Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information

Novel Electrospun Chitosan/PEO Membranes for More Predictive Nanoparticle Transport Studies at Biological Barriers

*Lea A. Furer, Ángela Díaz Abad, Pius Manser, Yvette Hannig, Simone Schuerle, Giuseppino Fortunato, Tina Buerki-Thurnherr**

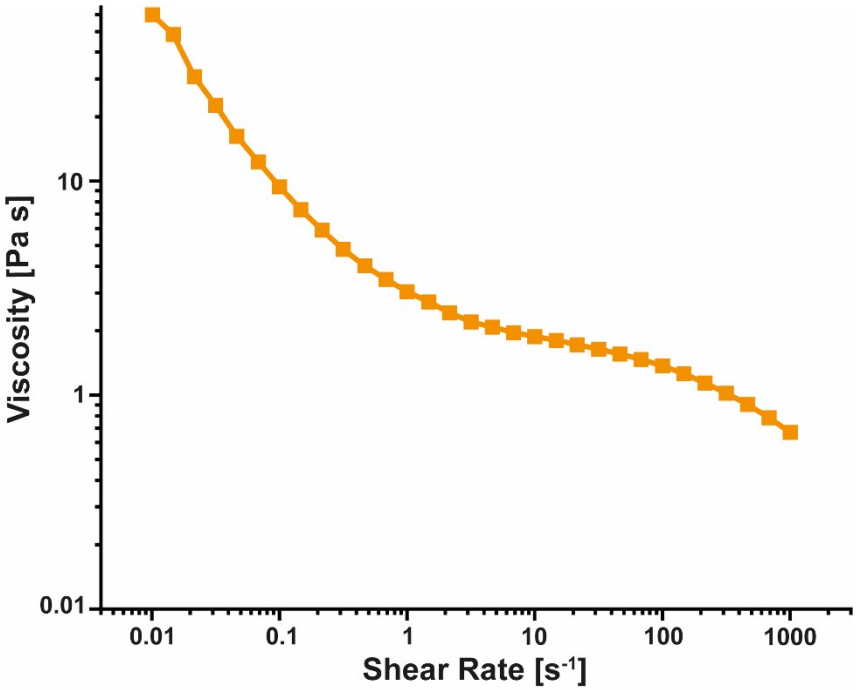

Figure S1. Viscosity of 4% chitosan/PEO (95:5 w/w) solution in 90% acetic acid. Shear-thinning behavior of the solution can be observed. Graph shows a representative measurement curve.

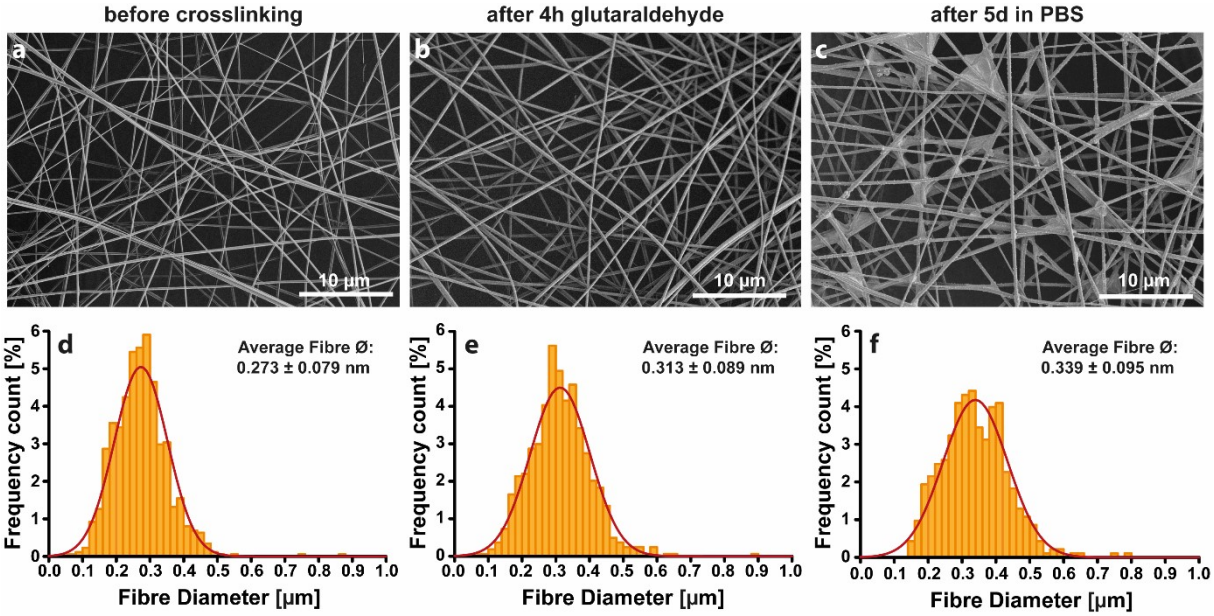

Figure S2. Development of fiber diameter and membrane stability. SEM images and average fiber diameter of 2 min chitosan/PEO membranes directly after electrospinning (a & d), after 4 h crosslinking in glutaraldehyde vapor (b & e) and after immersing the crosslinked membrane in PBS for 5 days (c & f).

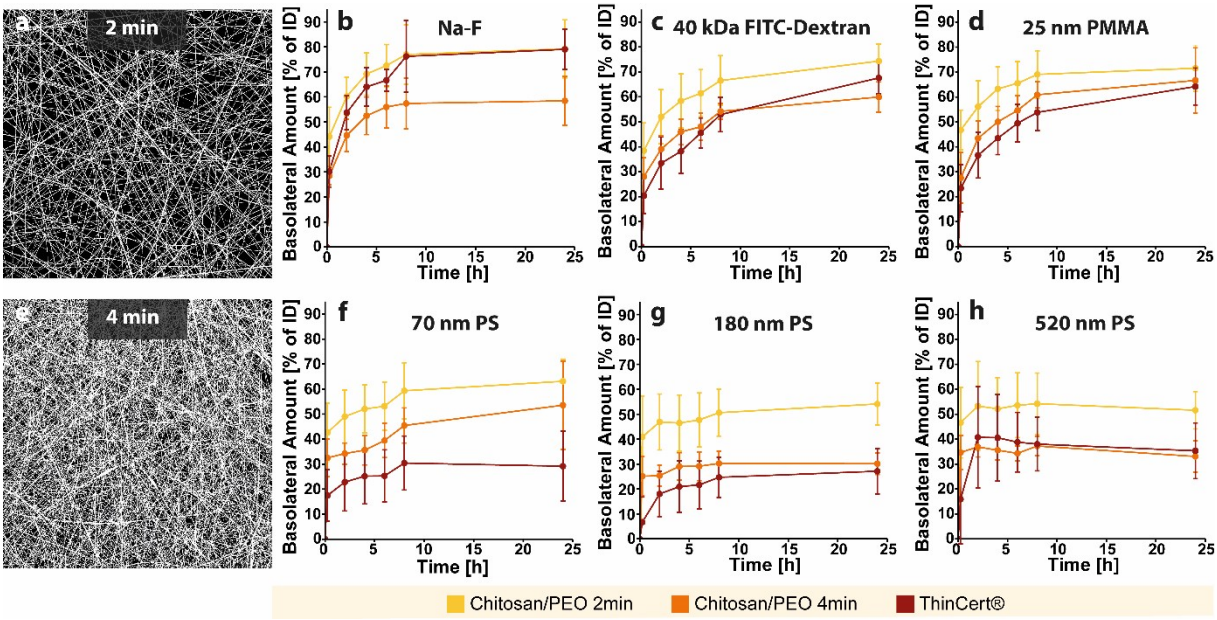

Figure S3. Comparison between 2 min and 4 min membranes. (a) and (e) show segmented images of the electrospun chitosan/PEO membranes that were used for porosity analysis. Additionally, transport of Na-F (b), 40 kDa FITC-Dextran (b), 25 nm PMMA (d), 70 nm PS (f), 180 nm PS (g) and 520 nm PS (h) across cell-free membranes was investigated to compare the permeability of the different membranes. Data represents the mean ± STD of 3 independent experiments with 3 technical replicates each.

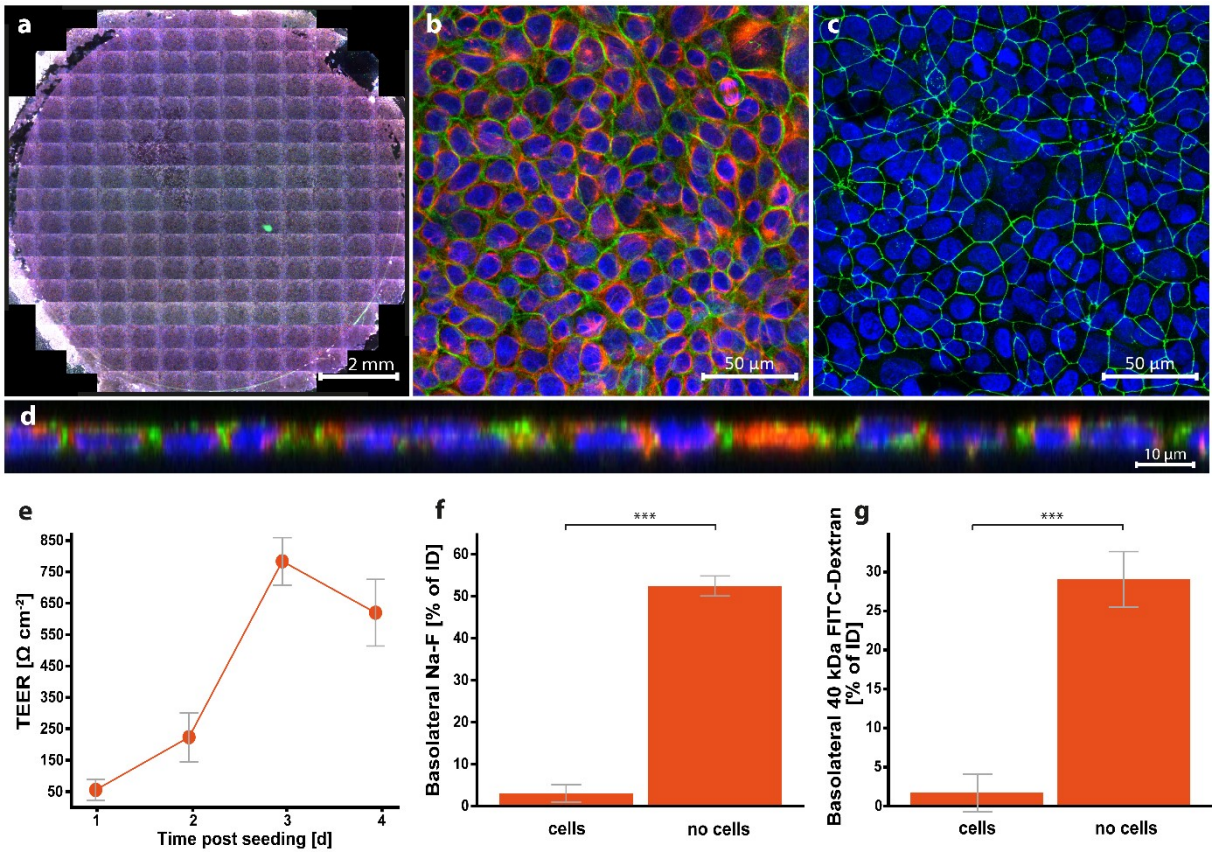

Figure S4. Formation of a tight placental barrier on ThinCert®. ICC staining of BeWo cells grown for 72 h on commercially available PET membranes (a-d). Whole membrane is shown in (a). Cell nuclei (Dapi; blue), tubulin (red) and γ-catenin (green) are stained. (b) CLSM image showing tubulin (red), adherens junctions (γ-catenin; green) and cell nuclei (Dapi; blue). (c) CLSM image showing cell nuclei (Dapi; blue) and tight junctions (ZO-1; green). (d) CLSM cross-section (blue = Dapi, red = tubulin, green = γ-catenin). Barrier formation was verified by TEER measurements (on days 1-4) (e), Na-F exclusion (on day 3) (f) and 40 kDa FITC-Dextran exclusion (on day 3) (g). Data represents mean ± STD from at least 3 independent experiments with 2 technical replicates each. *** p-value < 0.001

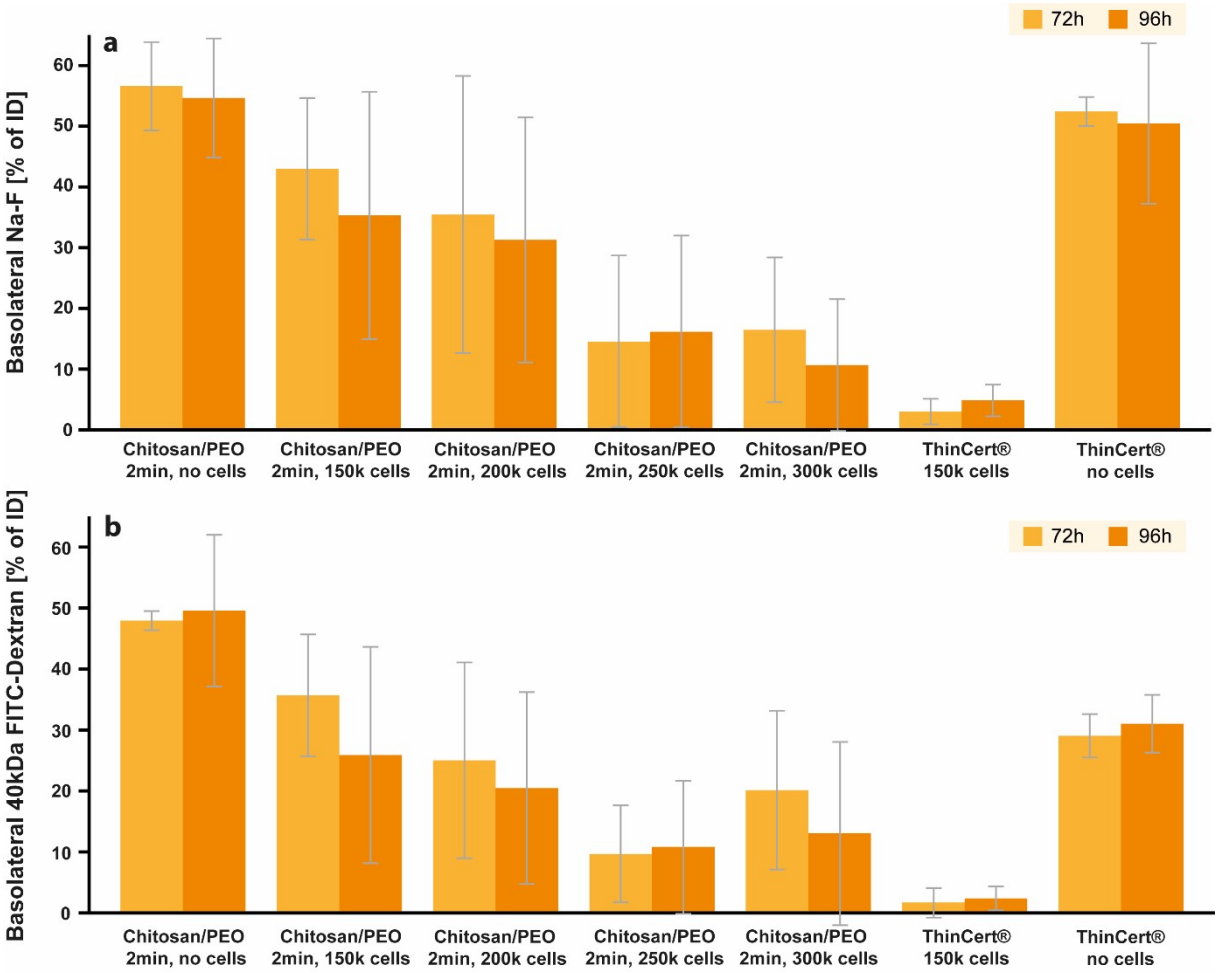

Figure S5. Barrier integrity after different cell culture conditions. Cells were seeded at different cell densities and cultured for 72 h or 96 h on the membranes. Na-F (a) and 40 kDa FITC-Dextran (b) exclusion assays were performed to find optimal cell culture conditions to establish a tight barrier. Data represents mean ± STD from 3 independent experiments with 2 technical replicates each.

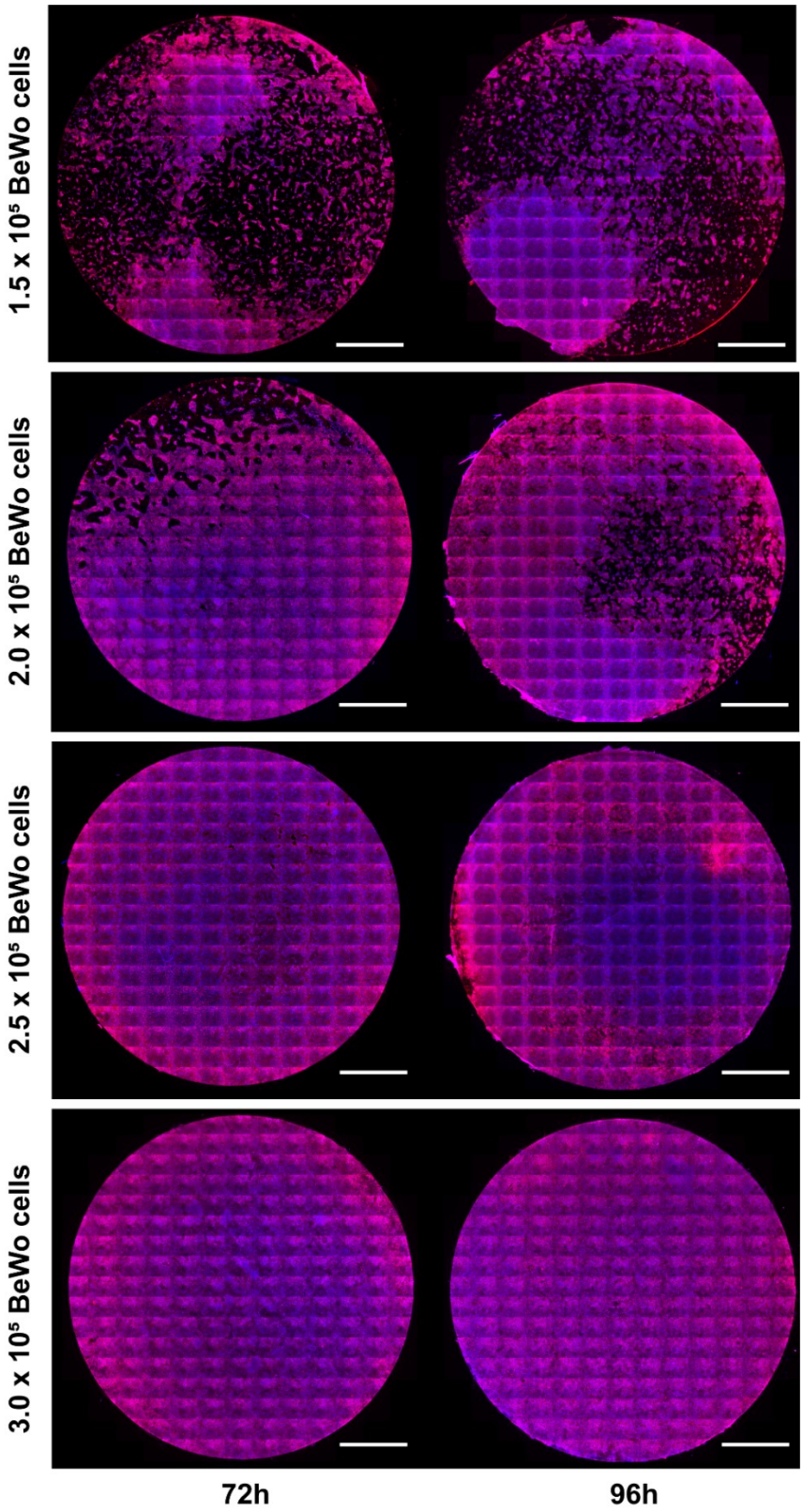

Figure S6. Confluent monolayer formation after different cell culture conditions. Tubulin (red) and Dapi (blue) staining of the whole chitosan/PEO membrane after seeding different cell numbers (1.5 x 10^5, 2 x 10^5, 2.5 x 10^5 and 3 x 10^5). Cells were cultured for 72 h or 96 h before ICC staining was performed. Black spots represent cell-free areas. Scale bar is 2 mm.

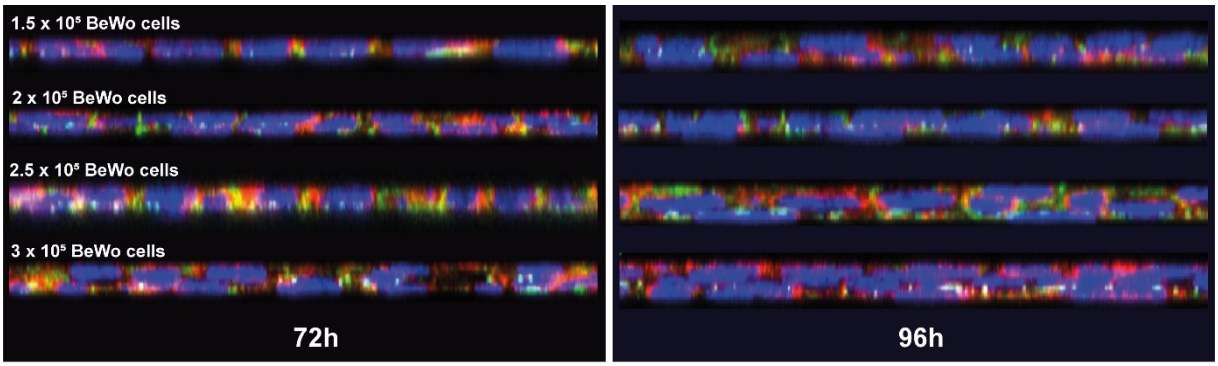

Figure S7. CLSM cross-sections after different cell culture conditions. 1.5 x 10^5, 2 x 10^5, 2.5 x 10^5 and 3 x 10^5 cells were seeded on the 2 min chitosan/PEO membranes and grown for 72 h or 96 h. Cell nuclei (Dapi; blue), tubulin (red) and γ-catenin (green) were stained and CLSM cross-sections were taken to assess monolayer formation.

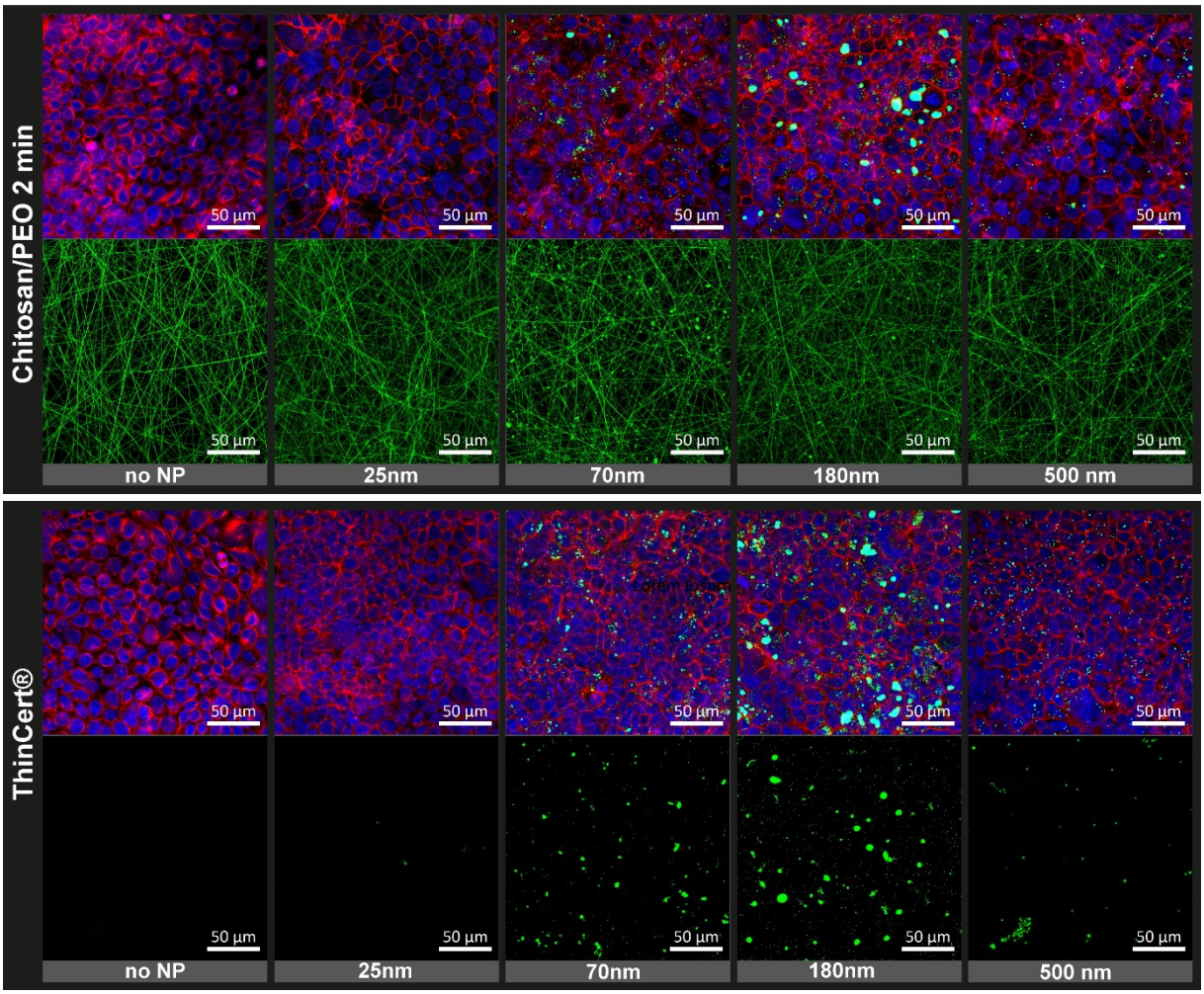

Figure S8. NP uptake and adsorption. After NP translocation studies, cell-free chitosan and ThinCert® membranes were imaged in a CLSM to investigate NP adsorption (lower rows of images). Green dots show NPs in the membrane. Additionally, BeWo cells were stained with Phalloidin to assess NP uptake into the cells after translocation studies (upper rows of images; cell nuclei (Dapi) are shown in blue, F-actin is shown in red, and NPs are shown in green). Results show representative images of 3 independent experiments with 2 technical replicates each.

Table S1. Comparison of different scaffolds used for cell culture applications

Membrane/scaffold	Advantages	Limitations
Track-etched membranes	Commercially available, easy to handle, biocompatible	Low porosity (< 14%), high thickness (~10 µm), artificial surface topography
PDMS membranes	Flexible, transparent, biocompatible, tunable mechanical properties and pore sizes	Usually high thickness (> 10 µm), hydrophobicity, absorption of small molecules and drugs, artificial surface topography
Silicon dioxide & silicon nitride membranes	Very thin (a few nm to > 1 µm), tunable pore size, porosity > 20%, optically transparent, better cell-cell communication	Brittle, difficult to handle, expensive, artificial surface topography
ECM derived membranes	Mimic structure and composition of ECM, easy to fabricate and handle	High thickness (> 10 µm) and low permeability
Electrospun membranes (e.g. nanofibrous chitosan/PEO)	Mimic fibrous structure of ECM, easy to fabricate, inexpensive, relatively thin (< 5 µm is possible), high porosity, good permeability, tunable pore size and mechanical properties, deliver predictive NP transport results	More difficult to handle than track-etched membranes & still absorb some larger NPs