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Supplemental Material

Evaluating the Effects of Chronic Oral Exposure to the Food Additive Silicon Dioxide on Oral Tolerance Induction and Food Sensitivities in Mice

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Figure S1. Transmission electron microscopy (TEM) analyses of the *fg*-SiO_2 particles. Representative transmission electron microscopy image of the *fg*-SiO_2 suspension in ultra-pure water (10 mg/mL).

Figure S2. Intestinal permeability in mice after oral exposure to food-grade SiO_2. *In vitro* paracellular FITC-dextran flux measured for 1h in Ussing chambers in ileum (A) or colon (B) segments from mice orally exposed to *fg*-SiO_2 (n=8 mice per group) or to the vehicle (n=10) for 60 days. The data are expressed as median with interquartile range and whiskers extending from minimum to maximum ± SEM. *$p<0.05$ by one-way ANOVA and *post-hoc* Tukey test (A) or Kruskal-Wallis test followed by a *post-hoc* Dunn's test (B). See Table S4 for data.

Figure S3. MLN cell viability and frequency after *ex vivo* treatment with food-grade SiO_2. (A) Cell suspension from MLN of untreated mice (n=4 mice per group) were exposed for 48h to various concentrations of *fg*-SiO_2 (0. 6.25. 12.5. 25 and 50 µg/mL). Frequency of viable cells was evaluated by flow cytometry analysis of propidium iodide staining and normalized to control. (B-F) Cell suspension from MLN of untreated mice (n=9 per group) were exposed for 48h to various concentrations of *fg*-SiO_2 (0. 6.25. 12.5. 25 and 50 µg/mL) before stimulation for 5h with phorbol 12-myristate 13-acetate (PMA) and ionomycin. Frequency of CD3+ CD4+ cells (B), of CD3+ CD4+ CD25+ cells (C), of CD3+ CD4+ CD25+ FoxP3+ cells (D), of CD45+ CD103+ CD11b+ cells (E), and of CD3+ CD4+ Tbet+ cells (F) was evaluated by flow cytometry. (G) Cell suspension from MLN of untreated mice (n=7 per group) were exposed for 3 days to concanavalin-A (a T-cell mitogen) in presence of various concentrations of *fg*-SiO_2 (0. 6.25. 12.5. 25 and 50 µg/mL). Frequency of dead T cells was evaluated by flow cytometry analysis of Viobility 488/520 Fixable Dye staining. The data are expressed as median with interquartile range and whiskers extending from minimum to maximum ± SEM. *$p<0.05$ by Kruskal-Wallis test followed by a *post-hoc* Dunn's test (A) or one-way ANOVA and *post-hoc* Tukey test (B-G). See Table S4 for data.

Figure S4. The gating strategy for analysis of T cell subpopulations from MLN *ex vivo* exposed to food-grade SiO_2. Flow cytometry gating strategy for quantification of T cell subpopulations from MLN of untreated mice (n=9 per group) *ex vivo* exposed for 48h to various concentrations of *fg*-SiO_2 (0. 6.25. 12.5. 25 and 50 µg/mL) before stimulation for 5h with phorbol 12-myristate 13-acetate (PMA) and ionomycin. The frequency of $CD3^+$ $CD4^+$ cells, $CD3^+$ $CD4^+$ $CD25^+$ cells, $CD3^+$ $CD4^+$ $CD25^+$ $Foxp3^+$ cells, $CD3^+$ $CD4^+$ $CD25^+$ $Foxp3^+$ IL-10^+ cells, $CD3^+$ $CD4^+$ $Tbet^+$ cells, and $CD3^+$ $CD4^+$ $Tbet^+$ IFN-γ+ cells was evaluated by flow cytometry. SS: side scatter. FS: forward scatter. FS-H: forward scatter height.

Figure S5. The gating strategy for analysis of $CD45^+$ $CD11b^+$ $CD103^+$ cells from MLN *ex vivo* exposed to food-grade SiO_2. Flow cytometry gating strategy for quantification of $CD45^+$ $CD11b^+$ $CD103^+$ cells (including DC and macrophage subpopulations) from MLN of untreated mice (n=9 per group) *ex vivo* exposed for 48h to various concentrations of *fg*-SiO_2 (0. 6.25. 12.5. 25 and 50 µg/mL) before stimulation for 5h with phorbol 12-myristate 13-acetate (PMA) and ionomycin. The frequency of $CD45^+$ $CD11b^+$ $CD103^+$ cells and $CD45^+$ $CD11b^+$ $CD103^+$ TGF-β^+ cells was evaluated by flow cytometry. SS: side scatter. FS: forward scatter. FS-H: forward scatter height.

Figure S6. The gating strategy for analysis of T cells proliferation. Flow cytometry gating strategy for quantification of $CD4^+$ proliferating cells isolated from MLN of untreated mice. Cell suspension from MLN of untreated mice were exposed for 3 days to concanavalin-A (a T-cell mitogen) in the absence or presence of various concentrations of *fg*-SiO_2 (0. 6.25. 12.5. 25 and 50 µg/mL). SS: side scatter. FS: forward scatter. FS-H: forward scatter height.

Figure S7. The gating strategy for analysis of T cells from colon *lamina propria*. Flow cytometry gating strategy for quantification of $CD3^+$ $CD4^+$ and $CD3^+$ $CD4^+$ $CD25^+$ subpopulations isolated from the colon *lamina propria* of OVA-immunized (PBS) or OVA-tolerized mice exposed to a vehicle or *fg*-SiO_2 through gavage (gav) or food. SS: side scatter. FS: forward scatter. FS-H: forward scatter height.

Figure S8. The gating strategy for analysis of $CD45^+$ $CD11b^+$ $CD103^+$ cells from colon *lamina propria*. Flow cytometry gating strategy for quantification of $CD45^+$ $CD11b^+$ and $CD103^+$ subpopulations isolated from the colon *lamina propria* of OVA-immunized (PBS) or OVA-tolerized mice exposed to a vehicle or *fg*-SiO_2 through gavage (gav) or food. SS: side scatter. FS: forward scatter. FS-H: forward scatter height.

Table S1. *fg*-SiO_2 sample characterization by DLS.

Table S2. Oral tolerance and challenge with OVA protocol.

Table S3. qPCR primers used for gene expression analysis.

Table S4. Corresponding numeric data for all figures. Reported is the measured data for all replicates.