Supplementary Data For:

The maternal-fetal transfer of passive immunity as a mechanism of transplacental nanoparticle drug delivery for prenatal therapies

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Table S1. Formulation of chitosan nanoparticles (CNPs)

Sample	Chitosan Concentration	Chitosan volume (mL)	STPP Concentration	STPP volume (mL)
CNP1 (Bare CNPs)	3 mg/mL	10	5 mg/mL	3
CNP2	2 mg/mL	15	3 mg/mL	5

Bare chitosan nanoparticles (bare CNPs) were synthesized by pouring 9 mL of a 5 mg/mL STPP solution into 30 mL of a 3 mg/mL chitosan solution. CNPs to be surface modified with IgG (CNP2) were synthesized by pouring 10 mL of a 3 mg/mL STPP solution into 30 mL of a 2 mg/mL chitosan solution. The as-synthesized NPs were centrifuged at 6500 rpm for 20 minutes and washed with deionized water thrice. CNPs were resuspended in deionized water via ultra-sonification on 30% power for 30 seconds.

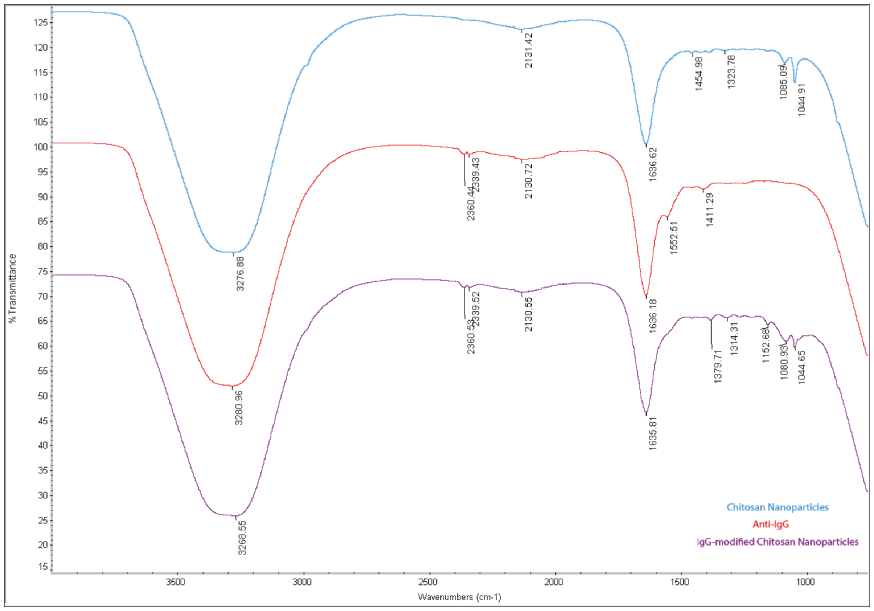

Figure S1. Bioconjugation of IgG to CNP2. Bioconjugation of IgG antibodies to CNP2 to form IgG-CNP was confirmed. FTIR spectrograph of IgG surface bioconjugation to CNP2. The combined spectra (purple) confirm the bioconjugation of unmodified CNP2 (blue) and IgG antibodies (red). Across all samples, a broad peak was detected around 3200 cm^{-1} attributed to the stretching of hydroxyl (-OH), and amino (-NH, $-NH_2$) groups. Stretching attributed to the interaction of water molecules with aromatic rings in the chitosan and amino acid structures was observed at 2130 cm^{-1} and amino bending (-NH) was observed at 1636 cm^{-1} across all samples. We observed stretching at 2340 and 2360 cm^{-1} which is attributed to the carbonate groups (-COO-) and nitro compound stretching (-NO) at 1550 cm^{-1} of peptide bonds in the anti-IgG. This also corresponds to the secondary structure of the IgG α-helices and β-sheets. C-O stretches were observed between 1044 to 1323 cm^{-1}. The 1550 cm^{-1} peak observed in the FITR spectrum of the IgG-modified CNPs indicating conjugation of IgG to the CNPs.

Table S2. Physical properties of chitosan nanoparticle formulations

Sample (n = 3)	**Size (nm)**	**Polydispersity Index**	**Zeta Potential (mV)**
CNP1 (bare CNP)	$375 \pm 17^{\delta}$	$0.23 \pm 0.01^{\varepsilon}$	0.24 ± 1.22
CNP2	$281 \pm 12^{\delta, \gamma}$	$0.19 \pm 0.02^{\varepsilon, \gamma}$	0.02 ± 0.72
CNP2-IgG (IgG-CNP)	$414 \pm 27^{\gamma}$	$0.30 \pm 0.02^{\varepsilon, \gamma}$	0.23 ± 0.73

A one-way ANOVA analysis with a Bonferroni's multiple comparison test was performed to compare effect of formulations on size, polydispersity index, and zeta potential. A significant difference in size was measured between CNP1 and CNP2 ($p \leq 0.01$; 95% C.I. = [42.2, 146.8]) and CNP2 and CNP2-IgG ($p \leq 0.001$; 95% C.I. = [-185.1, -80.47]). A significant difference in polydispersity index was measured between CNP1 and CNP2 ($p \leq 0.05$, 95% C.I. = [0.001503, 0.09650], CNP1 and CNP2-IgG ($p \leq 0.05$, 95% C.I. = [-0.1148, -0.01984]), and CNP2 and CNP2-IgG ($p \leq 0.001$, 95% C.I. = [-0.1638, -0.06884]). There was no significant difference in zeta potential ($p = 0.9171$).
ε represents $p \leq 0.05$; δ represents $p \leq 0.01$; γ represents $p \leq 0.001$.

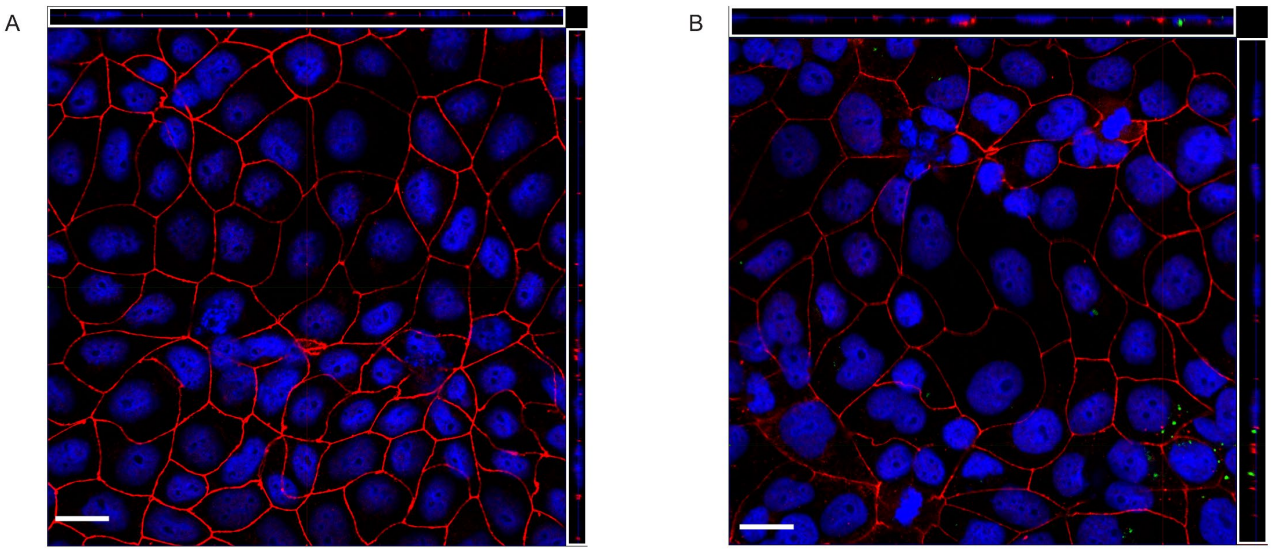

Figure S2. Orthographic projection of the established BeWo placental epithelial monolayer. Orthographic z-stack projections (white boxes) were obtained by laser scanning confocal microscopy. The establishment of zona occludens-1 (ZO-1, red) tight junction proteins was visualized by immunostaining. Nuclei were stained with DAPI (blue). Scale bar is 20 µm. A) Projection of the BeWo placental epithelial cell monolayer. B) ZO-1 tight junctions remained intact 18 hours post-exposure to FITC-tagged IgG-CNPs (green). IgG-CNPs were detected within the cell body.