Supporting information

Pomegranate Peel Carbon Dots Enriched Gum Arabic/ Guar Gum Multifunctional Film for Chicken Meat Preservation

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S1. Characterization

The FTIR spectra were recorded in a Thermo Nicolet iS5 FTIR spectrophotometer. In the wavelength range 4000 cm^{-1} to 400 cm^{-1} with 64 scan rates at the resolution of 4 cm^{-1}. The morphology of the films was visualized on a Carl Zeiss-Gemini FESEM 300 and the corresponding EDS spectra was recorded. The thermal analysis was performed in NETZSCH STA 449F3 under N_2 atmosphere (purging rate: 10 mL/ min) from room temperature to 900 ºC at a scanning rate of 5 ºC/min. The zeta potential was analyzed in an Anton Paar Litesizer 500 particle analyzer at a scattering angle of 90º and a temperature of 25 ºC. The UV spectra of pCDs were recorded with a UV−vis spectrophotometer (Cary 100, Agilent Technologies). The fluorescence spectrum of CDs was measured with a fluorescence spectrophotometer (Jasco, FP-8200). The TEM image was captured in a Jeol JEM-2100 Plus transmission electron microscope. Surface wettability of the hydrogels was determined from contact angle measurement using the sessile drop method in a Rame-Hart Tensiometer, USA. Data has been reported as an average of 20 measurements on random places of the films.

S2. Film properties

Mechanical Properties. The thickness of films was disordered with a micrometer (accurate to 0.001 mm). Four positions of the same composite film were randomly selected. The results were reported as the mean value. Elongation at break (EB) and tensile strength (TS) of films were analyzed in Instron 5567 (Instron, USA). TS and EB values were directly collected from the output data, and each sample was measured six times.

UV Blocking Properties of Films. The transmission spectrum of films was analyzed in a UV−Vis spectrophotometer (Cary 100, Agilent Technologies) to evaluate the UV blocking properties. Films were cut into 20 mm × 20 mm strip and then placed in a quartz cuvette for full wavelength scanning from 200 to 400 nm.

Antioxidant Capacities of Films. The free radical scavenging activities of different concentrations of pCDs were evaluated by DPPH.[1] In brief, different concentrations of pCDs (210 μL) were added to 100 μM methanolic solution of DPPH (1 mL). The mixtures were incubated for 30 min in the dark. After incubation, the absorbance was recorded at 517 nm. The scavenging of the DPPH free radicals was measured as follows:

$$DPPH\ scavenging\ activity = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \quad (1)$$

where $A_{control}$ and A_{sample} are the absorbance of the DPPH free radicals at 517 nm in the absence and presence of pCDs, respectively.

The free radical scavenging activity was determined by ABTS radical cation decolorization assay.[2] The ABTS solution was prepared via the reaction of 7 mM aqueous ABTS solution and 2.45 mM final concentration of potassium persulfate solution. After 16 h of storage in the dark, the radical cation solution was diluted to an optimal absorbance.[3] The dispersion was then treated with different concentrations of pCDs, and the absorbance was measured. The antioxidant activity of ABTS was calculated by using the following equation:

$$ABTS\ scavenging\ activity = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \quad (2)$$

where $A_{control}$ and A_{sample} are absorbance of the ABTS free radicals at 732 nm in the absence and presence of pCDs, respectively.

Water Vapor Permeability Measurement. Water vapour permeability (WVP) was measured gravimetrically.[4] 10 mL of distilled water was placed in a glass bottle with inner diameter of 18 mm. The mouth of the bottle was wrapped with a 30 mm diameter film sample and it was made airtight by using Teflon tape. The initial mass of the bottle was recorded and kept in the oven at 40 °C for 24 h. Mass of the bottle was recorded for 3 consecutive days. Then WVP was calculated with the following Eq. (3).

$$WVP = \frac{W \times x}{t \times A \times \Delta P} \quad (3)$$

Where W = mass gain of the sample (g), x = thickness of the film (m), t = 24 h, A = permeation area (m^2) and ΔP = difference in partial vapour pressure between the pure water and dry atmosphere at 25 °C. The result obtained was expressed as g $m^{-1}s^{-1}Pa^{-1}$. Three measurements were recorded for each film.

S3. pCDs release from films

Using food simulant solutions, the amount of pCDs released from the films was explored. A sample of the film measuring 2 cm × 2 cm was placed inside a conical flask containing four differnt food simulant solutions, including ethanol at concentrations of 10, 50, and 95%, as well as water, to simulate alcoholic and aqueous foods, respectively. The flask was maintained at a temperature of 25 C. The absorbance of the solution was measured with a UV−Vis spectrophotometer at different time intervals using 3 mL of aliquot taken from the solution. The kinetics of pCD release was assessed from the Koresemeyer-Peppas equation.[5]

S4. Cytotoxic studies

The cell viability of the synthesized nanocomposite hydrogels was performed by MTT assay. 3T3-L1 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% FBS, 100 U/mL streptomycin and 1% antibiotic/antimycotic solution kept in an incubator with 5% CO_2 at 37 °C. The films were sterilized with alcohol, exposed to

UV light and are kept in cell wells. The cell seeding is achieved at a confluence of 2×10^3 cells per well in 96 well plate and incubated for 24 h, 48h and 72 h with the films. After that, MTT solution of 0.5mg/ mL is added to the each well and further incubated for 3 h to generate formazan. Then, DMSO (100 µL) is added to soluble the formazan and absorbance is measured using a microplate reader (BioTek SYNERGY H1) at a wavelength of 570 nm. Data are presented as mean ± standard deviation (SD) from at least three independent experiments in triplicate. For dose dependant cell viability different concentration of hydrogels (10- 100 µg/ml) were taken and treated for 3days. The percentage of cell viability was calculated from Eq. 4:

$$\%\ Cell\ viability = \frac{ODt}{ODc} \quad (4)$$

where t stands for test sample and c for control.

The images of cytotoxicity assay are attained by a fluorescence microscope (Leica, Gremany) after staining with acridine orange/ ethidium dibromide (AO /EtBr) following fixing the cells with a 4% paraformaldehyde solution.

To evaluate the cell adhesion efficacy, the samples were put into 96 well plate and cells were seeded at a confluency of 2.5×10^3 per well onto the hydrogel surface and incubated in CO_2 atmosphere for 6 h. The cells were then fixed for 20 min with 4% para formaldehyde solution. Cell permeabilization is attained with 20 % methanol for 20 min and crystal violet (0.2%) staining has been executed for 20 min. Then cells were thoroughly washed with PBS to remove excess of dye and unused crystal violet is eluted using 10 % acetic acid. The absorbance was recorded in a microplate reader at a wavelength 570 nm. A phase- contrast microscope (Leica, Germany) is used to acquire the cell adhesion images after cells were fixed with 4% para formaldehyde solution. The concentration dependant cell adhesion was evaluated in the similar manner taking concentration from 10-100 µg/ml The percentage of cell adhesion was calculated from Eq. (4).

References

[1]. Murugesan, B.; Sonamuthu, J.; Pandiyan, N.; Pandi, B.; Samayanan, S.; Mahalingam, S. Photoluminescent Reduced Graphene Oxide Quantum Dots from Latex of Calotropis Gigantea for Metal Sensing, Radical Scavenging, Cytotoxicity, and Bioimaging in Artemia Salina: A Greener Route. *J. Photochem. Photobiol., B* 2018, *178*, 371−379.

[2]. Roy, S.; Ezati, P.; Rhim, J. W. Gelatin/Carrageenan-Based Functional Films with Carbon Dots from Enoki Mushroom for Active Food Packaging Applications. *ACS Appl. Polym. Mater.* 2021, *3*, 6437−6445.

[3]. Wojdyło, A.; Oszmiański, J.; Czemerys, R. Antioxidant Activity and Phenolic Compounds i 32 Selected Herbs. *Food Chem.* 2007, *105*, 940−949.

[4]. Standard, A. *E96/E96M-05 Standard Test Methods for Water Vapor Transmission of Materials*; ASTM Int: West Conshohocken (PA), 2005.

[5]. Korsemeyer, R. W.; Peppas, N. A. Effect of the morphology of hydrophilic polymeric matrices on the diffusion and release of water soluble drugs. *J. Membrane Sci.,* 1981, *9,* 211 – 227