zhang C.T., Strano M.S. (2011): Near-infrared fluorescent sensors based on single-walled carbon nanotubes for life sciences applications. ChemSusChem (Chemistry-Sustainability-Energy-Materials), 4: 848–863.

Chen J., Chen S., Zhao X., Kuznetsova L.V., Wong S.S., Ojima I. (2008): Functionalised single-walled carbon nanotubes as rationally designed vehicles for tumor-targeted drug delivery. Journal of the American Chemical Society, 130: 16778–16785.

Colon N., Nerin C. (2012): Role of catechins in the antioxidant capacity of an active film containing green tea, green coffee, and grapefruit extracts. Journal of Agricultural and Food Chemistry, 60: 9842–9849.

Elhissi A.M., Ahmed W., Hassan I.U., Dhanak V.R., D'Emanuele A. (2012): Carbon nanotubes in cancer therapy and drug delivery. Journal of Drug Delivery, 2012: 837327.

Fu D., Li L.J. (2010): Label-free electrical detection of DNA hybridisation using carbon nanotubes and grapheme. Nano Reviews, 1: 5354.

Ishibashi Y., Ito M., Homma Y., Umemura K. (2018): Monitoring the antioxidant effects of catechin using single-walled carbon nanotubes: Comparative analysis by near-infrared absorption and near-infrared photoluminescence. Colloids and Surfaces: B Biointerfaces, 161: 139–146.

Kim S.N., Kuang Z., Grote J.G., Farmer B.L., Naik R.R. (2008): Enrichment of (6, 5) single wall carbon nanotubes using genomic DNA. Nano Letters, 8: 4415–4420.

Koh B., Park J.B., Hou X., Cheng W. (2011): Comparative dispersion studies of single-walled carbon nanotubes in aqueous solution. The Journal of Physical Chemistry B, 115: 2627–2633.

Kurnosov N.V., Karachevtsev M.V., Leontiev V.S., Karachevtsev V.A., (2017): Tuning the carbon nanotube photoluminescence enhancement at addition of cysteine through the change of external conditions. Materials Chemistry and Physics, 186: 131–137.

Lei J., Ju H. (2010): Nanotubes in biosensing. WIREs Nanomedicine and Nanobiotechnology, 2: 496–509.

Tu X., Pehrsson P.E., Zhao W. (2007): Redox reaction of DNA-encased HiPco carbon nanotubes with hydrogen peroxide: A near infrared optical sensitivity and kinetics study. The Journal of Physical Chemistry C, 111: 17227–17231.

Wang L.J., Umemura K. (2019): Optical absorption spectroscopy of DNA-wrapped HiPco carbon nanotubes. Materials Science Forum, 943: 95–99.

Weisman R.B., Bachilo S.M. (2003): Dependence of optical transition energies on structure for single-walled carbon nanotubes in aqueous suspension: An empirical Kataura plot. Nano Letters, 3: 1235–1238.

Xu Y., Pehrsson P.E., Chen L., Zhang R., Zhao W. (2017): Double-stranded DNA single-walled carbon nanotube hybrids for optical hydrogen peroxide and glucose sensing. The Journal Physical Chemistry C, 111: 8638–8643.

Yum K., Mcnicholas T.P., Mu B., Strano M.S. (2013): Single-walled carbon nanotube-based near-infrared optical glucose sensors toward *in vivo* continuous glucose monitoring. Journal of Diabetes Science and Technology, 7: 72–87.

Zhao E.H., Ergul B., Zhao W. (2015): Caffeine's antioxidant potency optically sensed with double-stranded DNA-encased single-walled carbon nanotubes. The Journal of Physical Chemistry B, 119: 4068–4075.

Zheng M., Jagota A., Strano M.S., Santos A.P., Barone P., Chou S.G., Diner B.A., Dresselhaus M.S., Mclean R.S., Onoa G.B., Samsonidze G.G., Semke E.D., Usrey M., Walls D.J. (2003): Structure based carbon nanotube sorting by sequence-dependent DNA assembly. Science, 302: 1545–1548.

Received: May 21, 2020
Accepted: January 4, 2021