Supporting Information

for

Organic selenium derived from chelation of soybean peptides-selenium and its functional properties *in vitro* and *in vivo*

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1. Methods

1.1. Fluorescence spectroscopy

Fluorescence spectrometry was measured by Hitachi F-4600 fluorescence spectrophotometer (Hitachi Co., Japan) to investigate the conformational changes of SPIP and SPIP-Se. The excitation wavelength was set at 295 nm and the emission at wavelengths between 300 and 500 nm were recorded.

1.2. Cell viability assay

MTT cell proliferation assay was performed to determine the cell viability. The cells were seeded on 96-well plastic cell culture clusters at a concentration of 2×10^5 cell/mL, and incubated in the culture medium containing SPIP or SPIP-Se samples (0~16 μg/mL) at 37 °C in a 5 % CO_2 for 24 h and 48h. Each well was incubated with 20 μL MTT (5 mg/mL) for another 4 h, then the supernatant was removed and 150 μL DMSO was added to each well. After sufficient shaking, the absorbance of the mixture was measured at 570 nm.

2. Results and discussion

2.1. Fluorescence spectroscopy

Fluorescence spectroscopy was then used to detect the structural evolution of SPIP when chelated with Se. As shown in Fig. S1, the fluorescence intensity decreased successively with chelating selenium. Compared to the SPIP, the endogenous fluorescence of SPIP-Se decreased from 140 to 94, which indicated that the structural folding and aggregation of amino acids or oligopeptides was occurred during the chelation process.

2.2. Cell viability assay

To determine cytotoxic to Caco-2 cells, we first examined the Caco-2 cells viabilities after 24 and 48 h of incubation at various concentrations of SPIP and SPIP-Se using MTT assay. As shown in Fig. S2, the SPIP-Se reduced cell proliferation of Caco-2 cells both in a dose-dependent and time-dependent manner. The results showed that the security concentration of SPIP-Se and SPIP on Caco-2 cells was chosen at 10 μg/mL, where the cell viability was around 90 % .

Figure captions

Fig. S1

The characterization of SPIP and SPIP-Se. Fluorescence spectra of SPIP and SPIP-Se over the wavelength range from 300 to 500 nm.

Fig. S2

The cytotoxicity of SPIP and SPIP-Se. (A) Caco-2 cells were treated with various concentrations of SPIP and SPIP-Se for 24 h. (B) Caco-2 cells were treated with various concentrations of SPIP and SPIP-Se for 48 h.

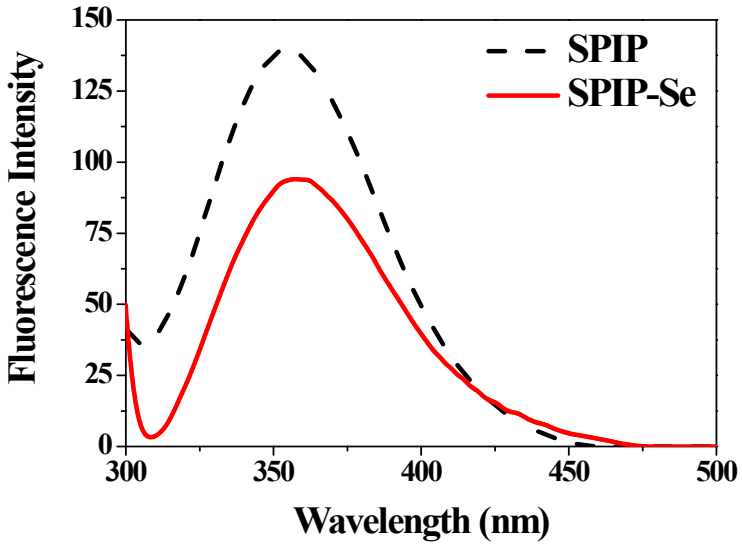
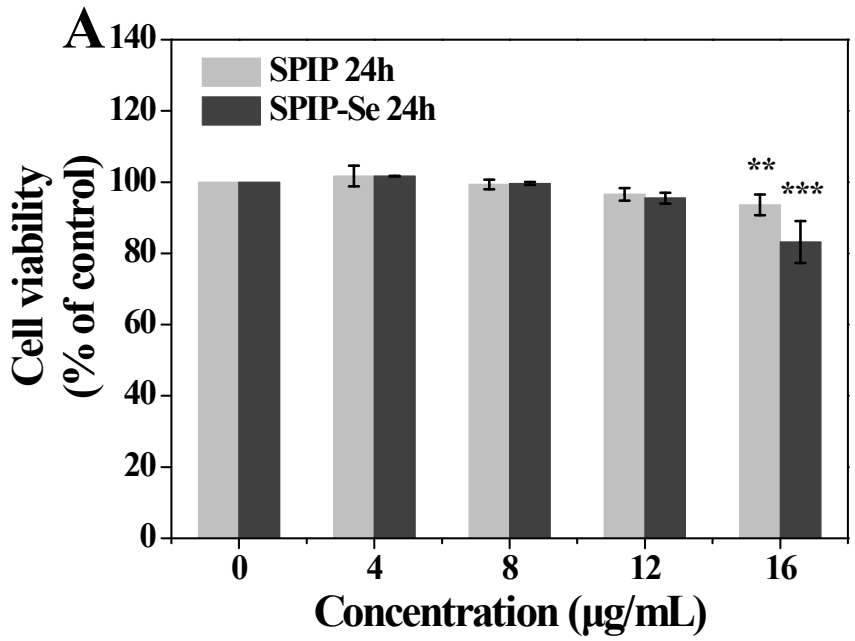

Fluorescence Intensity
Wavelength (nm)
SPIP
SPIP-Se
150
125
100
75
50
25
0
300
350
400
450
500

Fig.S1


A
SPIP 24h
SPIP-Se 24h
Cell viability (% of control)
Concentration (μg/mL)
0
4
8
12
16
0
20
40
60
80
100
120
140
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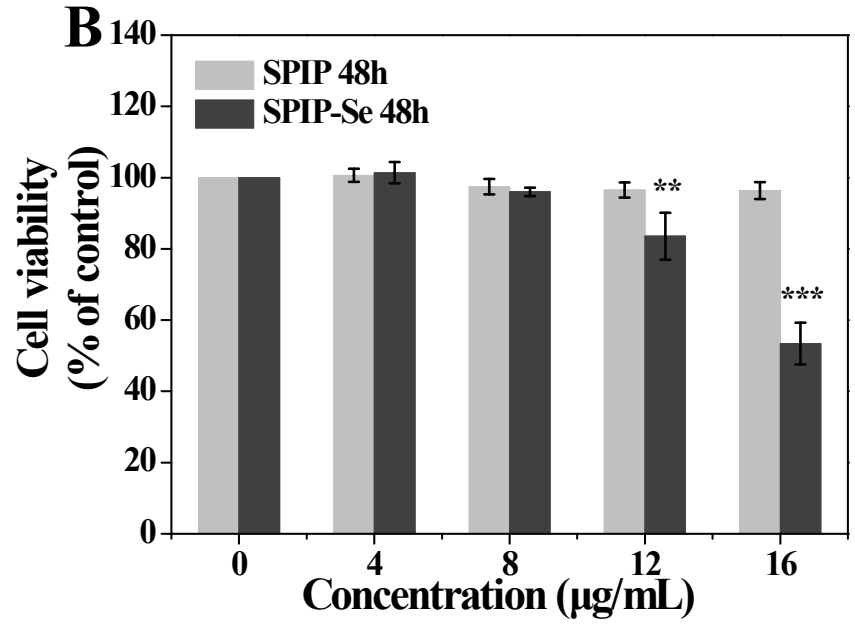
B
SPIP 48h
SPIP-Se 48h
Cell viability (% of control)
Concentration (μg/mL)
0
4
8
12
16
0
20
40
60
80
100
120
140
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Fig.S2