

Supporting Information

Development and Characterization of Fluorescently Tagged Nanocellulose for Nanotoxicological Studies

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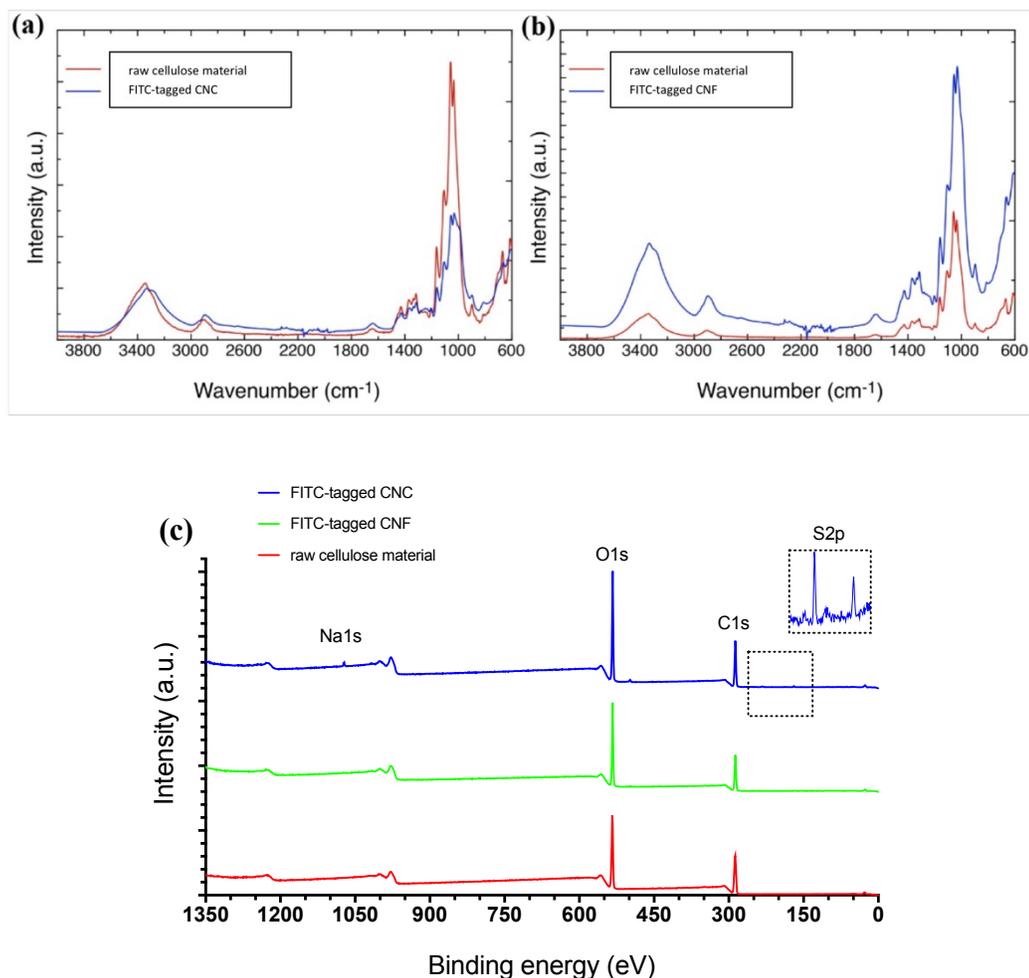


Figure S1. FTIR absorbance spectra of FITC-tagged CNC (a), FITC-tagged CNF (b), compared against the spectrum of raw cellulose material. (c) XPS elemental analyses of FITC-tagged CNF, FITC-tagged CNC, and raw cellulose material.

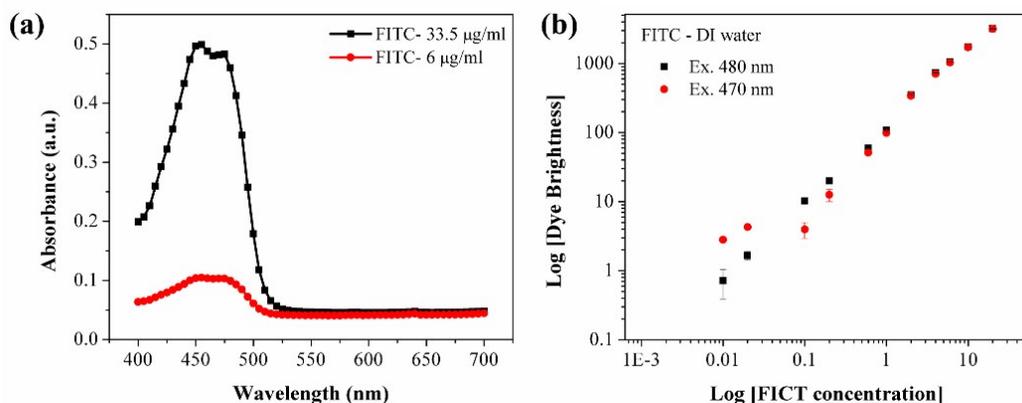


Figure S2. (a) UV-Vis absorption spectra obtained from pure FITC molecules in DI water. (b) Emission intensity at 520 nm of pure FITC at $\lambda_{\text{ex}} = 480\text{nm}$ (black rectangles) and at $\lambda_{\text{ex}} = 470\text{ nm}$ (red dots); the cut-off wavelengths were set at 495 nm and 475 nm, respectively.

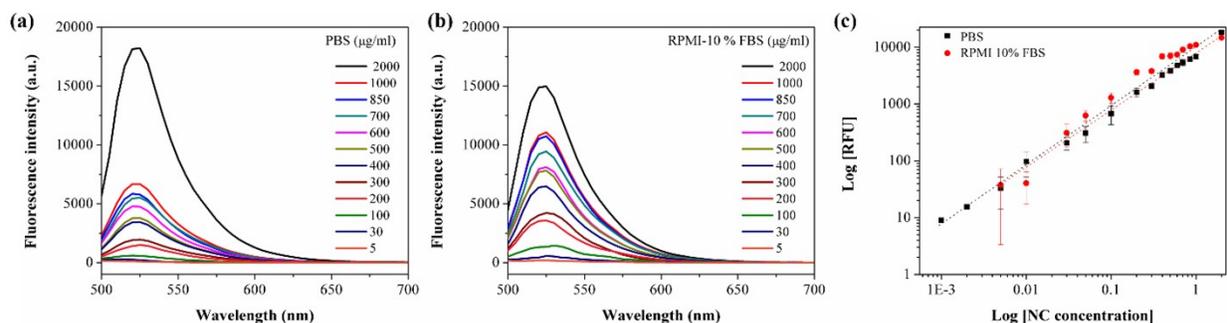


Figure S3. Fluorescence emission spectra of FITC-tagged CNF at $\lambda_{\text{ex}} = 470\text{nm}$ and cut-off at 475 nm dispersed at various concentrations in (a) PBS and (b) RPMI supplemented with 10% FBS. (c) Overlaid calibration curves at different buffers; standard deviations are obtained from 3 replicates ($n=3$).

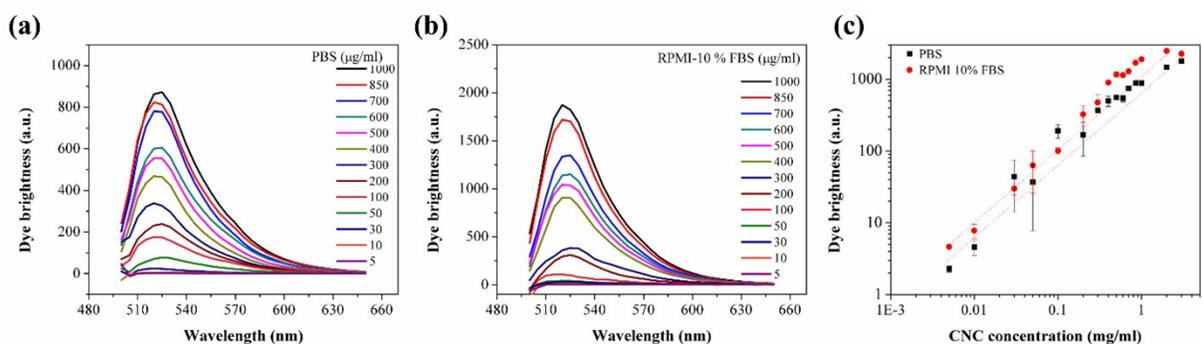


Figure S4. Fluorescence emission spectra of FITC-tagged CNC at $\lambda_{\text{ex}} = 480\text{nm}$ and cut-off at 495 nm dispersed at various concentrations in (a) PBS and (b) RPMI supplemented with 10% FBS. (c) Overlaid calibration curves at different buffers; standard deviations are obtained from 3 replicates ($n=3$).

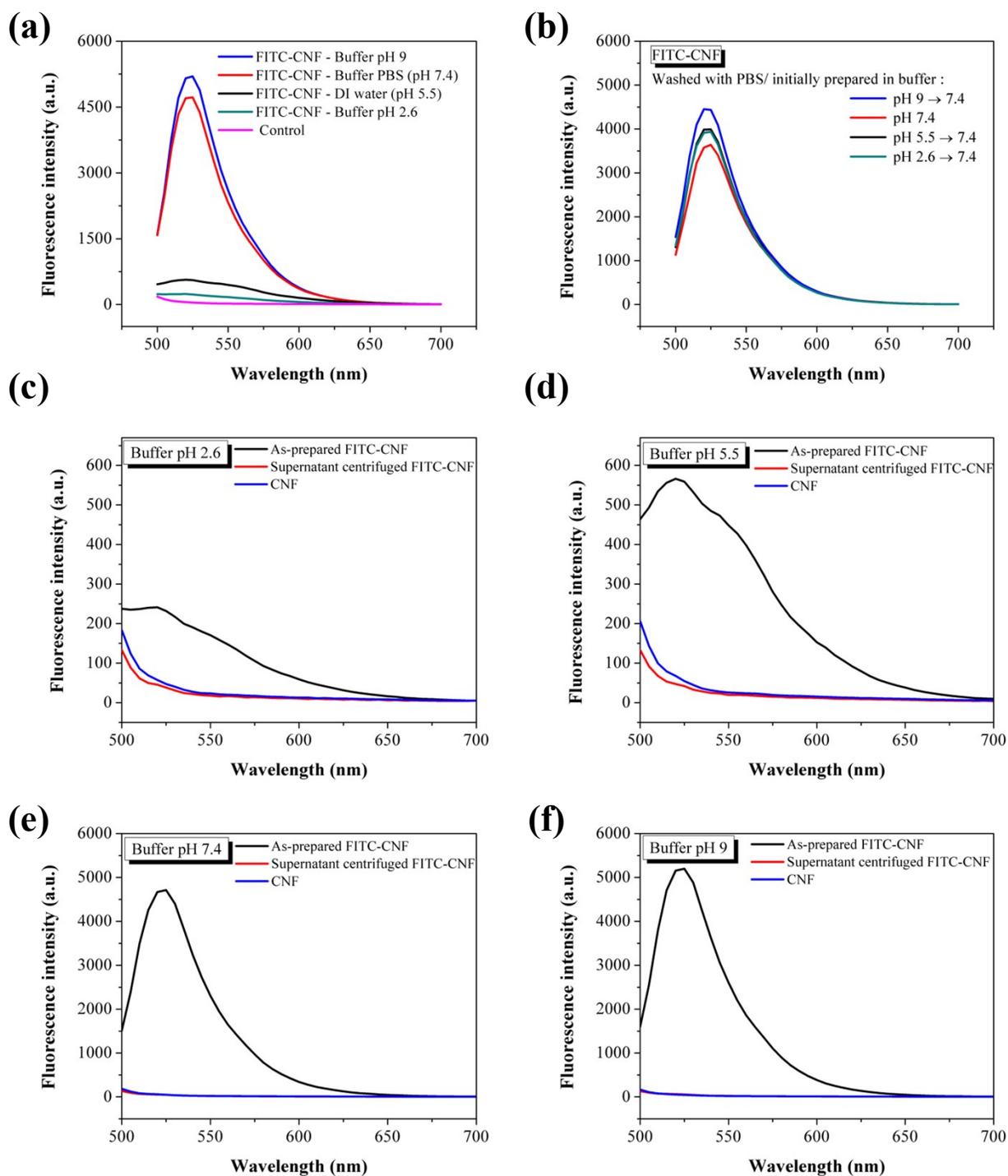


Figure S5. (a) Overlaid fluorescence spectra of 0.5 mg/ml FITC-CNF dispersions prepared at various pH values (2.6 - 9). (b) Recovery of fluorescence intensity once the pH of the suspension is brought back to neutral value. Variation in fluorescence intensity is attributed to slight changes in FITC-CNF concentration due to the washing process. (c-f) Evaluation of FITC detachment from CNF: as prepared 0.5 mg/ml FITC-CNF dispersed in different buffers, compared with their respective supernatants after centrifugation and with untagged CNF dispersed in same-pH buffers. $\lambda_{\text{ex}} = 470$ nm; cut-off wavelength was set at 475 nm.

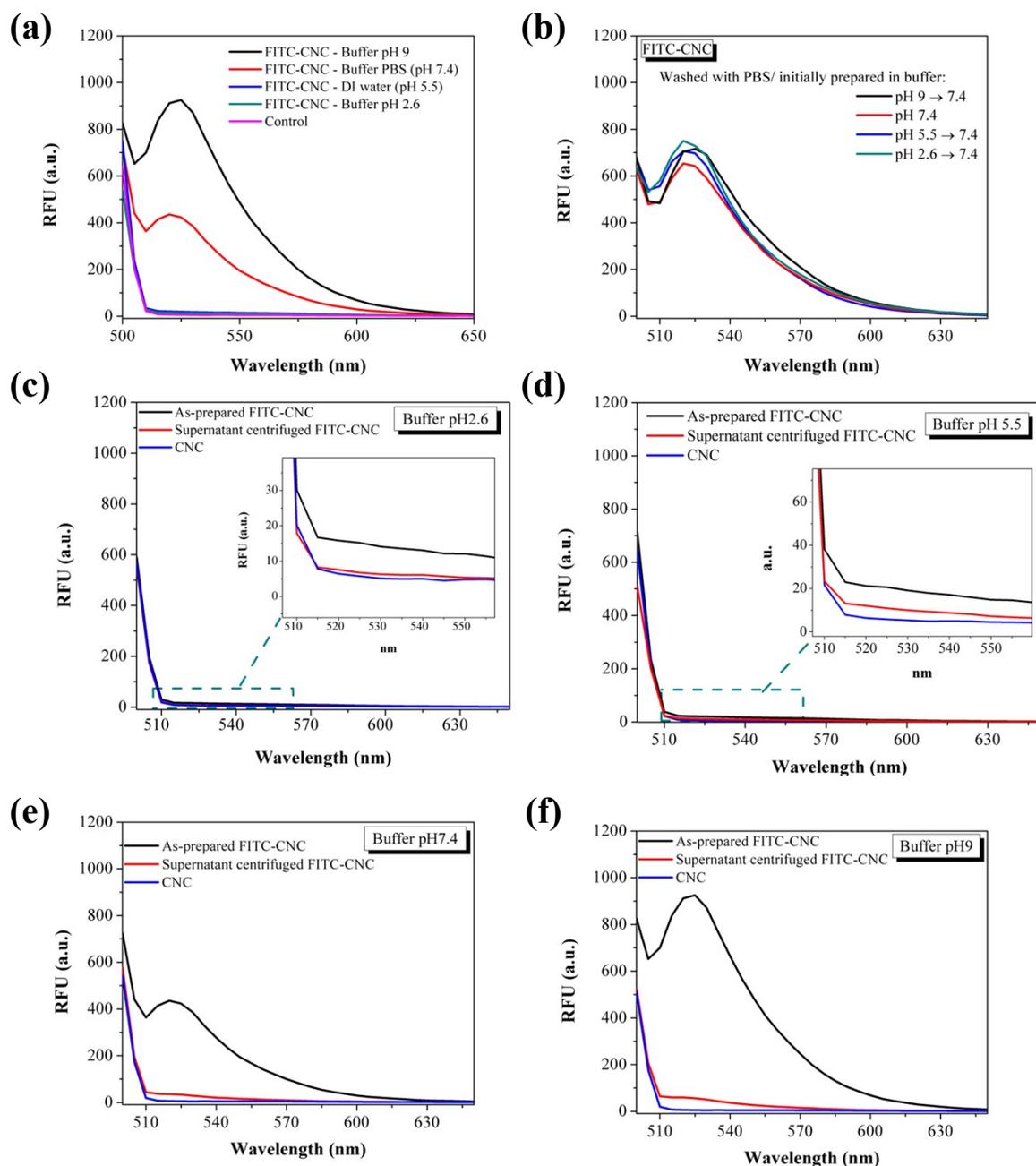


Figure S6. (a) Overlaid fluorescence spectra of 0.5 mg/ml FITC-CNC dispersions prepared at various pH values (2.6 - 9). (b) Recovery of fluorescence intensity once the pH of the suspension is brought back to neutral value. Variation in fluorescence intensity is attributed to slight changes in FITC-CNC concentration due to the washing process. (c-f) Evaluation of FITC detachment from CNC: as prepared 0.5 mg/ml FITC-CNC dispersed in different buffers, compared with their respective supernatants after centrifugation and with untagged CNF dispersed in same-pH buffers. $\lambda_{ex} = 480$ nm; cut-off wavelength was set at 495 nm.

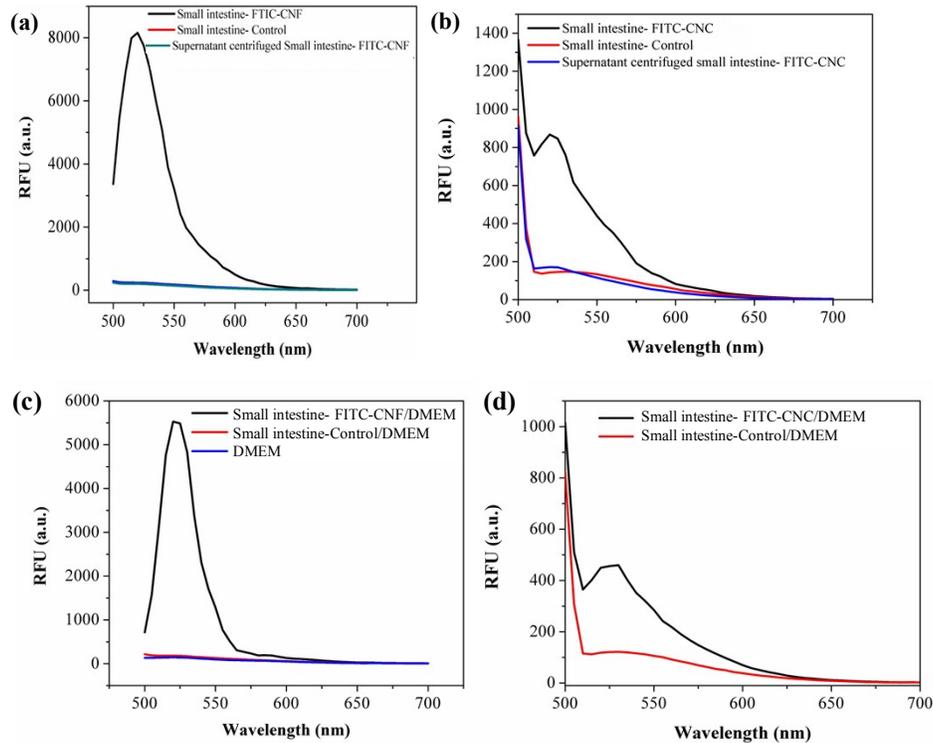


Figure S7. (a) Fluorescence emission spectra from FITC-CNF digesta (“Small-intestine FITC-CNF”), their supernatants digesta of untagged CNF in DI water (“Control”), and supernatants of FITC-CNF digesta. (b) Fluorescence emission spectra from FITC-CNC digesta, DI water (control), and supernatant of digested FITC-CNC digesta. Fluorescence emission spectra of small intestinal digesta of FITC-tagged CNF (c) and FITC-tagged CNC (d) mixed with cell culture media (DMEM; vol: vol 1: 3). Digestas of untagged CNM in DI water were used as control samples.

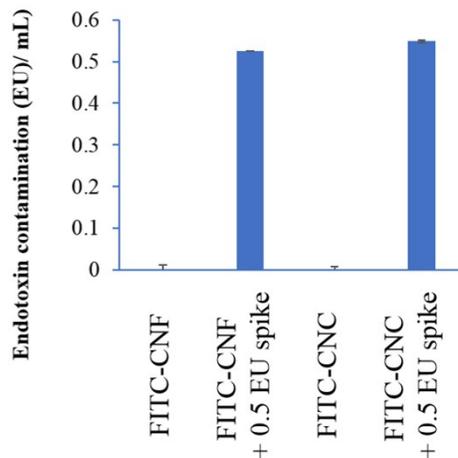


Figure S8: Endotoxin levels were non-detectable in FITC-CNF and FITC-CNC aqueous dispersions using the recombinant factor C assay. No assay interference was detected using 0.5 EU/mL spiked sample.