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PENETRATION OF STRATUM CORNEUM BY DOUBLE-LABELLED ULTRADEFORMABLE LIPOSOMES EMPLOYING A NON-OCCLUSIVE TECHNIQUE

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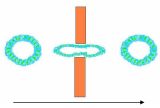
Purpose: *To compare the penetration depth into the stratum corneum of ultradeformable liposomes labelled with Rhodamine-phosphatidyl ethanolamine (Rho-PE) vs. conventional liposomes, employing a Saarbrücken penetration model modified ad-hoc for non occlusive conditions.*

Methods: *100 nm-sized unilamellar ultradeformable liposomes of soy phosphatidylcholine (SPC) and sodium cholate (NaChol) containing Rho-PE as membrane probe and the green label HPTS (pyranine) as aqueous marker, were non-occlusively applied to human skin explants and incubated 1h at 32ºC, followed by transversal cryosection and observation by confocal laser fluorescence microscope. Conventional (non ultradeformable) double-labelled liposomes were used as control.*

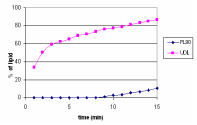
Results: *Confocal images revealed that Rho-PE from ultradeformable liposomes appeared in several stratum corneum layers, until a depth of 14 µm in average, while the HPTS was found in a deeper separate fraction into the viable epidermis, reaching a depth of 24 µm in average. On the contrary, the Rho-PE from conventional liposomes was observed only on the first superficial layer of stratum corneum while the HPTS signal remained absent.*

Conclusions: *The lipid matrix of ultradeformable liposomes deeply entered the stratum corneum, on the coast of losing their aqueous content along the penetration. However, the leaked hydrophilic label could deeply penetrate across the epidermis. On the other hand, only the lipid matrix of conventional liposomes was detected on the surface of the stratum corneum. Hence, only during squeezing of ultradeformable matrices across the stratum corneum, small hydrophilic drugs are released, to be found in deeper skin layers.*

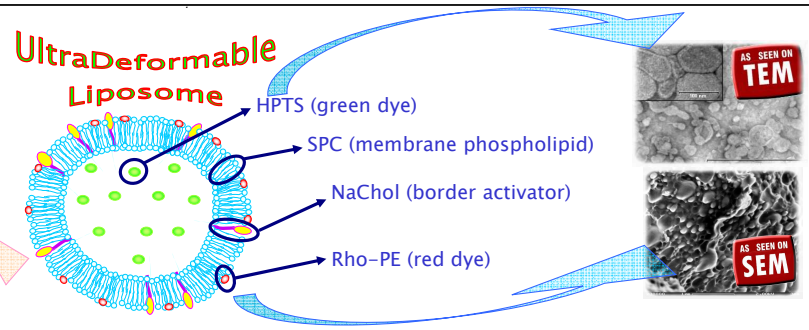
Double-labelled UDL were prepared by resuspension of a film made of a mixture of lipid formed by rotary evaporation of organic solutions of SPC, NaChol, and Rho-PE into a round bottom flask, followed by sonication and extrusion to render unilamellar UDL of 100 nm. Non-incorporated HPTS dye (dissolved in the resuspension buffer) was separated from UDL suspension by size exclusion cromatography



Ultradeformable Liposomes (UDL) are lipid bilayers of 100nm capable of changing their shape, being able of passing through pores of until a 10% of their diameter, experimenting spontaneous locomotion under the presence of a hydration gradient like the existent across the impermeable barrier of the skin

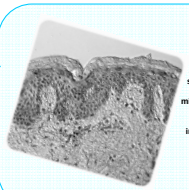


Ultradeformability test: UDL are able to pass through a 50nm pore membrane under low pressure (0,8 Mpa) while conventional liposomes (PL90) remain retained

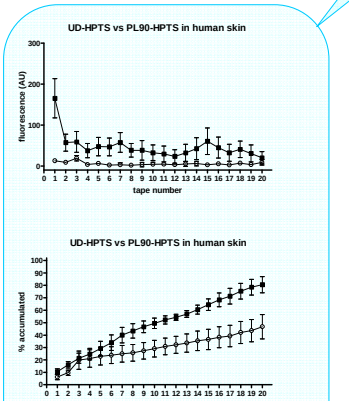


Like in the "Saarbrücken Penetration Model" routine, fat is separated from the full-thickness skin and the remaining stratum corneum, epidermis and dermis are kept freezed until use. The skin is cut in disks and placed into a teflon device where is incubated with the UDL suspension. Because of the special need of non-occlusive conditions for the formation of the hydration gradient across the skin, the disk is allowed to dry into a oven at 32ºC and then incubated to test penetration.

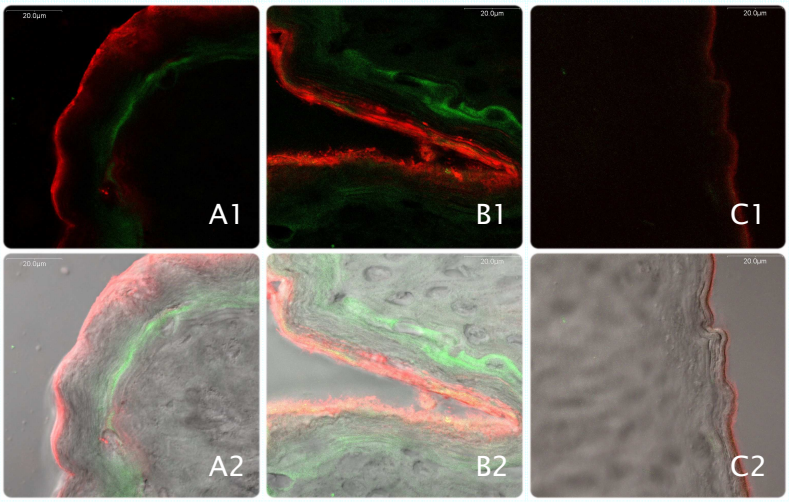
After the incubation time is reached, the skin disk can undergo tape stripping for probe quantitation, or transversal cryosectioning for image analysis by confocal laser scanning microscopy



8-micrometer thick skin slices are collected from the cryostate over a microscopy slide, following chemical fixation by methanal and physical immobilization by gelatin



20 tape strips of each sample were obtained, collected in separated flasks and submitted to an extraction process. Quantitation of HPTS by fluorometry after tape stripping showed a higher penetration of the hydrophilic dye into deeper strata, and an overall higher presence of the dye inside the skin



Confocal laser scanning microscopies of cryosections of human skin explants (from abdominal plastic surgery) non-occlusively incubated (1h after skin surface drying) with double-labelled UDL. Upper row shows confocal laser image only, while the lower row combines confocal laser and optical microscopy images. A and B columns show the presence of the labelled lipid (in red) into several layers of the stratum corneum and the hydrophilic dye (in green) located into deeper strata, even entering the viable epidermis. On the other hand, C column is a conventional (non-ultradeformable) double-labelled liposome control, where the lipidic label is located only at the outer surface of the stratum corneum (without entering it) and there is no presence of the green dye