Electronic Supplementary Material (ESI) for Analyst.

A TEM protocol for quality assurance of *in vitro* cellular barrier models and its application to the assessment of nanoparticle transport mechanisms across barriers

Dong Ye,[a] Kenneth A. Dawson,*[a] and Iseult Lynch*[b]

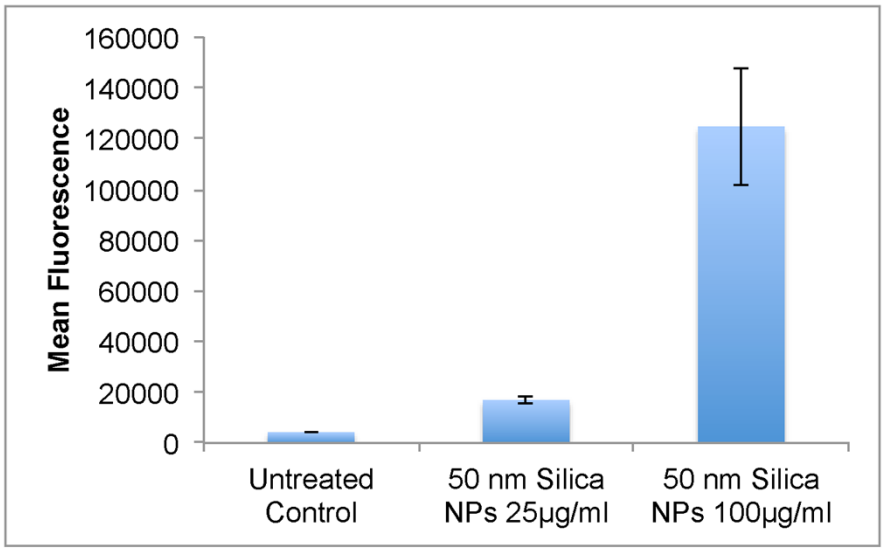
[a] Centre for Bio-Nano Interactions, School of Chemistry and Chemical Biology and UCD Conway Institute, University College Dublin, Belfield, Dublin 4, Ireland

[b] School of Geography, Earth and Environmental Science, University of Birmingham, Edgbaston, B15 2TT Birmingham, United Kingdom

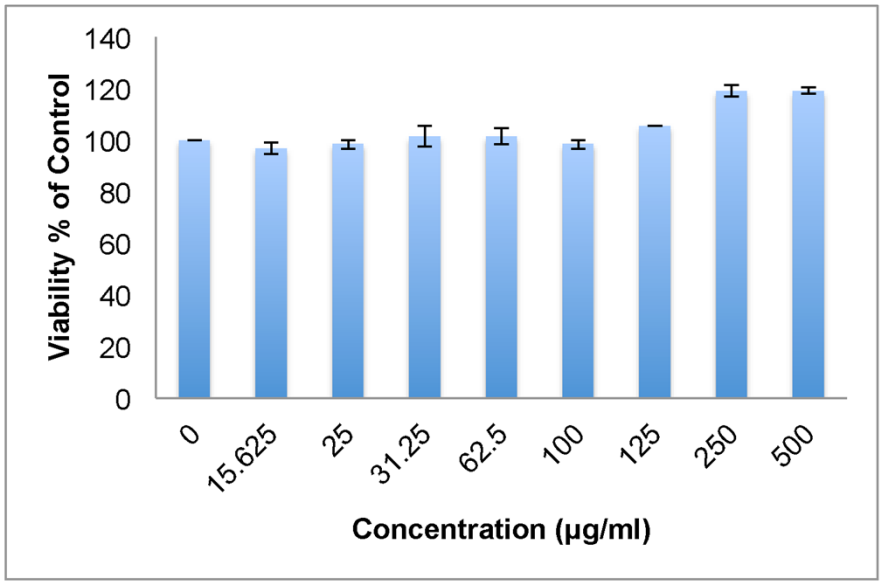
*E-mail: i.lynch@bham.ac.uk and kenneth.a.dawson@cbni.ucd.ie

Supplementary Figures

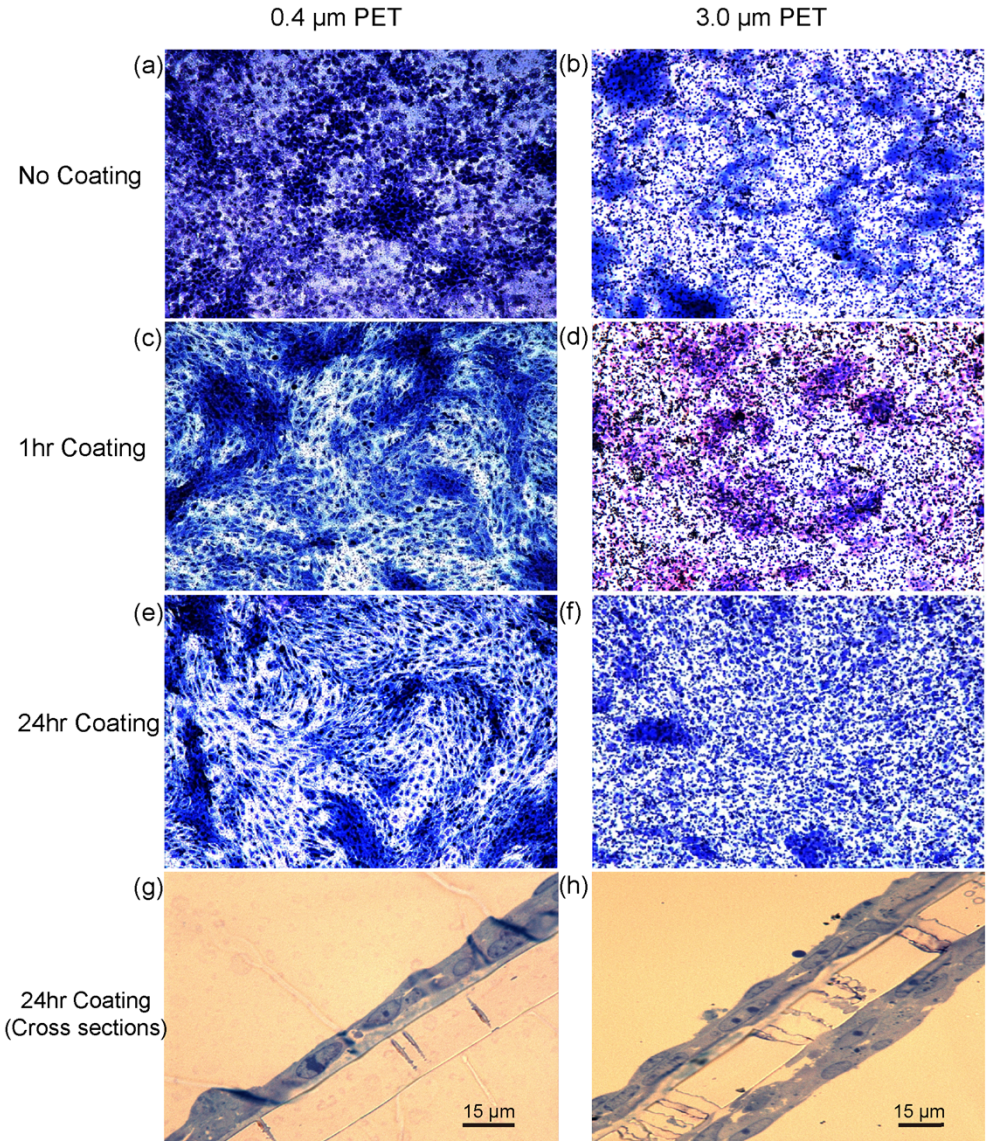
(a)



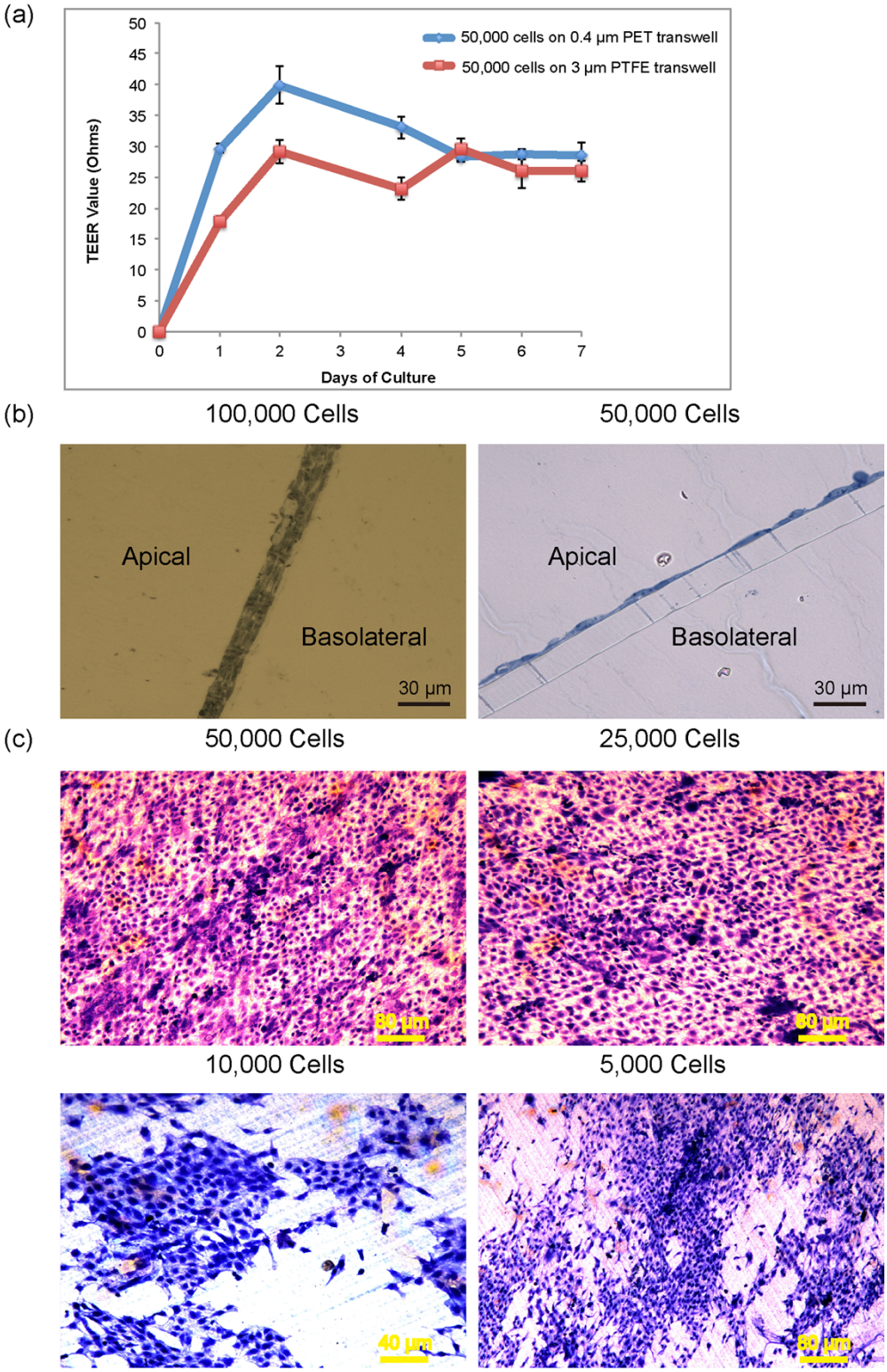
(b)



Supplementary Figure 1. Caco-2 barriers were exposed to different concentrations of 50 nm silica nanoparticles. (a) In flow cytometry, exposure to 100 µg/ml particles resulted in much higher uptake than exposure to 25 µg/ml with 21-day Caco-2 barriers after 6-hour incubation; (b) Cellular viability was investigated using the MTS assay by exposing a range of concentrations of silica nanoparticles to 4-day Caco-2 cells for 24 hours, where cytotoxicity was not significantly seen. Statistics were mean of three replicates with standard errors (SEM).



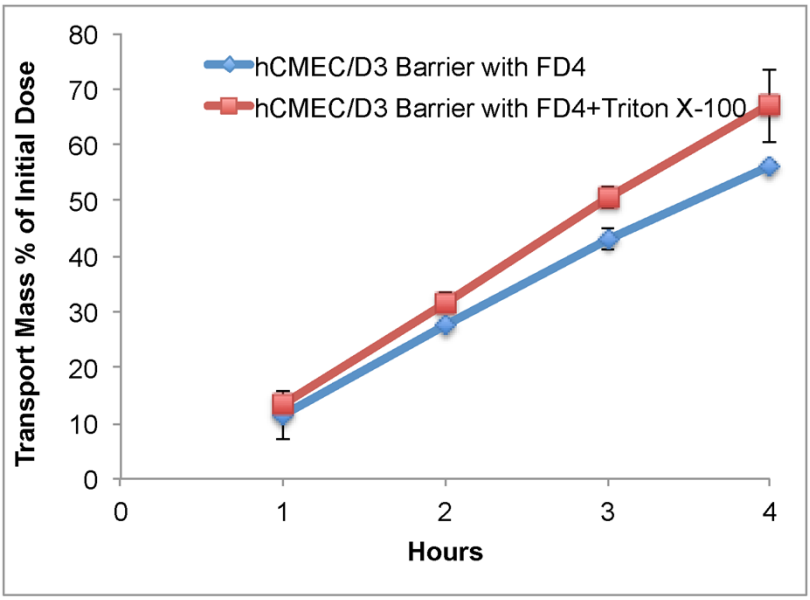
Supplementary Figure 2. Collagen coating and porosity of PET transwells was observed to affect the formation of hCMEC/D3 cell barriers under Light Microscopy. Morphological patterns of cell growth were compared with staining of toluidine blue among 0.4 and 3 µm PET transwells, which were coated without collagen (0.1 mg/ml) (a-b) and with collagen for 1 hr (c-d) and 24 hr (e-f) at 37 ºC, viewed using a 5 X lens. At 24 hours, collagen usually dried out and deposited on transwells, therefore the coating density was known to be higher than coating for just 1 hour. In addition, 3 µm PET transwells showed a poor quality of barrier formation in terms of their confluence and contact-inhibited pattern regardless of the variance of collagen coating, which contrasted with the morphology seen from 0.4 µm PET (c, e). Due to the bigger pore size, the cells on 3 µm PET lost confluence and their typical elongated morphological features due to their potential migration into the pores, which were seen as black dots in (b, d, f). Further cross sections proved the presence of cell migration in 24-hour collagen-coated 3 µm PET due to their bigger pore size, while a monolayer was perfectly formed on the 0.4 µm PET transwell.



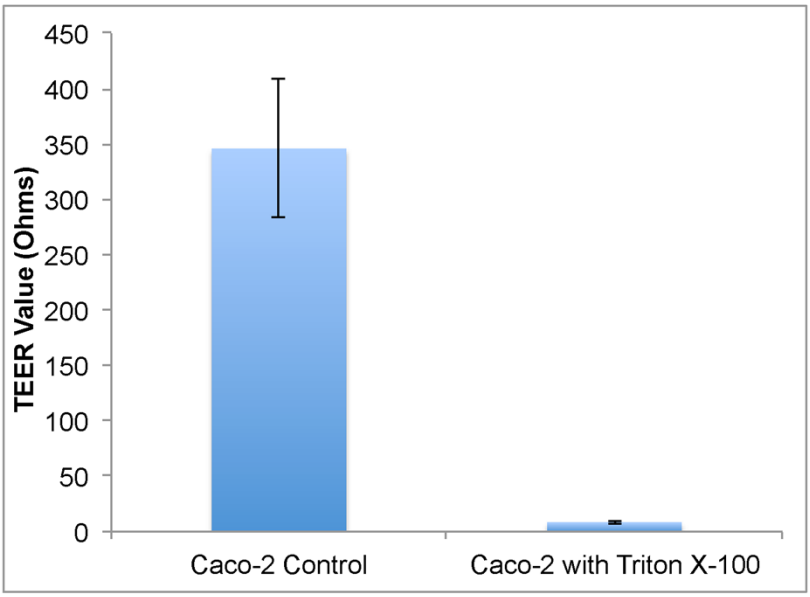
Supplementary Figure 3. Effect of seeding densities on cell overgrowth and confluence after 7 days of culture of hCMEC/D3 cells. (a) TEER values plateaued over 7 days culture similarly on 0.4 µm PET and 3 µm PTFE transwells with an optimal seending density at 50,000. (b) Cross sections showed the formation of multilayer on 0.4 µm PET transwells once the seeding density increased twofold,

from an optimal 50,000 to 100,000. (c) Confluent morphologies were compared among various seeding densities of cells on 3 μm PTFE transwells. Sub-optimal densities (from 25,000 to 5,000 cells) were seen progressively losing their confluence. Statistics were mean of three replicates with standard errors (SEM).

(a)



(b)



Supplementary Figure 4. Application of control chemical (0.1 % Triton X-100) to evaluate the quality of tight junctions of hCMEC/D3 and Caco-2 cell barriers after 7 days and 21 days of culture respectively. (a) Percentage of transport of 4 KDa FITC-Dextran (200 μg/ml) was changed before and after treatment with Triton X-100, which is known to destroy the integrity of the barriers. (b) TEER value was subject to a dramatic decrease after treatment with Triton X-100 in Caco-2 barrier. Statistics were mean of three replicates with standard errors (SEM).