Nanocarriers for Skin Applications: Where Do We Stand?

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Supporting Information

1. PENETRATION ENHANCERS

In addition to the previously discussed nanoparticles, a widely studied and established method for enabling the penetration of active molecules is the use of so-called penetration enhancers. Penetration enhancers are compounds or methods which can either change the solubility of a transported therapeutic or disrupt the SC, therefore enabling the penetration of NPs into the deepest layers of the skin. Penetration enhancers are most commonly used in transdermal therapies (Figure S1) and are classified into: physical and chemical penetration enhancers.

![Figure S1](image)

**Figure S1.** Different penetration enhancers for drug delivery of across the skin.

1.1 Physical Penetration Enhancers

Physical disruption of SC can be achieved by employing techniques like ultrasound, electroporation, iontophoresis, microneedles or lasers (Figure S1). These techniques can be further classified into methods that do not puncture the skin (e.g., electroporation, iontophoresis and sonophoresis), and methods that puncture the skin (e.g., microneedles). Methodologies that do not puncture the skin, can enhance the penetration by permeabilizing the lipid bilayers within the skin. These strategies are usually associated with the use of short duration and high intensity electric pulses (electroporation), continuous low intensity electric fields at a constant field strength (iontophoresis) and low frequency ultrasound (sonophoresis). Although popular, these methods are usually expensive, have a limited application area and rather limited penetration depths. An attractive alternative is to use methods which create additional channels (pores) within the skin. In this regard, the most popular methods that gained considerable interest over the past decades include the use of microporation and microneedles. In microporation, an electric current is pulsed through an array of resistors placed on the skin resulting in localized ablation of the corneocytes in the SC. Intradermal injections using microneedles is another popular technique for the direct, size independent delivery of therapeutics to the viable tissue layers of
the skin by employing micrometer sized needles.\cite{6} This easy and cost-effective technique for transdermal delivery has experienced significant developments due to the advancements in various fields like polymer- and material-chemistry, leading to the creation of various kinds of microneedles including solid, coated, dissolving and hollow.\cite{7} Microneedles that are degradable under specific conditions, assist in the controlled release of the transported cargo such as therapeutics alone or loaded into NPs.\cite{6b,8} As example, Li et al. reported the fabrication of a reversible contraceptive microneedle patch for the slow release of levonorgestrel, a contraceptive hormone. It was demonstrated that a slow and sustained release of levonorgestrel could be achieved by the degradation of poly(lactic-co-glycolic) (PLGA) acid needles over a month in rats.\cite{9} Moreover, Fakhraei et al. investigated the delivery of small and macromolecular biomolecules across the skin by employing dissolving microneedles (DMNs) based on a hyaluronic acid backbone (Figure S2). In this study, the authors evaluated the effect of the shape of the microneedles to achieve tissue interlocking (TI) (ultimately achieved by using microneedles with a sharp tip, narrow neck and wide tip geometry) as well as enhance the penetration of Rhodamine B (employed as a drug model) under in vitro and in vivo conditions.\cite{10} From the drug release profile in Figure S2A, it is evident that TI-DMN induced a faster Rhodamine B accumulation compared to conventional DMN when applied on pig cadaver skin in a diffusion cell apparatus. This was explained by the complete dissolution of TI-DMN over a period of 120 min (Figure S2C) compared to the conventional DMN, which did not completely dissolve over the patch (Figure S2B). Furthermore, the authors investigated the skin penetration properties of the TI-DMNs in in vivo on dorsal skin of mice (Figure S2D). The mice treated with TI-DMN showed a complete array of spots (Figure S2E) compared to the conventional DMNs wherein the spots were barely visible (Figure S2F). The penetration success rate of TI-DMNs on mice skin was found to be 98±1.1% which was considerably higher than for conventional DMNs at 74±5.2% (Figure S2G). Finally, permeation efficiency studies measuring the volume of the permeated Rhodamine B in mice skin showed that TI-DMN treated groups received a higher amount of Rhodamine B at 4.56±0.19 µg/cm² compared to conventional DMNs at 2.85±0.29 µg/cm² (Figure S2H).
**Figure S2.** Analysis of the penetration and permeation of tissue interlocking (TI) dissolving microneedles (DMNs) (TI-DMNs) compared to conventional DMNs. (A) Cumulative transcutaneous drug release profile of TI-DMN and conventional DMN over two hours. (B) Large, undissolved amount of encapsulated Rhodamine B over the patch for a DMN post-application. (C) Small, undissolved amount of Rhodamine B over patch for TI-DMNs post-application. (D) Pre-application on back skin of mice. (E) Diffusion spots on DMN-treated skin. (F) Diffusion spots (TI-DMN- treated skin). (G) Comparison of Rhodamine B penetration between TI-DMN and DMNs. (H) Comparison of Rhodamine B permeation efficiency between TI-DMN and DMNs. Scale bars in (B) and (C): 300 μm. Scale bar in (E) and (F): 5 mm. Figure adapted with permission from reference [10]

1.2 Chemical Penetration Enhancers

Another commonly used strategy to enhance the penetration of NPs into the skin is co-formulation with chemical agents which disturb the SC. Various chemical penetration enhancers have been studied in the past to disrupt the uppermost layer of the SC and create a passage for the delivery of NPs (Table S1). Their mode of action is dependent on their chemical class and their specific interaction with the skin.[11]

**Table S1:** List of selected chemical penetration enhancers and their chemical class

| Chemical Class | Enhancer                                      | Ref. |
|---------------|----------------------------------------------|------|
| Alcohols      | Ethanol, propanol, isopropyl alcohol, decanol, octanol, oleyl alcohol | [12] |
| Fatty acids   | Lauric acid, linoleic acid, stearic acid      | [13] |
| Surfactants   | Sodium lauryl sulphate, Tween 80, alkyl trimethyl ammonium halides | [14] |
Short chain alcohols, like ethanol and isopropyl alcohol, are known to have skin penetration features; however, their exact mode of action still needs to be clarified. In a comparative study by Fujii et al. the penetration enhancement of ethanol, propanol and iso-propanol were investigated on Yucatan micropig skin, using p-hydroxy benzoic acid methyl ester (HBM) as a drug model. This study showed that all three alcohols were able to enhance the penetration of HBM, and differently affect the metabolism of HBM in the skin. It was found that each alcohol produced different species and amounts of metabolites which influenced the skin permeation and retention of the drug. Other chemical classes that enjoy great representation in commercially available products are fatty acids and surfactants. Most of the investigations regarding the penetration enhancement features of fatty acids are performed in the presence of a co-solvent, hence the analysis of the sole properties of the fatty acid in question are difficult to extrapolate. In an effort to address this issue, Ibrahim and Li investigated the penetration enhancement properties of commonly used fatty acids to transport estradiol (E2β) through the human epidermal membrane (HEM) by using volatile solvents as co-solvents. The uptake results on the SC described in this study, hinted that the penetration enhancement mechanism (induced by the fatty acids) could be mostly attributed to an increase in the penetration partitioning into the SC intercellular lipid domain. As another class of chemical penetration enhancers, surfactants are among the most commercially used, as part of many formulations in various products ranging from cosmetics to pharmaceutics. Surfactants disrupt the lipid and protein layers in the SC, thus enhancing the penetration of formulated molecules. Furthermore, surfactants can self-assemble into micelles that can increase the solubility of strongly hydrophobic compounds leading to stable formulations. However, high amounts of surfactants are an increasing concern due to their poor biodegradability and high irritation potential. Oligosaccharides are another class of well-known chemical penetration enhancer, especially cyclodextrins (CDs), have been extensively investigated. A typical characteristic of these cyclic oligosaccharides is their hydrophilic outer surface combined with a hydrophobic inner void that can be used for the entrapment of small hydrophobic molecules. In addition, CDs act as a mild cutaneous penetration enhancer by increasing the solubilization and the partition coefficient of drugs in the skin. Thus, they are explored as interesting building blocks of various drug delivery carriers. Giulbudagian et al. developed tNGs based on dPG as a cross-linker and linear poly(glycidyl methyl ether-co-ethyl glycidyl ether) (p(GME-co-EGE)) as thermoresponsive polymer. The thermoresponsive NGs were formed using a strain-promoted click reaction between (1R,8S,9S)-bicyclo[6.1.0]non-4-ylmethyl (4-nitrophenyl) carbonate (BCN) functionalized dPG (dPG-BCN) and the thermoresponsive polymer. This method has the advantage of avoiding the use of copper, as well as allowing the facile functionalization of the formed NGs by adding azide modified β-CD at the end of the reaction, exploiting the unreacted dPG-BCN segments. The β-CD decorated NGs showed good encapsulation of...
the corticosteroid dexamethasone (DXM) and could transport it into deep skin layers, outperforming the commercial formulation DXM LAW® cream.\textsuperscript{15b} Although CD is known to be a penetration enhancer, the exact permeability mechanism has not been revealed yet. Reports suggest that the CDs could extract some of the lipophilic components of the SC, thus disrupting the otherwise highly ordered barrier.\textsuperscript{15c}

Among chemical penetration enhancers, dimethylsulphoxide (DMSO) is one of the most effective and hence extensively used molecule in topical pharmaceutical formulations.\textsuperscript{20} Although unclear, the penetration mechanism is hypothesized to involve the sulphoxide groups in DMSO causing the denaturation of skin proteins leading to the disruption of the lipid bilayer. Thus, DMSO is able to accelerate the penetration of molecules regardless of their hydrophilicity. Due to the disruption of the protein and lipid structure in the skin, the use of DMSO poses a risk as it could lead to skin irritation and long-term damage.\textsuperscript{21} Recent work published by Isik et. al. discusses an interesting potential replacement of DMSO for dermal delivery of therapeutics by using biocompatible NGs functionalized with the sulphoxide moieties mimicking the penetration properties of DMSO.\textsuperscript{21} Another important chemical penetration enhancer is water. Exposure to water can induce strong skin hydration which results in a disturbance of the lipid and protein structures within the skin, hence enabling the penetration of substances across the skin.\textsuperscript{17} This effect can be exploited for treating various skin disorders by applying certain materials (e.g. nanocarriers) on the skin.

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