ChemComm

Supplementary Information

Nanochemoprevention by Encapsulation of (–)-Epigallocatechin-3-Gallate with Bioactive Peptides/Chitosan Nanoparticles for Enhancing its Bioavailability

Bing Hu[a,b], Yuwen Ting[b], Xiaoqing Yang[b], Wenping Tang[b],

Xiaoxiong Zeng[*a] and Qingrong Huang[*b]

Characterization and Analysis of Interactions among CS, CPP and EGCG by QCMD

The binding between peptides and EGCG was monitored by QCMD (Q-Sense D300 electronic unit, Q-sense AB, Sweden) at 25.00 ± 0.02 ℃, and the frequency signal was considered stable when the frequency change was less than 1.0 Hz in 5 min of the third overtone. Firstly, 0.01 M phosphate buffer solution (PBS, pH6.2) was injected into the QCMD chamber with an AT-cut quartz crystal coated with gold. After the frequency signal was stabilized, 1.0 mg/ml CS solution was injected and followed by rinsing with the same PBS buffer to remove the redundant CS. When CS was immobilized on the crystal surface, EGCG solutions with different concentrations, 1.0 mg/ml CPP solution and PBS buffer were injected respectively.

If the deposited molecules form a rigid layer on the crystal surface, the absorbed mass of CPP onto EGCG could be calculated by the Sauerbrey equation:

$$\Delta m = -C\Delta f/n \quad (1)$$

where C is the mass sensitivity constant related to the physical properties of the quartz crystal (C = 17.7 ng cm^{-2} Hz^{-1} for a 5 MHz gold crystal), n is the overtone number. However, when the deposited layer is elastic, the adsorbed molecules cannot fully couple the oscillation of the quartz crystal thus a more complex model is needed to calculate Δm. QCMD has the advantage to monitor the change of an energy dissipation factor (ΔD), and when ΔD > $1E10^{-6}$, the Voigt model will be used to calculate the Δm.

Table SI-1

Absorbed mass of caseinophosphopeptides with different EGCG concentrations (overtone numbers n = 3, 5, 7 were used in the calculation).

EGCG concentrations (mg/mL)	Δm (ng/cm^2) By Sauerbrey equation	Δm (ng/cm^2) By Voigt model
0.25	141.8 ± 4.486	141.0 ± 0.3577
0.5	160.6 ± 14.31	154.0 ± 11.25
1	164.8 ± 9.045	160.0 ± 0.9259
1.5	178.1 ± 2.362	177.1 ± 0.1352
2.5	275.7 ± 9.958	279.4 ± 2.865

Determination of Cytotoxicity of CS-CPP or CS-TPP Nanoparticles

In the present study, cell viability was evaluated by MTT-based colorimetric method. Briefly, Panc-1 cells at a density of 4×10^4 cells/mL were seeded into a 96-well plate and cultured in 200 μL of DMEM in a humidified 5% CO_2 incubator for 24 h. The spent medium was replaced with CS-CPP or CS-TPP nanoparticles and diluted with culture medium to give a CS concentration

from 0.063 to 1.0 mg/mL (pH 6.2). After overnight incubation at 37 ℃, the CS-CPP and CS-TPP nanoparticles were replaced with 100 μL of MTT solution (0.5 mg/mL in PBS, pH 7.4), and the cells were incubated for a further 2.5 h at 37 ℃. Finally, the solution was decanted, and 100 μL of DMSO was added for the dissolution of formazan crystals. Absorbance at 570 nm of the resultant solutions was measured by a microplate reader (Thermo Lab systems, Franklin, MA), and cell viability was expressed as percentage of absorbance relative to control (the control comprising cells not exposed to CS materials). Experiments were performed in triplicate with six replicate wells for each sample and control per assay.

Transport of EGCG through Caco-2 Cell Monolayer

Procedures for determination of the apparent permeation rate (P_{app}) of EGCG encapsulated in CS-CPP nanoparticles across Caco-2 cell monolayer were generally followed the detailed protocol reported previously[1]. Caco-2 cells were maintained in DMEM with 10% FBS, 1X nonessential amino acids and 1X penicillin and streptomycin at 37 ℃ with 5% CO_2. Cells of passage 37-45 to keep relatively constant cellular phenotypes were used in the present study. To generate Caco-2 cell monolayers in the insert filters of 12-well plates, 0.5 mL Caco-2 cells were plated onto the insert (the apical compartment) at a density of 6×10^5 cell/mL and 1.5 mL culture media were subsequently added in the lower (basolateral) compartment of each well. The culture media were renewed every 2 days. Permeation experiments were performed after 29-31 days of plating. In the permeation experiments, HBSS + 10 mM hydrochloric acid (pH 6.2) was used as the transport solution. EGCG at constant concentration (50 μM) was encapsulated by various CS-CPP nanoparticle concentrations at 0.063, 0.125 and 0.250 mg/mL as donor media. In the permeation direction of apical to basolateral (A-B) compartment, 0.4 mL donor media was added

to the apical compartment and 1.2 mL receiving media (same as transport solution) were added to the basolateral compartment. Plates were then put in a shaker at 100 rpm at 37 ℃. At 30, 60, 90, and 120 min of permeation, half volumes of the receiving media were removed and the same volumes of fresh media were replenished. The removed receiving media were diluted to 1.0 mL by HPLC grade water and then analyzed by HPLC for EGCG quantification according to our previous method[2]. Cumulative quantity of EGCG permeated at each time interval was calculated and plotted against time. The initial slope was then used to calculate *P*app using the following equation:

$$P_{app} = \left(\frac{dQ}{dt}\right)\left(\frac{1}{AC_0}\right) \quad (2)$$

where dQ/dt is the rate of EGCG permeation. A is the surface area of the insert (1.1 cm^2), C_0 is the EGCG concentration.

To ensure the integrity of Caco-2 monolayers, the value of transepithelial electrical resistance (TEER) was determined. TEER value was measured before each experiment by using Evohm2 epithelial voltmeter (World Precision Instruments, New Haven, CT, USA) and calculated as:

$$\text{TEER value } (\Omega \cdot cm^2) = (\text{TEER } (\Omega) - \text{TEER}_{\text{background}} (\Omega)) \times \text{Area } (cm^2) \quad (3)$$

where TEER (Ω) is the electrical resistance across Caco-2 monolayers directly read from the Evohm2 epithelial voltmeter, $\text{TEER}_{\text{background}}$ (Ω) is that across the insert only (without cells). Area is the area of the insert, 1.1 cm^2.

1. I. Hubatsch, E. G. E. Ragnarsson and P. Artursson, Nature Protocols, 2007, **2**, 2111-2119.

2. B. Hu, L. Wang, B. Zhou *et al*. Journal of Chromatography A, 2009, **1216**, 3223-3231.