Supporting Information

Glutenin protein corona ameliorated TiO_2 nanoparticle-induced gut barrier dysfunction and altered gut microbiota composition

Methods:

ABTS Radical Scavenging Assay. The detection of ABTS radical scavenging ability was performed according to the literature with slight changes.[1] The ABTS mother solution was obtained by mixing 10 mL of 7 mmol/L ABTS solution with 178 μL of 140 mmol/L potassium persulfate solution in the dark for 12 hours. The working solution with $OD_{734\ nm}$=0.7 was obtained by diluting the stock solution. The absorbance was measured at 734 nm after mixing 1 mL of the working solution with 1 mL of digestive solution in the dark at room temperature for 1 h.

DPPH Radical Scavenging Assay. The measurement of DPPH scavenging activity was performed with some changes according to the previous literature.[2] Samples were mixed with DPPH (0.04 mg/mL in absolute ethyl alcohol) at the ratio of 1:1 in the 96-well plate and incubated at 37°C in the dark for 30 min. The absorbance was read at 517 nm.

Hydroxyl radical scavenging Assay. The hydroxyl radical scavenging activity was measured according to the method reported in the literature.[3] One milliliter of samples, 1 mL 6 mmol/L $FeSO_4$, 1 mL 6 mmol/L H_2O_2, and 1 mL 6 mmol/L of ethanol salicylic acid solution were added consecutively and incubated at 37°C for 1 h. After incubated, the reaction solution was transferred to a 96-well plate and the absorbance was

measured at 510 nm.

Total reducibility Assay. The total reducibility assay was slightly modified according to the method reported previously.[4] Mix 2.5 mL samples, 2.5 mL 0.02 mol/L PBS, 2.5 mL 1% (w/v) potassium ferricyanide and incubate at 55^{o}C for 20 minutes. Then add 2.5 mL 10% (w/v) trichloroacetic acid followed by a 1000 g centrifugation for 15 min. The supernatant (2.5 mL) was mixed with 2.5 mL of distilled water and 0.5 mL 0.1% (w/v) ferric chloride, and the absorbance at 700 nm was measured after 10 min of incubation at room temperature in the dark.

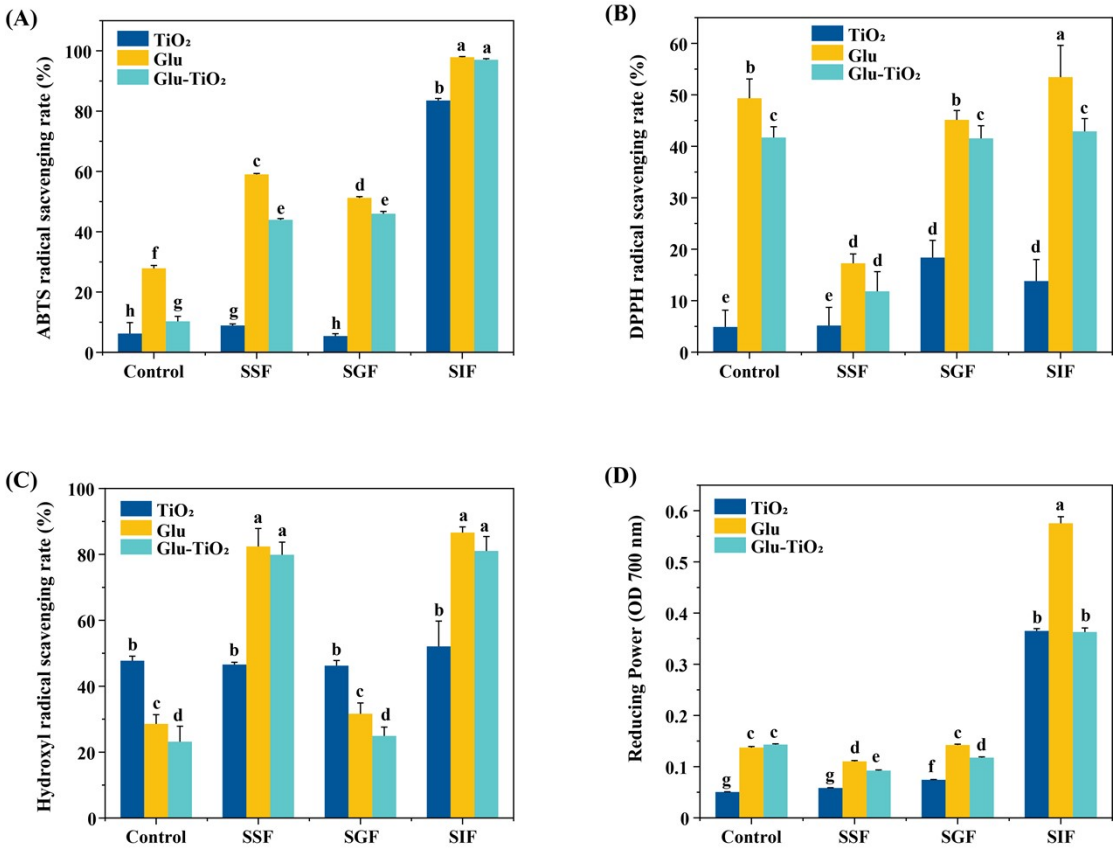
Supplementary Figures

Supplementary Figure 1. The antioxidant activity of digestive fluid with different treatments. (A-D) The ABTS radical scavenging rate (A), DPPH radical scavenging rate (B), Hydroxyl radical scavenging rate (C) and Reducing power (D) of digestive fluids with TiO_2 nanoparticles, glutenin, and Glu-TiO_2 corona during simulated digestion. Bars assigned different lowercase letters are significantly different ($p < 0.05$).

Supplementary Table 1. Primers used in qRT-PCR experiments.

Supplementary Table 2. The visceral index of mice with different groups.

Supplementary Figure 1.



Supplementary Table 1. Primers used in qRT-PCR experiments.

Gene	Forward Primer	Reverse Primer
Mouse Cldn1	5′-TGCCCCAGTGGAAGATTTACT-3′	5′-CTTTGCGAAACGCAGGACAT-3′
Mouse Tjp1	5′-GAGCGGGCTACCTTACTGAAC-3′	5′-GTCATCTCTTTCCGAGGCATTAG-3′
Mouse Ocln	5′-TTGAAAGTCCACCTCCTTACAGA-3′	5′-CCGGATAAAAAGAGTACGCTGG-3′
Mouse Cldn7	5′-AGGGTCTGCTCTGGTCCTT-3′	5′-GTACGCAGCTTTGCTTTCA-3′
Mouse Cldn2	5′-GATTGGAGAGGCTCTGTACTTG-3′	5′-TAGTTGGTACGATTGCCCTG-3′
Mouse Muc2	5′-GGCTCGGAACTCCAGAAAGAAG-3′	5′-CTCGGCAGTCAGACGCAAAG-3′
Mouse Gapdh	5′-TGAATACGGCTACAGCAACA-3′	5′-AGGCCCCTCCTGTTATTATG-3′
Human Cldn1	5′-GATGAGGTGCAGAAGATGAG-3′	5′-GGACAGGAACAGCAAAGTAG-3′
Human Ocln	5′-CGCTGCTGTAACGAGGCT-3′	5′-CCAATGTCGAGGAGT-3′
Human Tjp1	5′-ATCAGGGACATTCAATAGCGTAGC-3′	5′-CAAGATAGTTTGGCAGCAAGAGATG-3′
Human Gapdh	5′-CGGAGTCAACGGATTTGGTC-3′	5′-GACAAGCTTCCCGTTCTCAG-3′

Supplementary Table 2. The visceral index of mice with different groups.

	Heart (mg/g)	Liver (mg/g)	Spleen (mg/g)	Lung (mg/g)	Kidney (mg/g)	Brain (mg/g)
Control	$5.91 \pm 0.72^{a,*}$	48.83 ± 3.90^{a}	5.32 ± 0.77^{a}	7.56 ± 1.19^{a}	12.79 ± 0.56^{a}	14.88 ± 3.41^{a}
TiO_2 100 mg/kg	6.28 ± 1.56^{a}	44.18 ± 4.61^{a}	3.93 ± 0.86^{a}	7.16 ± 0.56^{a}	12.84 ± 0.66^{a}	19.49 ± 4.98^{a}
TiO_2 500 mg/kg	6.35 ± 0.93^{a}	45.87 ± 2.08^{a}	3.86 ± 0.85^{a}	6.38 ± 0.94^{a}	12.99 ± 0.60^{a}	16.43 ± 1.77^{a}
Glu-TiO_2 Corona 100 mg/kg	5.82 ± 0.39^{a}	45.41 ± 2.84^{a}	4.26 ± 1.37^{a}	7.84 ± 1.43^{a}	12.95 ± 0.65^{a}	19.99 ± 1.53^{a}
Glu-TiO_2 Corona 500 mg/kg	6.34 ± 1.09^{a}	47.18 ± 2.63^{a}	3.41 ± 0.39^{a}	6.49 ± 0.54^{a}	12.39 ± 0.96^{a}	18.36 ± 1.00^{a}

* Values with different inline letters within a row were significantly different ($p < 0.05$).

References:

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