Polymersome Macromolecule Delivery across Intact Human Skin

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ABSTRACT

For any drug or therapeutic molecule to cross skin it must possess specific size, charge, hydro- and lipophilic constraints. Very few do this without the assistance of delivery vehicles, such as the one we report here based on flexible nano-sized polymeric vesicles (otherwise known as polymersomes) formed from high molecular weight, amphiphilic, pH-sensitive block copolymers. Our polymersome formulation exhibits both flexibility and shape deformability that allows them to cross very small pores without fragmenting. Our experiments show that they are able to cross 50 nm sized pores and *ex vivo* skin without rupturing and that they can efficiently transport high molecular weight dextran across both synthetic and biological barriers.

Keywords: Polymersomes, Transdermal, Drug Delivery.

1 INTRODUCTION

Transdermal drug delivery (TDD) offers many advantages compared to the more traditional administration routes but it has to contend with skin impermeability. Skin is a highly stratified tissue with specific size, charge, hydrophilic-lipophilic restraints on the kind of molecule that can diffuse across it [1]. The uppermost layer of skin, the stratum corneum (SC), consists of flat, tightly packed, dead or dying, dehydrated, highly keratinized cells, called *corneocytes*, surrounded by a lipid-rich matrix [2-4]. This structure is responsible for 80% of skin transport resistance, even though the SC represents only 10% of all skin. The space between corneocytes is 50-100 nm thick and has a diameter of 30-50nm. Apart from nicotine there are very few drugs that can passively diffuse across skin. To overcome this many researchers have used chemical enhancers, macro- and microscopic skin poration techniques or micro-needle arrays, which can however irritate or damage the skin [5-8]. An alternative way of obtaining transdermal drug delivery is using drug delivery carriers such as micelles, liposomes, dendrimers, niosomes, etc. These systems however do not possess an adequate mechanical stability that would allow them to permeate across skin intact without loosing their payload and would allow them to reach the viable skin layers in which a therapeutic effect could be achieved. It is evident that both size and the ability to deform as a function of the shear rate is an important factor in transdermal delivery. Soft nanoparticles such as vesicles have the necessary requisites to deform so as to fit though small pores. In the last decade a new alternative has been proposed by using amphiphilic block copolymers that assemble into vesicles. Due to the much higher molecular weight of the polymer chains compared to lipids, the membrane structures are held together by a combination of hydrophobic forces and the entanglement between chains [9]. Polymer vesicles, or *polymersomes*, have therefore superior mechanical properties and can withstand higher mechanical stresses compared to liposomes, which render them ideal for transdermal applications [10, 11].

Here we present a pH-sensitive, flexible polymersome formulation based on a poly(2(methacryloyloxy) ethylphosphorylcholine)- co –poly(2- (diisopropylamino) ethylmethacrylate) ($PMPC_{25}$–$PDPA_{70}$) block copolymer. This block copolymer can form stable vesicles whose hydrophobic membranes comprise PDPA chains; this polybase can switch from being hydrophobic at physiological pH to being hydrophilic at acidic pH due to the protonation of its tertiary amine groups [12]. This polymersome formulation can encapsulate hydrophilic, hydrophobic and amphiphilic drugs and can deliver its payload to many different cell types via endocytocis [13]. We demonstrate that our polymersome formulation can cross nanoscopic porous synthetic barriers without losing mechanical stability and that it is able to deliver a model biopolymer across *ex vivo* skin.

2 RESULTS AND DISCUSSION

The polymersome-transport mechanism across a porous synthetic barrier was studied using an adapted Franz diffusion cell, which allowed us to change the hydration pull, the concentration of the permeant and the size of the pores. The porous barrier is placed at an air-liquid-interface between a donor and an acceptor chamber in the same way that skin is naturally exposed to air whilst the deeper layers have a high water content. This creates a similar hydration gradient to that existing for skin *in vivo*. The acceptor

chamber is connected to a peristaltic pump that creates a controllable laminar flow at the air-liquid-interface. This flow is necessary for the correct simulation of the hydration pull that would be found *in vivo*. Our studies show that by increasing the flow rate and the concentration of the polymersome solution, transport across a homogeneously porous polycarbonate membrane with an average pore size of 50 nm and a pore density of about 10% is enhanced dramatically. If we look carefully at the flux across the membrane as a function of concentration and pressure difference created by the flow rate we can see that the relationship is not a linear one, indicating that the process is not purely a diffusive one.

2.1 Transport of large polymersomes across narrow pores

A factor that strongly influences the rate of polymersome transport is the pore size compared to the polymersome dimensions (Fig.1a). Experimental results show that larger polymersomes are more flexible than smaller polymersomes and hence are able to deform more easily across 50 nm pores. Both 400 and 200 nm sized polymersomes have a fast initial transport rate (67.6% and 40.0% respectively of the initial solution has crossed after 3h) and after 11 h of transport 95% of the larger polymersome solution has permeated across the membrane compared to only 69.4% of the smaller polymersome solution. The energy required to deform a large spherical vesicle into a smaller pore is proportional to the bending rigidity k and the ratio between vesicle and pore radii. Polymersomes possess a bending rigidity that is much higher than simple thermal fluctuations and must therefore be pushed across the pore by a driving force. If the force is small it will pull the vesicle towards the pore and the vesicle will find itself in a state of equilibrium. If however the force is enough to extend the vesicle into the pore a distance comparable to radius of the pore then it will spontaneously be sucked into the pore due to the lower potential energy. Permeation under these circumstances becomes proportional to the driving force.

Dynamic Light Scattering (DLS) analysis shows that both sized polymersomes are mechanically stable and that their average size does not change before or after transport (Fig.1b-c), within the experimental error. Polymersomes deform sufficiently to permeate across pores that are up to eight times smaller than their average. Confirmation of polymersomes integrity was obtained by comparing the trans-barrier flow of free FITC labelled dextran, of FITC-labelled dextran loaded polymersome and of empty polymersome solutions across a 50 nm pore sized membrane (Fig.1d). Compared to the encapsulated dextran the free dextran solution had a slower initial release rate and after 3 h it reached a maximum transport of 33%. The encapsulated dextran transport rate follows very closely that of the empty polymersomes, with a maximum of 58.67%. This co-migration of polymersomes and encapsulated biopolymer confirms that transport occurs and does not involve vesicle fragmentation.

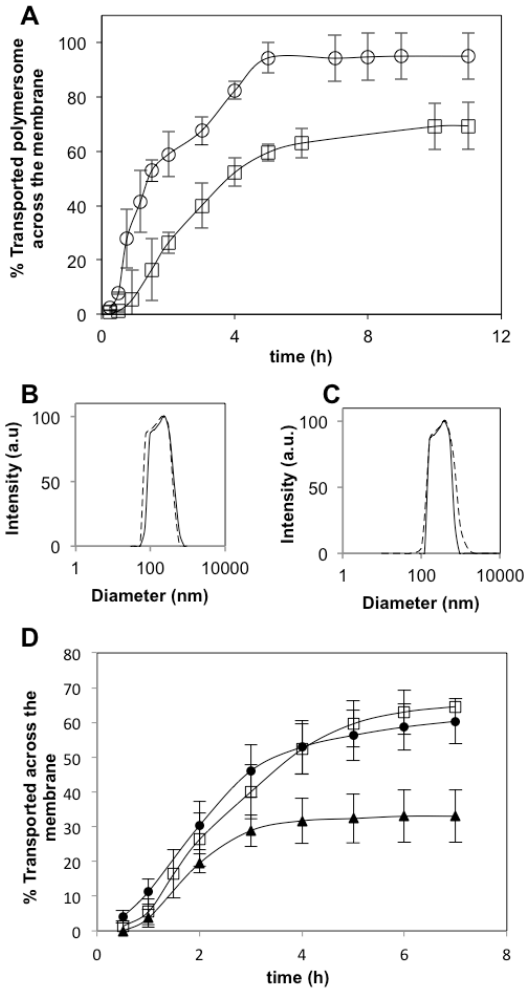

Figure 1: Polymersome transport across synthetic porous barriers. (a) Transport of 400 nm (○) and 200 nm (□) sized polymersomes across a 50 nm pore sized membrane as a function of time (n=3). (b-c) Size distribution analysis using Dynamic Light Scattering (DLS) of 400 nm and 200 nm polymersomes before (−) and after (−−). (d) Transport of 200 nm empty polymersomes (□), of FITC-labelled dextran encapsulated in 200nm polymersomes (●) and of free FITC-labelled dextran (▲), (n=3).

2.2 Transport and delivery across intact human skin

Confocal Scanning Laser Microscopy (CSLM) analysis of skin sections shows that 400nm sized polymersomes crossed the SC and reached the viable epidermis after 48 h (Fig.2a). The 3D reconstructed images of permeation after 6, 24 and 48h show an increasing depth of permeation as a function of time (Fig.2b-e). Analysis of the acceptor

chamber fluorescence shows that polymersome transport across skin has a very fast release rate in the first few hours and that after 48 h the 400 and 200 nm vesicles diffused to a maximum of 73.6 % and 64.5% respectively (Fig.2f).

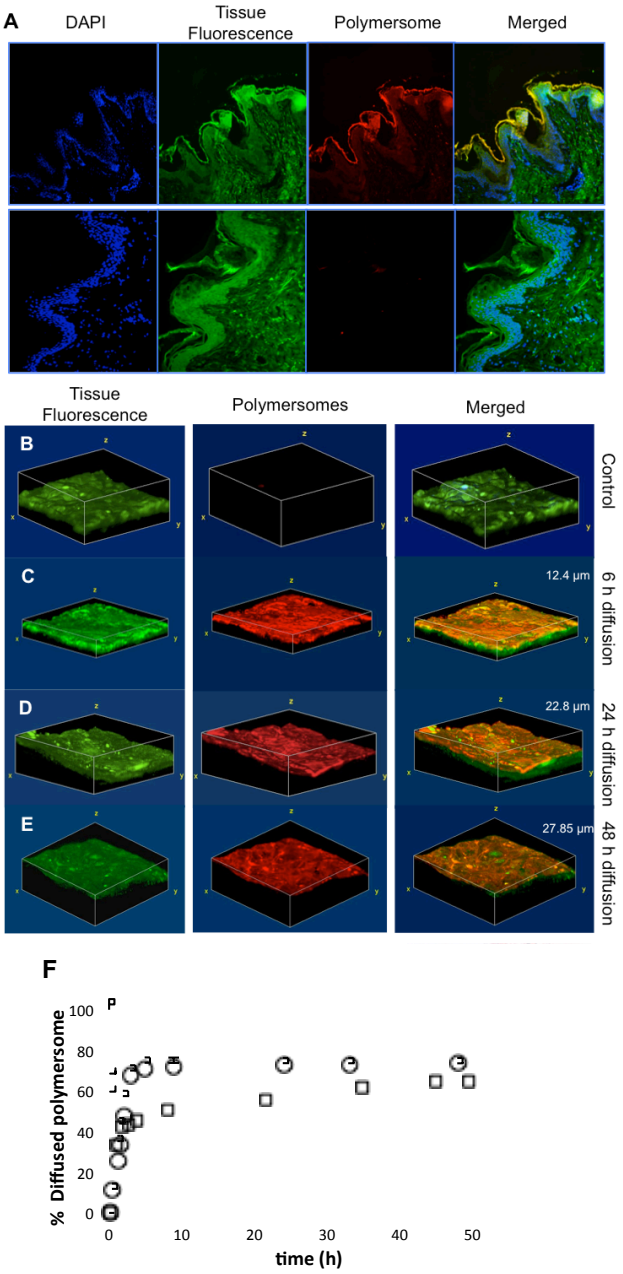

Figure 2: Polymersome transport across intact skin. (a) Fluorescent microscope images of a section of *ex vivo* skin after transport of 400nm sized fluorescently labelled polymersomes for 48 h. (b-e) 3D reconstructed images of transport across skin (n=3). (f) Permeation of 400 (○) and 200 (□) nm sized polymersomes as a function of time.

CLSM of the sections of *ex vivo* skin after 48 h transport of free dextran show no or very little penetration (Fig.3). Dextran remains mostly on the skin surface and only reaches the deeper layers of skin via the skin appendages. The encapsulated dextran was carried across the SC by polymersomes and delivered all the way across the epidermis to the level of the basal membrane. Polymersome fluorescence is evident in the top layers of skin where the PMPC block of the polymersomes may have interacted with the highly keratinized cells but not along the basal layer where dextran appears to have collected. This suggests that the encapsulated dextran has been delivered during transport as a consequence of the polymersome-cell interaction.

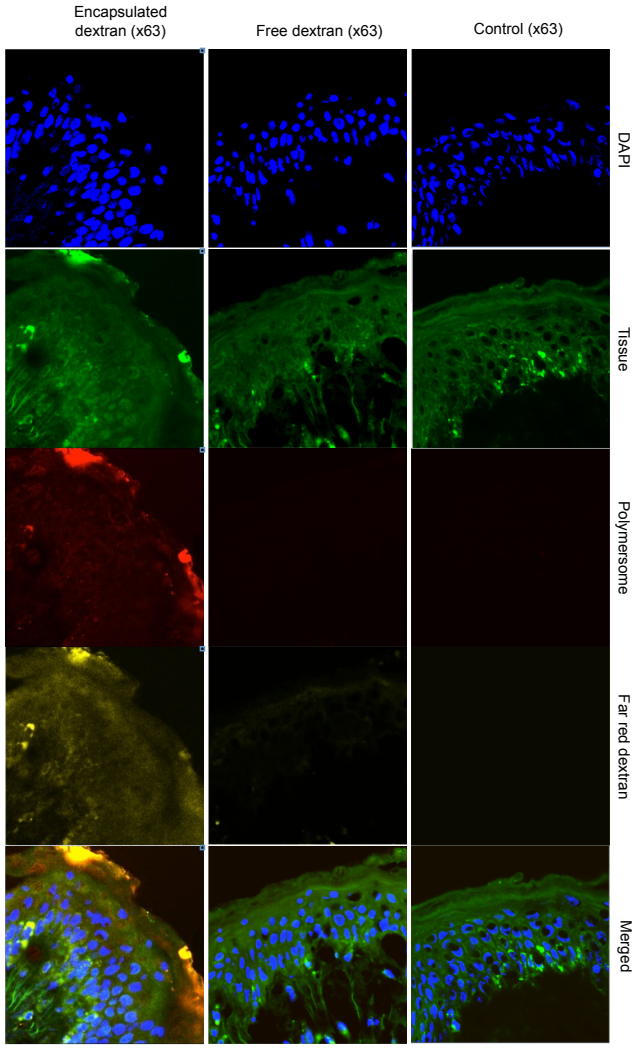

Figure 3: CLSM images of *ex vivo* skin after 48 h of dextran-loaded polymersome transport (n=5). The blue channel shows the cell nuclei stained with DAPI, the green channel the tissue fluorescence due to collagen, the red channel the polymersome fluorescence and the yellow channel the dextran fluorescence.

Analysis of the acceptor chamber fluorescence shows that free dextran has an extremely slow release rate with a very long lag time (it reaches a plateau after 4 h with a maximum transport of 4.3%). Encapsulated dextran has a

relatively faster release rate, which also attains a plateau after 4 h with 17.8%. The release rate within the first 4 h is similar to that of polymersomes. It then decreases rapidly confirming, as was suggested by the CLSM analysis, that polymersomes have delivered dextran into the tissue and have kept on crossing the skin.

3 CONCLUSIONS

PMPC-PDPA polymersomes exhibit flexibility and good shape deformability which allow them to traverse very small pores without losing their mechanically stability or rupturing. Polymersomes of 200 and 400 nm diameter are able to diffuse across 50 nm polycarbonate membrane pores as well as across *ex vivo* skin. Due to their greater surface area relative to surface tension, larger polymersomes are able to deform more easily and cross the porous barriers more quickly than smaller polymersomes. Compared to the passive diffusion of free dextran, the permeation of this biopolymer across polycarbonate membranes was improved by a factor of 2 using dextran-loaded polymersomes as delivery vehicles. A comparable improvement was observed across *ex vivo* skin. CLSM studies confirmed that dextran was transported across the SC by polymersomes and delivered to the viable epidermis up to the basal membrane.

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