Electronic Supplementary Material (ESI) for Biomaterials Science.

Translocation of flexible polymersomes across pores at the nanoscale

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Translocation dependence on flow and concentration gradients. The influence of flow and of the concentration gradient within the perfusion system is shown in Figure S1. Under static conditions 200 nm $PMPC_{25}$-$PDPA_{70}$ polymersome transport was predominantly influenced by the concentration gradient, which only achieved 28% permeation. By increasing the flow rate between 2 and 20ml/hr, permeation increased very rapidly reaching plateau within the first few hours. The concentration gradient was altered by placing 10mg/ml, 5mg/ml and 2mg/ml solutions of $PMPC_{25}$-$PDPA_{70}$ polymersomes in the donor chamber. Compared to the standard 10mg/ml experiments discussed in the main text, the lower concentrations achieved permeations

that were less efficient by a factor 4 and 11 respectively. Previous studies have shown that with a hydration gradient across a porous barrier liposome transport is increased two fold. In our system the hydration gradient was recreated by placing the porous barrier between the donor and acceptor chambers. This last chamber was connected to a peristaltic pump that created a controllable flow. It is clear that with the absence of flow and only the influence of the concentration gradient, transport was not successful. By increasing the flow rate, permeation was rapidly improved, thus demonstrating the effect hydration has on vesicle transportation (we observed a five-fold increase in permeation). With the higher flow rates PMPC-PDPA polymersomes do fragment but these hydration pull conditions are not found in transdermal delivery applications. The same gradient dependence was observed for changes in the concentration difference across the barrier. With increasing concentration gradients the permeation profiles become faster.

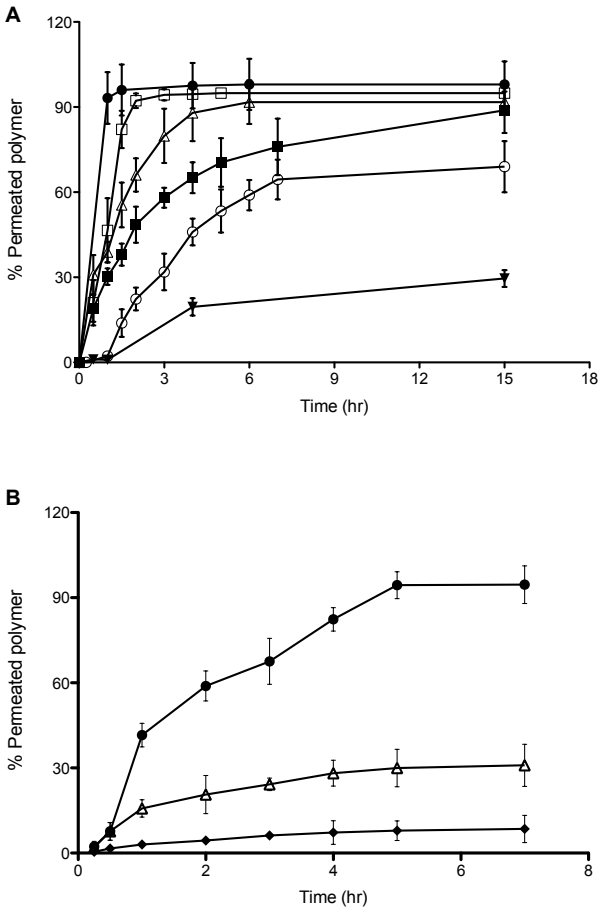

Figure S1. (**A**) Permeation of 200 nm sized $PMPC_{25}$-$PDPA_{70}$ polymersomes across a 50 nm pore sized membrane as a function of flow rate in the acceptor chamber: (●) 20 ml/hr, (□) 15 ml/hr, (△) 10 ml/hr, (■) 5ml/hr, (○) 2ml/hr and (▼) 0 ml/hr. **(B**) Concentration gradient dependence of permeation. Permeation of 400 nm sized $PMPC_{25}$-$PDPA_{70}$ polymersomes across a 50 nm pore sized membrane as a function of concentration in the donor chamber: (●) 10 mg/ml, (△) 5 mg/ml, (◆) 2 mg/ml.

Human skin samples were obtained from breast reductions and

abdominoplasties from the Sheffield Teaching Royal Hallamshire Hospital. It is general practice that all cell extraction must be done within three days of the skin's arrival from the operating theatre. All of the permeation experiments were performed and concluded within a week of skin arrival. If the skin were less viable or less mitotically active it could affect polymersome transport across the barrier (a dying layer of skin is more permeable than a fresh one). For this reason it was decided to see for how long the skin could still be reasonably considered viable. The MTT assay of treated and untreated ex vivo skin samples are shown in figure S2A. MTT application times were adapted due to the thickness of the tissue samples (average thickness 1 mm). The graph in figure S2B shows the skin viability as a percentage of the control (ex vivo skin day 0) as a function of time. It can be clearly seen that the viability remained relatively steady during the first 3 days and that up to 12 days the viability was still above 50%. From 14 days onwards the viability drastically declined as was to be expected. All experiments were begun 1 or 2 days after arrival from the operating theatre and were never longer than 48 hr. We have now added these data in the supporting information

The loss of water across the skin barrier gives an indication of epidermal damage and is often used to measure the effects of certain chemicals, such as drugs or even creams, for dermal applications Netzlaff F. et al (European Journal of Pharmaceutics and Biopharmaceutics 63 (2006) 44–50). The change in TEWL of ex vivo skin was measured as a function of the difference in electrical resistance over time compared to day 1 (arrival from the operating theatre). Figure 2 shows that after 2 weeks there was a minimal increase in resistance suggesting perhaps a very minor loss of impermeability and therefore of barrier properties

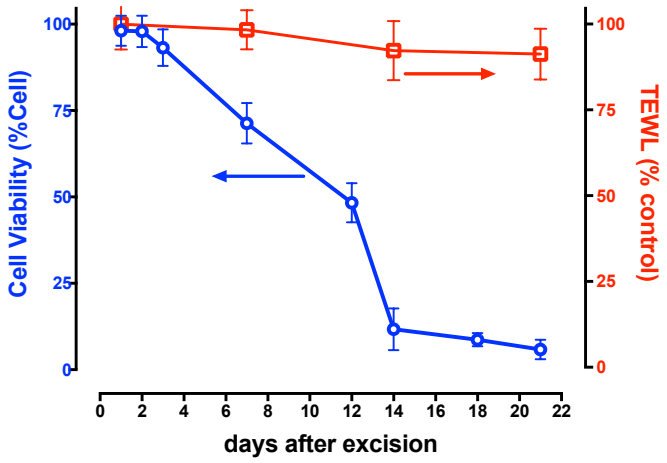

Figure S2. Skin viability. Ex vivo skin samples from days 1 to 18 before and after the addition of the MTT solution and incubation in the dark at 37°C (A). Results of the MTT assay as a function of time and Transepidermal water loss (TEWL) normalized with respect to day zero.

Furthermore, by sectioning the skin samples it was possible to study the morphology of the tissue and determine if there were any noticeable differences with the passage of time that would imply a loss in barrier properties. Both fluorescent and H&E staining did not show any evident change in morphology during the course of two weeks (fig. 3-4).

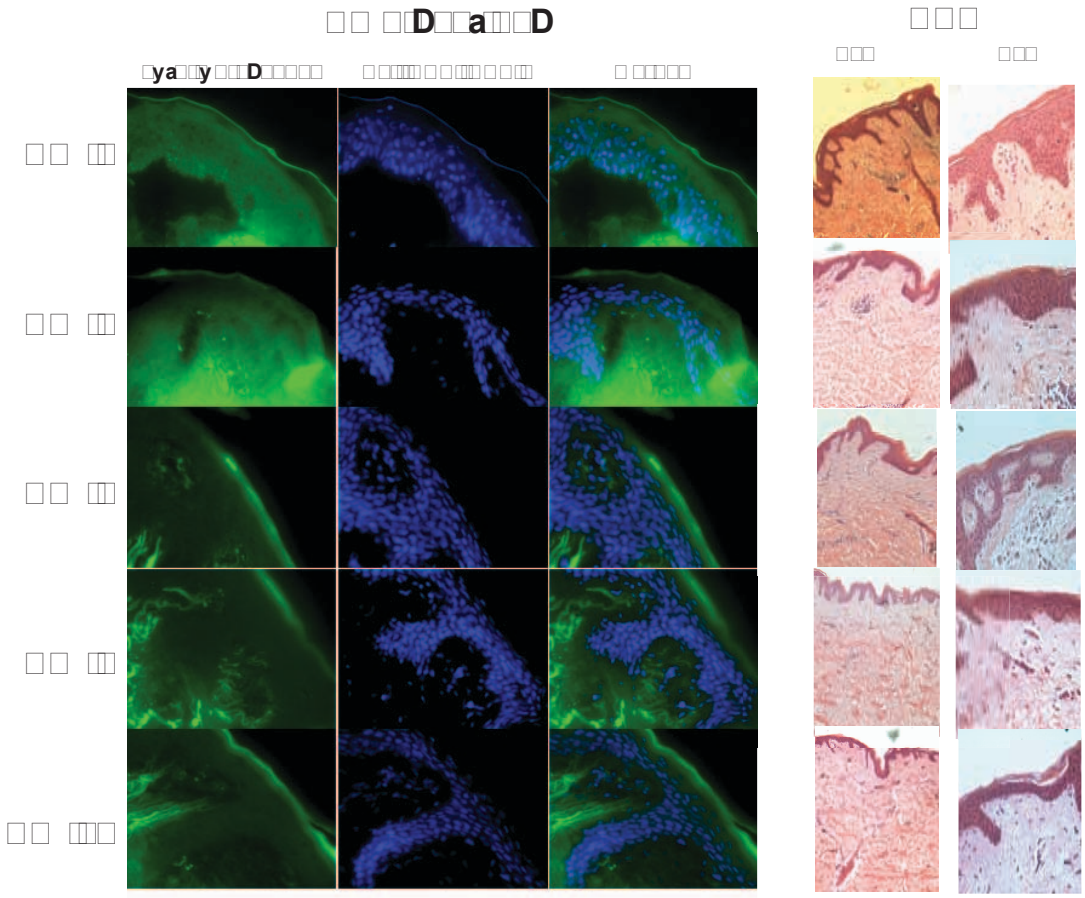

Figure S3. Epifluorescent images of cryo-sectiones of ex vivo skin stained with DAPI showing the morphology as a function of time and H&E stained sections of ex vivo skin stained showing the morphology as a function of time (10 and 40 times magnification).

The results from the experiments showed above, clearly demonstrate that the skin model that have been used was still maintaining the original structure and water content. This have been shown before in other studies from K. Kleszczyn´ski et al, Arch Dermatol Res (2012) 304:579–587. In this work they test the skin capability to keep the initial conditions. What they shows is that the skin start to show damage after 48 hours with an increased LDH release after 72 hours.

Polymersome delivery across *ex vivo* skin. Figure S2 clearly shows the co-localisation of dextran and cell nuclei (image analysis performed using ImageJ software). In blue we have those areas of skin where cell nuclei are on their own, in yellow where dextran is on its own and in white where they are

together. The image shows that a considerable amount of dextran is localized within the cell cytosol surrounding the area around the nuclei. Co-localization between dextran and polymersomes is negligible everywhere apart from the top layer of skin. Both of these observations are a further confirmation of our previous supposition: polymersome interact with the viable cells in the deeper layers of the epidermis, they enter the cytosol via endocytosis, they deliver their payload and then escape the cell environment to continue their permeation across the tissue, thereby achieving transdermal delivery. Co-localization of free dextran and DAPI and dextran and tissue is limited and only occurs in the skin appendages. CLSM clearly show that: (i) dextran has been released and delivered by $PMPC_{25}$-$PDPA_{70}$ polymersomes to the viable and endocytoticly active cells in the deeper epidermal and dermal layers and that (ii) polymersomes continued permeating across the barrier into the acceptor chamber. This explains the permeation profile of encapsulated dextran shown in figure 7b, in which, compared to the membrane model dextran kinetics do not follow as closely those of polymersomes. The permeation profile of encapsulated dextran is faster than that of free dextran because without the polymeric vesicles this molecule cannot cross skin but it is less efficient than that of polymersome since it has been delivered and remains within the cell cytosol.

Figure S3 shows the TEM micrographs of normal untreated human *ex vivo* skin and topically delivered free gold labelled antibodies across the biological barrier. Free IgG is clearly visible only on the top surface of skin.

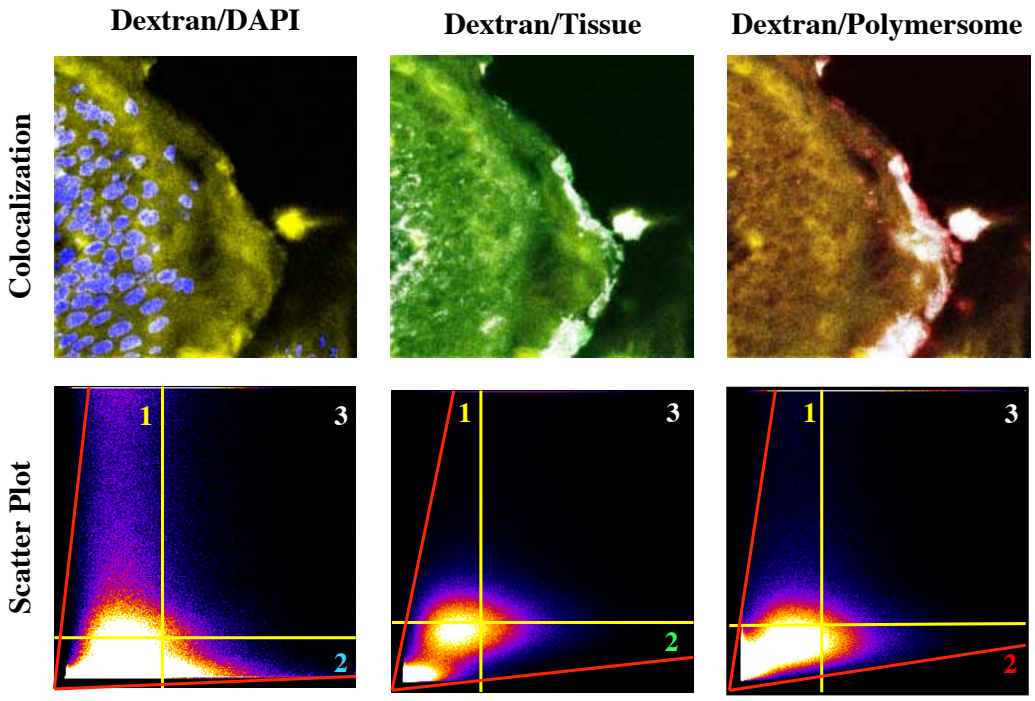

Figure S2. Co-localization of dextran (yellow) with DAPI (blue), with tissue (green) and with polymersomes (red) with the relevant Scatter Plots.

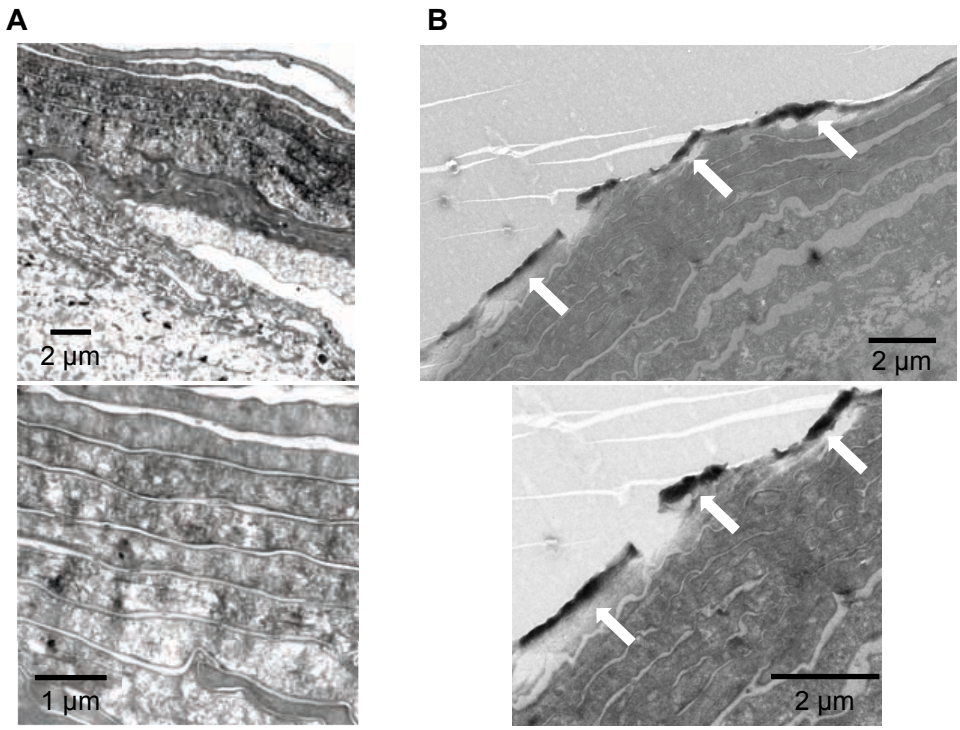

Figure S3. Polymersome delivery across *ex vivo* skin. (A)TEM micrograph of control *ex vivo* human skin. (**B**) TEM micrograph of *ex vivo* human skin after 48hr translocation of free gold nanoparticle. The white arrows indicate where the gold nanoparticles are in the tissue.