FINAL

Send Orders for Reprints to reprints@benthamscience.ae

Current Gene Therapy, 2017, *17*, 000-000

REVIEW ARTICLE

Recent Advances in Skin Penetration Enhancers for Transdermal Gene and Drug Delivery

Morteza Amjadi[a,b,§,*], Babak Mostaghaci[a,§] and Metin Sitti[a,b]

[a]*Physical Intelligence Department, Max Planck Institute for Intelligent Systems, Stuttgart, 70569, Germany;* [b]*Max Planck-ETH Center for Learning Systems, Max Planck Institute for Intelligent Systems, Stuttgart, 70569, Germany*

ARTICLE HISTORY

Received: April 01, 2017
Revised: April 23, 2017
Accepted: May 01, 2017

DOI:
10.2174/1566523217666170510151540

Abstract: There is a growing interest in transdermal delivery systems because of their noninvasive, targeted, and on-demand delivery of gene and drugs. However, efficient penetration of therapeutic compounds into the skin is still challenging largely due to the impermeability of the outermost layer of the skin, known as stratum corneum. Recently, there have been major research activities to enhance the skin penetration depth of pharmacological agents. This article reviews recent advances in the development of various strategies for skin penetration enhancement. We show that approaches such as ultrasound waves, laser, and microneedle patches have successfully been employed to physically disrupt the stratum corneum structure for enhanced transdermal delivery. Rather than physical approaches, several non-physical route have also been utilized for efficient transdermal delivery across the skin barrier. Finally, we discuss some clinical applications of transdermal delivery systems for gene and drug delivery. This paper shows that transdermal delivery devices can potentially function for diverse healthcare and medical applications while further investigations are still necessary for more efficient skin penetration of gene and drugs.

Keywords: Skin penetration enhancers, Transdermal delivery, Gene and drug delivery, Skin barrier.

1. INTRODUCTION

Transdermal delivery is described as delivery of therapeutic compounds across the skin layer [1, 2]. The large surface area of the human skin tissue offers a convenient, selective, and noninvasive route for gene and drug delivery [3]. Transdermal administration of gene and drug not only can be used for the skin specific maladies but also could be applied for systemic therapeutic delivery and vaccination [4]. Furthermore, transdermal delivery overcomes the first-pass hepatic metabolism and can reduce harmful side effects that are found in oral, nasal, and/or intravenous administrations [1, 2]. Additionally, transdermal delivery devices are simply accessible, replaceable, controllable, and could be self-administered in several cases [1, 5, 6]. Despite several advantages of transdermal gene and drug delivery systems, efficient transdermal delivery of pharmacological agents is still challenging largely due to the impermeability of the outermost layer of the skin called stratum corneum (SC) [7]. This issue is even more severe in the case of drugs with large molecular weight and hydrophilic properties [8, 9].

In order to increase the skin penetration of therapeutic agents and boost the efficiency of transdermal delivery systems, pharmaceutical scientists have come up with various methods to enhance the permeability of the skin barrier. Some of these methods rely on the physical disturbance of the SC layer (*e.g.* using microneedles (MNs) [10] or electroporation [11]) whereas some others deal with chemical modifications of therapeutic agents or the SC layer for enhanced skin penetration [7, 12, 13]. Hair follicles have also been explored as another transport channel for transdermal delivery of gene and drugs [14, 15].

This paper aims to review recent advances in the skin penetration enhancers for transdermal gene and drug delivery applications. The paper is organized as follows: first, the multilayered structure of the skin is described. Herein, we show that the SC layer is the main skin barrier for efficient transdermal gene and drug delivery. Second, physical routes for transdermal delivery enhancement are detailed. Third, we explain different gene and drug nanocarriers for enhanced transdermal delivery. Fourth, we show that cell-penetrating peptides (CPPs) can assist therapeutic compounds or carriers to penetrate into the skin. Fifth, we summarize recent progress of using hair follicles as transdermal delivery channels followed by some important clinical applications of transdermal gene and drug delivery systems. Last section provides our conclusions on skin penetration enhancers.

*Address correspondence to this author at the Physical Intelligence Department, Max Planck Institute for Intelligent Systems, Heisenbergstr. 3, 70569, Stuttgart, Germany; Fax: +497116893412; E-mail: amjadi@is.mpg.de

§ *These authors contributed equally to this work.*

1566-5232/17 $58.00+.00

2. MULTILAYERED STRUCTURE OF THE SKIN

The multilayered structure of the skin is illustrated in (Fig. **1**). The human skin is mainly composed of epidermis, dermis, and hypodermis layers [1, 2, 16]. The epidermis layer is in direct contact with the environment and controls the water release rate of the body. The dermis layer is comprised of dense irregular connective tissue and maintains sensors (*e.g.* mechanoreceptors and thermoreceptors) and blood vessels. Furthermore, the hypodermis layer is the deepest layer of the skin and serves as skin energy supplier. The 10-20 μm thick outermost layer of the skin, SC, is known as the main barrier for transdermal gene and drug delivery because of its extracellular structure [2, 16]. To overcome this barrier, there have been significant efforts to physically or chemically enhance the permeability of the SC layer for efficient gene and drug delivery [1, 13, 16]. On the other hand, nanoscale delivery systems capable of passing the intercellular or follicular routes have actively been studied as an alternative or complementary strategy to promote the transdermal delivery efficiency [17, 18].

3. SKIN PENETRATION ENHANCERS

As mentioned in the introduction section, numerous strategies have been explored to enhance the skin penetration of therapeutic agents. In this section, we summarize some representative examples of various physical and non-physical enhancers for transdermal gene and drug delivery:

3.1. Physical Routes

There are several physical methods to enhance the transdermal gene and drug delivery [7, 12]. These methods are mostly based on the ablation of the SC layer or application of an external force to facilitate the drug penetration [19]. Fig. **2a** depicts the most important physical routes have been investigated for enhancement of the drug penetration into the skin. Most of these physical methods including ultrasound, electroporation, and MNs are applicable to transdermal gene delivery as well.

External physical forces can be used to increase the drug penetration depth by forming a temporary change in the structure of the SC layer or enhancing the diffusion rate of drug molecules [20]. To date, different sources have been employed to apply external physical forces to the SC layer [21]. For example, ultrasonic waves can enhance the transdermal drug penetration by means of different underlying mechanisms (Fig. **2b**) [22, 23]. Thermal effect and cavitation are the main consequences of using ultrasound waves that can facilitate the drug penetration. Upon ultrasound treatment of the skin, it partially absorbs the energy from ultrasonic waves, leading to a local increase in the skin temperature which in turn significantly enhances the diffusivity of the skin toward drug molecules [24]. A significant enhancement (35 times) in the permeation of mannitol as a model drug has been observed when 20 kHz ultrasound waves were applied to the skin. The enhanced drug penetration was postulated to be the increase of the skin temperature. However, it was found that increasing the skin temperature without using ultrasound could also enhance the drug penetration, but not as high as that of the ultrasound-treated skin. This finding have suggested that there should be other mechanisms that cooperate with raised temperature in order to boost the drug permeation [25]. Studies have shown that another assisting mechanism is formation of micro-gaseous cavities which could be created in the biological medium (*e.g.* skin layers) upon acoustic pressure cycles. These types of microcavities cause shear force over skin cell layers through two mechanisms as microstreaming and inertial cavitation. The "microstreamings" can be originated from the unidirectional flow of fluid due to the movement of bubbles under the ef-

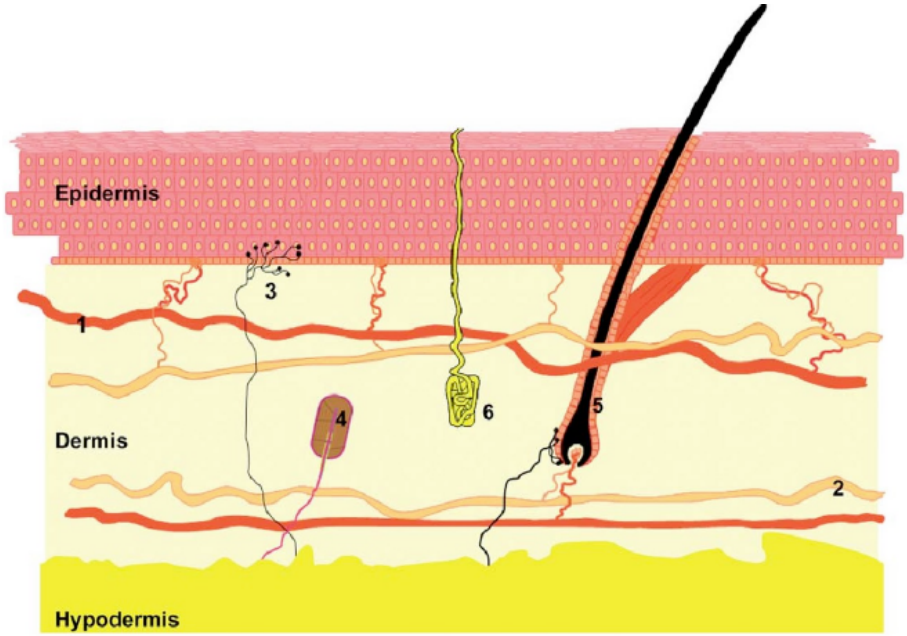

Fig. (1). Schematic illustration of the skin at a cross-section. The skin is composed of epidermis, hypodermis, and dermis containing blood vessels (1), lymph vessels (2), nerve endings (3), mechanoreceptors (4), hair follicles (5), and sweat glands (6). Reproduced with permission from ref. [1] Copyright 2012 Royal Society of Chemistry.

fect of ultrasonic waves and inertial cavitation can be produced due to sudden growth and collapse of microbubbles (Fig. **2b**) [22, 26, 27].

Although low-frequency ultrasound is being studied for its application in transdermal drug delivery as well as other medical applications, there still are many open questions for safety issues of this method. The biggest efforts are toward minimizing the side effects associated with skin temperature change and cavitation during ultrasonic treatments [28, 29].

Another well-studied source of external force for the enhancement of drug permeation is laser (Fig. **2c**) [30, 31]. Application of laser can lead to the ablation of the SC layer as well as increase of the skin diffusivity due to temperature increase. Both scenarios can result in increasing the drug permeation depth. Different types of laser (CO_2, Nd:YAG, Erb:YAG, *etc*.) have been employed for drug delivery applications. Particularly, commercial products are already available in the market for delivery of macromolecular drugs and vaccination [32, 33]. Additionally, laser technology has been investigated for increasing the permeability of oligonucleotides for vaccination purposes. Furthermore, this technology has been used for transdermal administration of both DNA and RNA based vaccines [34, 35].

Another common permeation assistant strategy is the use of electroporation [36-39]. Electroporation employs a pair of electrodes than can be attached to the skin (Fig. **2d**). When an electric filed is applied to electrodes, the energy needed for water molecules to enter the cells is reduced, due to the rearrangement of the molecular structure in the cell membrane. Subsequently, a higher number of pores can be formed on the cell membrane due to excess entrance of water, leading to an increase of the cell permeability [40]. Although the biophysical concept is complex, this enhancement method has been exploited thoroughly over the last decades to increase the penetration through cell membranes or biological barriers including the skin [41, 42].

With respect to transdermal drug delivery, it has been shown that electroporation can create an aqueous pathway through the SC layer, thereby leading to an increase of the skin permeability. This method can be used for different types of therapeutic molecules with different size and surface characteristics (*e.g.* low/high molecular weight, hydrophilic or lipophilic, proteins or oligonucleotides). The main drawback of this method, however, is its low reproducibility. Additionally, this method should be utilized with extreme caution due to the application of high voltage pulses. When the voltage or application time exceed certain limits, skin irritation or damage, edema, and muscle contraction can occur [43-46].

Bypassing the SC layer of the skin could be performed by MN arrays (Fig. **2e**) [10, 47-51]. MNs fabricated on a substrate or patch support can piece the epidermis layer and form microchannels for efficient gene and drug delivery. Therapeutic drug or (oligo)nucleotides could be administrated using MN patches [50]. MNs can be hollow and com-

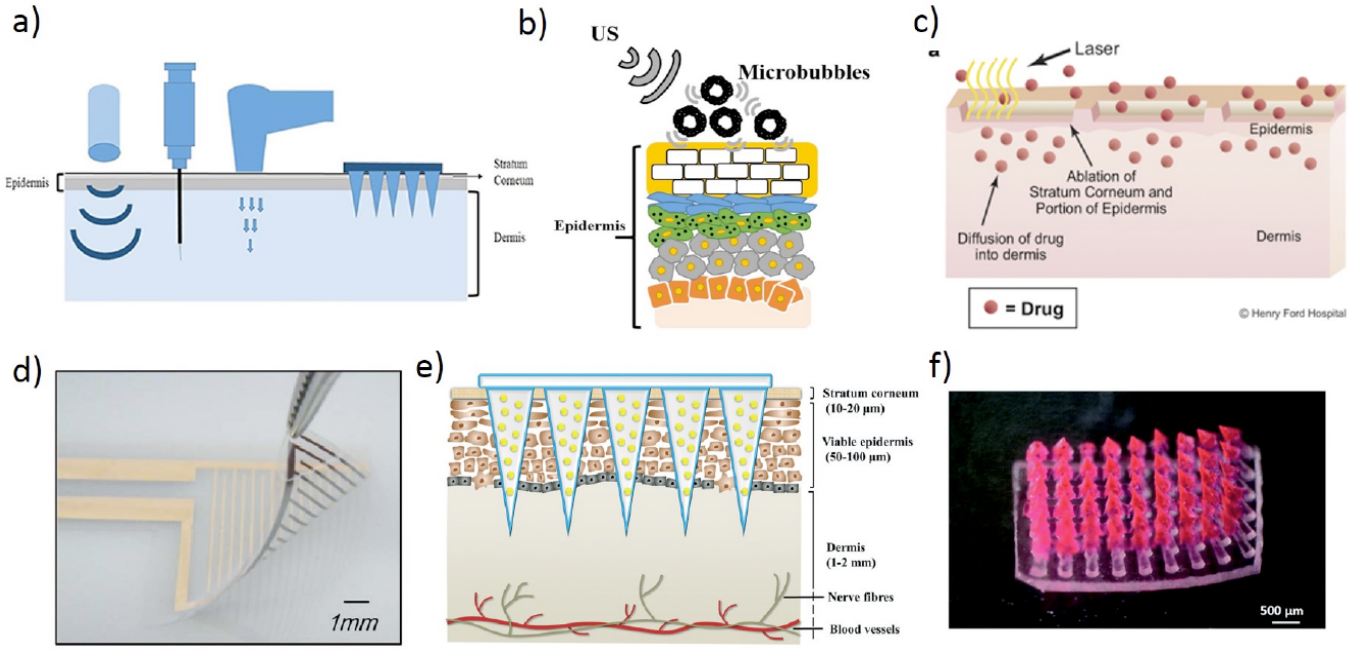

Fig. (2). Physical enhancers for transdermal gene and drug delivery. (**a**) Schematic illustration of physical administration routes for transdermal gene delivery. Reproduced with permission from Ref. [21]. (**b**) Enhancement of the drug penetration depth using microbubbles in combination with ultrasound. Reproduced with Permission from ref. [23] Copyright 2016 Elsevier Taiwan LLC and the Chinese Taipei Society of Ultrasound in Medicine. (**c**) Effect of laser on enhancing the drug delivery by increasing the drug diffusion and ablation of the SC layer. Reproduced with permission from Ref. [30] Copyright 2014 John Wiley and Sons. (**d**) A flexible electroporation patch for transdermal delivery of DNA and siRNA. Reproduced with permission from Ref. [37] Copyright 2015 Nature Publishing Group. (**e**) The use of MN patches for transdermal gene and drug delivery. Reproduced with permission from Ref. [51] Copyright 2017 Royal Society of Chemistry. (**f**) Photograph of a flexible MNs-based patch for drug delivery. Reproduced with permission from Ref. [49] Copyright 2015 Royal Society of Chemistry.

pletely solid structure. Hollow MNs direct the therapeutic drug from a reservoir through the skin layer. In the case of solid MNs, the drug loaded in MNs can often be released upon continuous biodegradation of MN materials in a controlled rate (Fig. **2f**) [10]. This platform is straight forward for transdermal gene and drug delivery due to its painless, noninvasive, and controlled delivery profile. Besides transdermal delivery of MN patches, they can potentially function as biosensing devices for feedback controlled gene and drug delivery [51, 52].

3.2. Gene and Drug Carriers

Numerous drugs have inefficient permeation rate into the skin. To enhance the gene or drug permeation across the skin, one approach is to encapsulate or condense the therapeutic compounds or (oligo)nucleotide inside nano-scale carriers with proper surface chemistry [16, 53-58]. These types of nanocarriers not only increase the penetration efficiency but also protect the gene or drug during the administration process and consequently reduce the immunogenicity [59]. Moreover, nanocarriers with tunable degradation properties can add another layer of control to the drug release rate.

Different nanocarrier systems have been employed for transdermal gene and drug delivery including carbon nanotubes, liposomes, chitosan tripolyphosphate (CS/TPP) (pDNA), peptides, and dendrimers for the delivery of different (oligo)nucleotides such as pDNA and siRNA [59-63]. Notably, liposomal systems are among the most common carriers for transdermal gene delivery.

Liposomal systems are also very popular for transdermal drug delivery [64, 65]. Fig. **3a** shows a representative example of a transdermal drug delivery system using liposomal carriers [66]. As schematically depicted in the figure, free curcumin nanoparticles cannot penetrate into the deep layers of skin due to the impermeability of the SC layer. Encapsulating curcumin in nano-metric liposomes, however, increased the permeation due to lipophilic feature of liposomes. Moreover, incorporation of carbon-dots in the liposome structure improved their stability and permeability. It has been shown that functionalization of liposome with a CPP can further increase the skin permeation depth [66]. We have detailed the application of CPPs in the next section.

3.3. CPPs

CPPs are certain peptides on the surface of some pathogens that can help pathogens penetrate into the cells and colonize them. These peptides have been extensively studied regarding their role in emerging of infections. The discovery of CPPs was studied with TAT, a transactivating factor belonging to HIV virus. Since then, such peptides have been recognized for transdermal delivery. They are generally short peptides consisting of less than 40 amino acids and can recruit different mechanisms to internalize into the cells. CPP family mainly includes TAT, penetratin, and polyarginine [67, 68].

In drug delivery, these peptides have been isolated and attached to drugs or drug carriers in order to give them the cell penetrating feature and increase their therapeutic efficiency [69]. In addition, they have been used to increase the transfection efficiency in gene delivery applications. Diverse types of CPPs have been attached to different types of (oligo)nucleotide or gene carriers in order to increase their competence to internalize into the cells. Although CPPs have been studied extensively, the mechanism behind their role in cell internalization is not fully understood yet. We suggest the reader the review from Boisguérin *et al.* for a full discussion on the application of CPPs in gene delivery [70]. CPPs have been used also in combination with the recently discovered gene delivery systems such as Transcription activator-like effector nucleases (TALENs) [71] and CRISPR/Cas9 system for the purpose of gnome editing [72].

It has been shown that liposomes attached to an arginine rich CPP have better permeability and subsequently drug delivery efficiency in a transdermal drug delivery experiment [70]. This type of CPP can even increase the skin permeation of naked drugs. In a recent study, a novel type of CPPs (IMT-P8) was conjugated to KLA (a pro-apoptotic peptide).

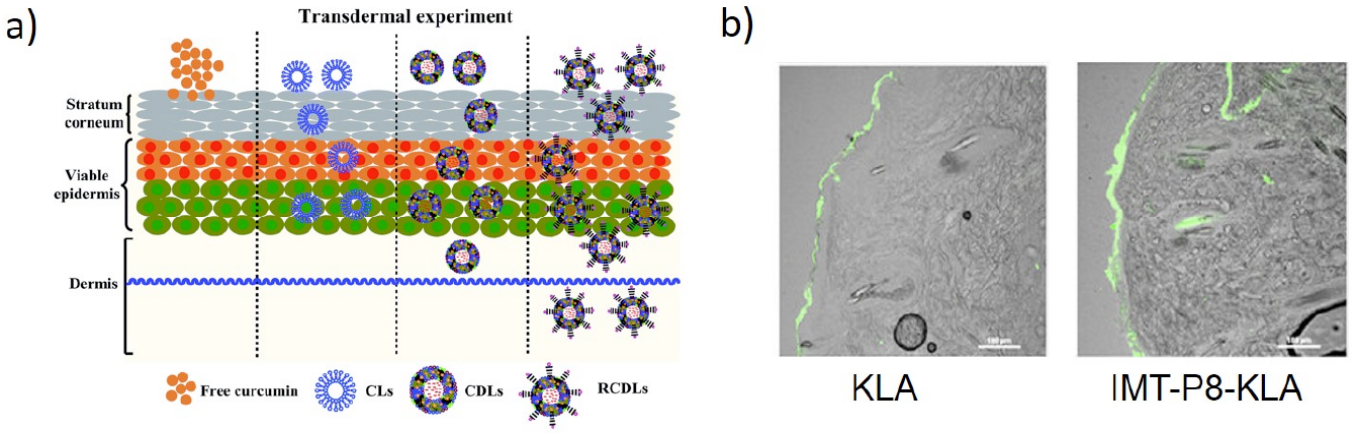

Fig. (3). Non-physical enhancers for transdermal gene and drug delivery. (**a**) Schematic illustration of transdermal drug delivery using different forms of nano-liposome carriers. CL: Conventional nano-liposome, CDL: Carbon dot modified nano-liposome, RCDL: R9 conjugated CDL. R9 is a CPP. Reproduced with permission from Ref. [66] Copyright 2015 Royal Society of Chemistry. (**b**) Penetration enhancement of KLA protein by conjugating to an arginine-rich CPP (IMT-P8). Reproduced with permission from Ref. [73] Copyright 2016 Nature Publishing Group.

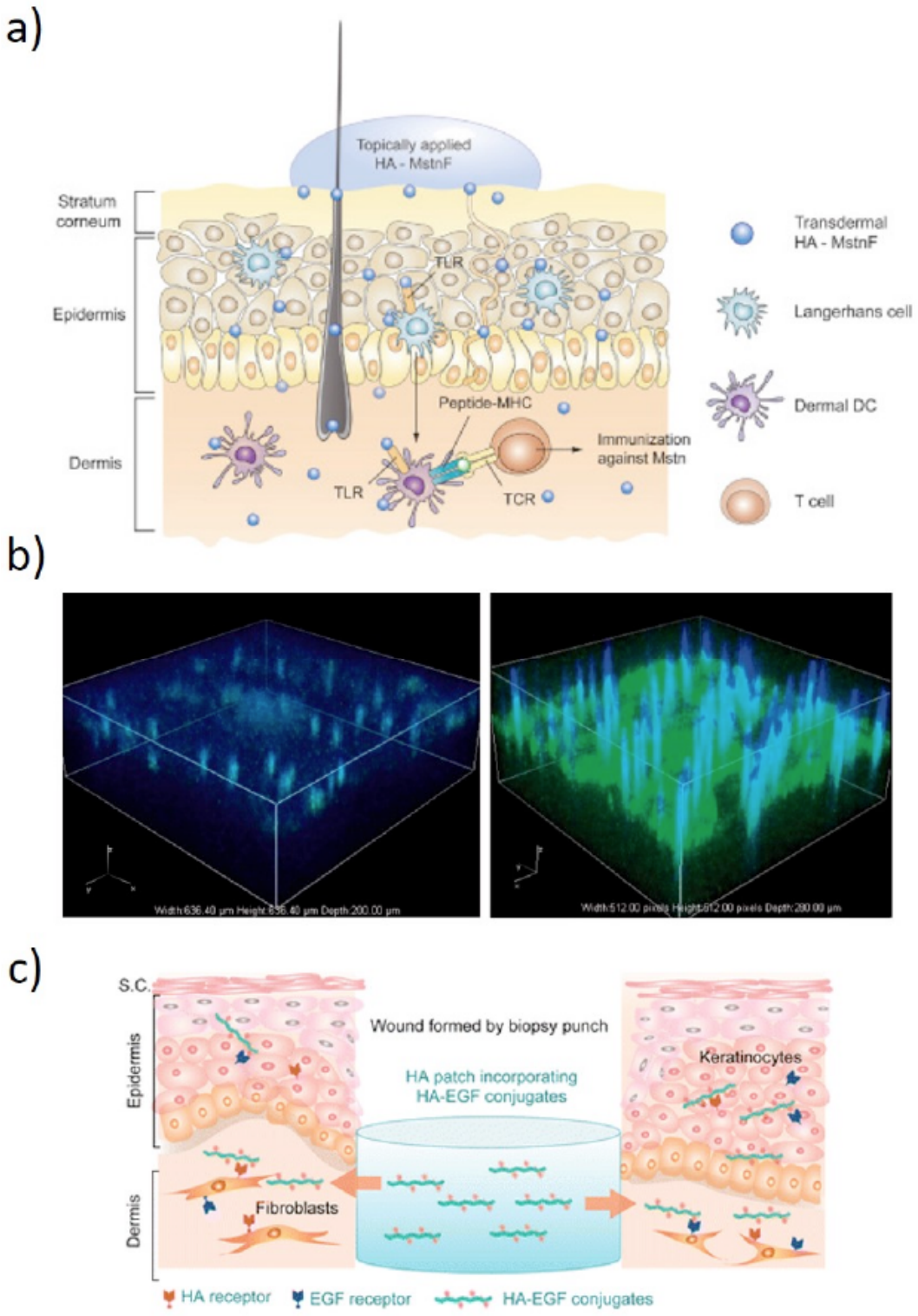

Fig. (4). Clinical applications of transdermal gene and drug delivery. (**a**) Peptide-based vaccination: this schematic illustration shows the delivery of self-adjuvanted hyaluronate (HA)-antigenic peptide (myostatin fragment, MstnF) conjugate and the following immunization against myostatin. Reproduced with permission from Ref. [86] Copyright 2016 Elsevier. (**b**) (oligo)nucleotide-based vaccination: expression of green fluorescent protein (GFP) in skin after 1 and 4 day administration of GFP plasmid DNA using MNs. The method has been exploited more to be used as a vaccination strategy against Hepatitis B. Reproduced with permission from Ref. [88] Copyright 2015 Elsevier. (**c**) Skin disease and wounds: sustained release of growth factors to stimulate wound healing. Reproduced with permission from Ref. [85] Copyright 2016 American Chemical Society.

The KLA peptide conjugated with CPP had a significantly higher skin permeation (Fig. **3b**) [73]. Also, it was shown that CPPs can increase the transdermal permeation rate of oligonucleotides. For example, TAT was conjugated to

gold/PEI particles to enhance the transdermal gene delivery for a topical application [74]. In recent years, another group of peptides (SPACE peptides) has been discovered through phage display, which is getting increasing interest due to its skin penetration competence. SPACE peptides have successfully been used for transdermal drug delivery, as well as transdermal oligonucleotide delivery [75, 76]. In addition to different nanocarriers discussed here, biohybrid nanosystems could also be utilized for transdermal delivery and enhancement of the therapeutic penetration depth [77-79].

3.4. Hair Follicles

As discussed above, the SC layer of the skin is a very strong barrier for any external material including therapeutic drugs. However, it has been shown that certain drugs can pass through hair follicles in a more efficient way. Although this is not a real enhancer, hair follicles can be recognized as a strategy to increase the drug permeation efficiency [14, 80-82]. In fact, hair follicles themselves could be targeted for treatment of certain hair and skin disorders [83]. The other aspect making hair follicles even more interesting is the presence of certain cells like immune cells. These cells can be used as a target for transdermal vaccination [82]. Additionally, blood vessels and stem cells near hair follicles can be other destinations for therapeutic genes and drugs [81].

4. APPLICATIONS

The main goals in drug delivery research are to make the drug administration more comfortable for patients, control the administrated doses, and guide the drugs to the site of disease in order to increase the therapeutic efficiency and reduce the side effects. Considering these facts, transdermal drug delivery can bring the opportunity to administrate multiple doses of drugs with precise control in a noninvasive and painless manner. Besides that, there are certain skin problems which could rather be reached through transdermal drug delivery, much easier than the systemic administration of drugs. Some common applications of transdermal drug delivery systems are indicated in Fig. **4** [84-88]. For example, smoking-quitting patches [84], insulin delivery [89], delivery of parathyroid hormone (PTH) for treatment of osteoporosis [90], and vaccination [91] are among the important applications of transdermal delivery.

CONCLUSION

Gene therapy is considered as one of the most promising therapeutic routes. Different methods have been developed for transferring the (oligo)nucleotides to the site of disease. In this paper, we reviewed some representative examples of skin penetration enhancers for efficient transdermal gene and drug delivery. Physical and chemical routes, together with functional nanocarriers and hair follicles have been proposed to improve the skin penetration of therapeutic compounds. Among these methods, MN patches are getting more attention for transdermal gene and drug delivery due to their painless procedure, controlled release, and capability of delivering various drugs.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

Declared none.

REFERENCES

[1] Pegoraro C., MacNeil S., and Battaglia G. Transdermal drug delivery: From micro to nano. Nanoscale 2012; 4 (6): 1881-1894.

[2] Prausnitz M. R., Mitragotri S., and Langer R. Current status and future potential of transdermal drug delivery. Nat. Rev. Drug Discovery 2004; 3 (2): 115-124.

[3] Watkinson A. C., Kearney M. C., Quinn H. L., Courtenay A. J., and Donnelly R. F. Future of the transdermal drug delivery market - have we barely touched the surface? Expert Opin. Drug Del. 2016; 13 (4): 523-532.

[4] Jain A., Jain P., Kurmi J. *et al.* Novel strategies for effective transdermal drug delivery: A review. Crit. Rev. Ther. Drug Carrier Syst. 2014; 31 (3): 219-272.

[5] Riahi R., Tamayol A., Shaegh S. A. M., Ghaemmaghami A. M., Dokmeci M. R., and Khademhosseini A. Microfluidics for advanced drug delivery systems. Curr. Opin. Chem. Eng. 2015; 7 101-112.

[6] Merino S., Martín C., Kostarelos K., Prato M., and Vázquez E. Nanocomposite hydrogels: 3d polymer–nanoparticle synergies for on-demand drug delivery. ACS Nano 2015; 9 (5): 4686-4697.

[7] Parhi R., Suresh P., and Patnaik S. Physical means of stratum corneum barrier manipulation to enhance transdermal drug delivery. Curr. Drug Delivery 2015; 12 (2): 122-138.

[8] Liu S., Jin M. N., Uuan Y. S. Q. *et al.* Transdermal delivery of relatively high molecular weight drugs using novel self-dissolving microneedle arrays fabricated from hyaluronic acid and their characteristics and safety after application to the skin. Eur. J. Pharm. Biopharm. 2014; 86 (2): 267-276.

[9] Mueller J., Oliveira J. S. L., Barker R. *et al.* The effect of urea and taurine as hydrophilic penetration enhancers on stratum corneum lipid models. Biochim. Biophys. Acta, Biomembr. 2016; 1858 (9): 2006-2018.

[10] Indermun S., Luttge R., Choonara Y. E. *et al.* Current advances in the fabrication of microneedles for transdermal delivery. J. Controlled Release 2014; 185 130-138.

[11] Becker S., Zorec B., Miklavcic D., and Pavselj N. Transdermal transport pathway creation: Electroporation pulse order. Math. Biosci. 2014; 257 60-68.

[12] Alexander A., Dwivedi S., Giri T. K., Saraf S., Saraf S., and Tripathi D. K. Approaches for breaking the barriers of drug permeation through transdermal drug delivery. J. Controlled Release 2012; 164 (1): 26-40.

[13] Wong T. W. Electrical, magnetic, photomechanical and cavitational waves to overcome skin barrier for transdermal drug delivery. J. Controlled Release 2014; 193 257-269.

[14] Patzelt A. and Lademann J. Drug delivery to hair follicles. Expert Opin. Drug Del. 2013; 10 (6): 787-797.

[15] Raber A. S., Mittal A., Schafer J. *et al.* Quantification of nanoparticle uptake into hair follicles in pig ear and human forearm. J. Controlled Release 2014; 179 25-32.

[16] Vogt A., Wischke C., Neffe A. T., Ma N., Alexiev U., and Lendlein A. Nanocarriers for drug delivery into and through the skin—do existing technologies match clinical challenges? J. Controlled Release 2016; 242 3-15.

[17] Grimaldi N., Andrade F., Segovia N. *et al.* Lipid-based nanovesicles for nanomedicine. Chem. Soc. Rev. 2016; 45 (23): 6520-6545.

[18] Roberts M., Mohammed Y., Pastore M. *et al.* Topical and cutaneous delivery using nanosystems. J. Controlled Release 2016;

[19] Mitragotri S. Devices for overcoming biological barriers: The use of physical forces to disrupt the barriers. Adv. Drug Delivery Rev. 2013; 65 (1): 100-103.

[20] Bhatnagar S., Kwan J. J., Shah A. R., Coussios C. C., and Carlisle R. C. Exploitation of sub-micron cavitation nuclei to enhance ultrasound-mediated transdermal transport and penetration of vaccines. J. Controlled Release 2016; 238 22-30.

[21] Chen W., Li H., Shi D., Liu Z. G., and Yuan W. E. Microneedles as a delivery system for gene therapy. Front. Pharmacol. 2016; 7

[22] Azagury A., Khoury L., Enden G., and Kost J. Ultrasound mediated transdermal drug delivery. Adv. Drug Delivery Rev. 2014; 72 127-143.

[23] Liao A.-H. Ultrasound in biomedical engineering: Ultrasound microbubble contrast agents promote transdermal permeation of drugs. J. Med. Ultrasound 2016; 24 (3): 86-88.

[24] Park D., Park H., Seo J., and Lee S. Sonophoresis in transdermal drug deliverys. Ultrasonics 2014; 54 (1): 56-65.

[25] Merino G., Kalia Y. N., Delgado-Charro M. B., Potts R. O., and Guy R. H. Frequency and thermal effects on the enhancement of transdermal transport by sonophoresis. J. Controlled Release 2003; 88 (1): 85-94.

[26] Polat B. E., Hart D., Langer R., and Blankschtein D. Ultrasound-mediated transdermal drug delivery: Mechanisms, scope, and emerging trends. J. Controlled Release 2011; 152 (3): 330-348.

[27] Singh A. V., Baylan S., Park B.-W., Richter G., and Sitti M. Hydrophobic pinning with copper nanowhiskers leads to bactericidal properties. PLOS ONE 2017; 12 (4): e0175428.

[28] Ahmadi F., McLoughlin I. V., Chauhan S., and Ter-Haar G. Bioeffects and safety of low-intensity, low-frequency ultrasonic exposure. Prog. Biophys. Mol. Bio. 2012; 108 (3): 119-138.

[29] Lammertink B. H., Bos C., Deckers R., Storm G., Moonen C. T., and Escoffre J.-M. Sonochemotherapy: From bench to bedside. Front. Pharmacol. 2015; 6 138.

[30] Sklar L. R., Burnett C. T., Waibel J. S., Moy R. L., and Ozog D. M. Laser assisted drug delivery: A review of an evolving technology. Laser Surg. Med. 2014; 46 (4): 249-262.

[31] Lee W.-R., Shen S.-C., Chen W.-Y., Aljuffali I. A., Suen S.-Y., and Fang J.-Y. Noninvasive delivery of sirna and plasmid DNA into skin by fractional ablation: Erbium: Yag laser *versus* co 2 laser. Eur. J. Pharm. Biopharm. 2014; 86 (3): 315-323.

[32] Haak C. S., Bhayana B., Farinelli W. A., Anderson R. R., and Haedersdal M. The impact of treatment density and molecular weight for fractional laser-assisted drug delivery. J. Controlled Release 2012; 163 (3): 335-341.

[33] Bachhav Y. G., Summer S., Heinrich A., Bragagna T., Bohler C., and Kalia Y. N. Effect of controlled laser microporation on drug transport kinetics into and across the skin. J. Controlled Release 2010; 146 (1): 31-36.

[34] Probst J., Brechtel S., Scheel B. *et al.* Characterization of the ribonuclease activity on the skin surface. Genet. Vaccines Ther. 2006; 4 4.

[35] Zeira E., Manevitch A., Manevitch Z. *et al.* Femtosecond laser: A new intradermal DNA delivery method for efficient, long-term gene expression and genetic immunization. FASEB J. 2007; 21 (13): 3522-33.

[36] Lakshmanan S., Gupta G. K., Avci P. *et al.* Physical energy for drug delivery; poration, concentration and activation. Adv. Drug Delivery Rev. 2014; 71 98-114.

[37] Wei Z., Huang Y., Zhao D., Hu Z., Li Z., and Liang Z. A pliable electroporation patch (ep-patch) for efficient delivery of nucleic acid molecules into animal tissues with irregular surface shapes. Sci. Rep. 2015; 5 7618.

[38] Wei Z., Zheng S., Wang R. *et al.* A flexible microneedle array as low-voltage electroporation electrodes for *in vivo* DNA and sirna delivery. Lab Chip 2014; 14 (20): 4093-4102.

[39] Golzio M., Escoffre J.-M., Portet T. *et al.* Observations of the mechanisms of electromediated DNA uptake-from vesicles to tissues. Curr. Gene Ther. 2010; 10 (4): 256-266.

[40] Escoffre J.-M., Bellard E., Faurie C. *et al.* Membrane disorder and phospholipid scrambling in electropermeabilized and viable cells. Biochim. Biophys. Acta, Biomembr. 2014; 1838 (7): 1701-1709.

[41] Yarmush M. L., Golberg A., Sersa G., Kotnik T., and Miklavcic D. Electroporation-based technologies for medicine: Principles, applications, and challenges. Annu. Rev. Biomed. Eng. 2014; 16 295-320.

[42] Chen C., Smye S. W., Robinson M. P., and Evans J. A. Membrane electroporation theories: A review. Med. Biol. Eng. Comput. 2006; 44 (1-2): 5-14.

[43] Pavselj N., Preat V., and Miklavcic D. A numerical model of skin electropermeabilization based on *in vivo* experiments. Ann. Biomed. Eng. 2007; 35 (12): 2138-44.

[44] Kang T. H., Monie A., Wu L. S., Pang X., Hung C. F., and Wu T. C. Enhancement of protein vaccine potency by *in vivo* electroporation mediated intramuscular injection. Vaccine 2011; 29 (5): 1082-9.

[45] Blagus T., Markelc B., Cemazar M. *et al. In vivo* real-time monitoring system of electroporation mediated control of transdermal and topical drug delivery. J. Control Release 2013; 172 (3): 862-71.

[46] Ita K. Perspectives on transdermal electroporation. Pharmaceutics 2016; 8 (1):

[47] Kang S. M., Song J. M., and Kim Y. C. Microneedle and mucosal delivery of influenza vaccines. Expert Rev. Vaccines 2012; 11 (5): 547-560.

[48] Zaric M., Ibarzo Yus B., Kalcheva P. P., and Klavinskis L. S. Microneedle-mediated delivery of viral vectored vaccines. Expert Opin. Drug Deliv. 2016; 1-11.

[49] Xue P., Zhang X. Y., Chuah Y. J., Wu Y. F., and Kang Y. J. Flexible pegda-based microneedle patches with detachable pvp-cd arrowheads for transdermal drug delivery. RSC Adv. 2015; 5 (92): 75204-75209.

[50] Ita K. Transdermal delivery of drugs with microneedles: Strategies and outcomes. J. Drug Deliv. Sci. Tec. 2015; 29 16-23.

[51] Wang M., Hu L., and Xu C. Recent advances in the design of polymeric microneedles for transdermal drug delivery and biosensing. Lab Chip 2017; 17: 1373-1387.

[52] Cahill E. M. and O'Cearbhaill E. D. Toward biofunctional microneedles for stimulus responsive drug delivery. Bioconjugate Chem. 2015; 26 (7): 1289-1296.

[53] Khan N. R., Harun M. S., Nawaz A., Harjoh N., and Wong T. W. Nanocarriers and their actions to improve skin permeability and transdermal drug delivery. Curr. Pharm. Design 2015; 21 (20): 2848-2866.

[54] Kurmi B. D., Tekchandani P., Paliwal R., and Paliwal S. R. Transdermal drug delivery: Opportunities and challenges for controlled delivery of therapeutic agents using nanocarriers. Curr. Drug. Metab. 2017; DOI: 10.2174/1389200218666170222150555

[55] Mura S., Nicolas J., and Couvreur P. Stimuli-responsive nanocarriers for drug delivery. Nat. Mater. 2013; 12 (11): 991-1003.

[56] Mostaghaci B., Loretz B., Haberkorn R., Kickelbick G., and Lehr C.-M. One-step synthesis of nanosized and stable amino-functionalized calcium phosphate particles for DNA transfection. Chem. Mater. 2013; 25 (18): 3667-3674.

[57] Mostaghaci B., Susewind J., Kickelbick G., Lehr C.-M., and Loretz B. Transfection system of amino-functionalized calcium phosphate nanoparticles: *In vitro* efficacy, biodegradability, and immunogenicity study. ACS Appl. Mater. Interfaces 2015; 7 (9): 5124-5133.

[58] Mostaghaci B., Loretz B., and Lehr C.-M. Calcium phosphate system for gene delivery: Historical background and emerging opportunities. Curr. Pharm. Design 2016; 22 (11): 1529-1533.

[59] Zakrewsky M., Kumar S., and Mitragotri S. Nucleic acid delivery into skin for the treatment of skin disease: Proofs-of-concept, potential impact, and remaining challenges. J. Controlled Release 2015; 219 445-456.

[60] Ozbas-Turan S. and Akbuga J. Plasmid DNA-loaded chitosan/tpp nanoparticles for topical gene delivery. Drug Deliv. 2011; 18 (3): 215-222.

[61] Siu K. S., Chen D., Zheng X. *et al.* Non-covalently functionalized single-walled carbon nanotube for topical sirna delivery into melanoma. Biomaterials 2014; 35 (10): 3435-42.

[62] Bahadoran A., Moeini H., Bejo M. H., Hussein M. Z., and Omar A. R. Development of tat-conjugated dendrimer for transdermal DNA vaccine delivery. J. Pharm. Pharm. Sci. 2016; 19 (3): 325-338.

[63] Hsu T. and Mitragotri S. Delivery of sirna and other macromolecules into skin and cells using a peptide enhancer. Proc. Natl. Acad. Sci. 2011; 108 (38): 15816-15821.

[64] Benson H. A. E. Elastic liposomes for topical and transdermal drug delivery. Methods Mol. Biol. 2010; 605 77-86.

[65] Palac Z., Engesland A., Flaten G. E., Skalko-Basnet N., Filipovic-Grcic J., and Vanic Z. Liposomes for (trans)dermal drug delivery: The skin-pvpa as a novel *in vitro* stratum corneum model in formulation development. J. Liposome Res. 2014; 24 (4): 313-322.

[66] Patra S., Roy E., Madhuri R., and Sharma P. K. The next generation cell-penetrating peptide and carbon dot conjugated nanoliposome for transdermal delivery of curcumin. Biomater. Sci. 2016; 4 (3): 418-429.

[67] Copolovici D. M., Langel K., Eriste E., and Langel U. Cell-penetrating peptides: Design, synthesis, and applications. ACS Nano 2014; 8 (3): 1972-1994.

[68] Bechara C. and Sagan S. Cell-penetrating peptides: 20 years later, where do we stand? Febs Lett. 2013; 587 (12): 1693-1702.
[69] Jin E. L., Zhang B., Sun X. R. *et al.* Acid-active cell-penetrating peptides for *in vivo* tumor-targeted drug delivery. J. Am. Chem. Soc. 2013; 135 (2): 933-940.
[70] Boisguerin P., Deshayes S., Gait M. J. *et al.* Delivery of therapeutic oligonucleotides with cell penetrating peptides. Adv. Drug Delivery Rev. 2015; 87 52-67.
[71] Liu J., Gaj T., Patterson J. T., Sirk S. J., and Barbas C. F., 3rd Cell-penetrating peptide-mediated delivery of talen proteins *via* bioconjugation for genome engineering. PLOS ONE 2014; 9 (1): e85755.
[72] Suresh B., Ramakrishna S., and Kim H. Cell-penetrating peptide-mediated delivery of cas9 protein and guide rna for genome editing. Methods Mol. Biol. 2017; 1507 81-94.
[73] Gautam A., Nanda J. S., Samuel J. S. *et al.* Topical delivery of protein and peptide using novel cell penetrating peptide imt-p8. Sci. Rep. 2016; 6 26278.
[74] Niu J., Chu Y., Huang Y. F. *et al.* Transdermal gene delivery by functional peptide-conjugated cationic gold nanoparticle reverses the progression and metastasis of cutaneous melanoma. ACS Appl. Mater. Interfaces 2017; 9 (11): 9388–9401.
[75] Chen M., Gupta V., Anselmo A. C., Muraski J. A., and Mitragotri S. Topical delivery of hyaluronic acid into skin using space-peptide carriers. J. Controlled Release 2014; 173 67-74.
[76] Chen M., Zakrewsky M., Gupta V. *et al.* Topical delivery of sirna into skin using space-peptide carriers. J. Controlled Release 2014; 179 33-41.
[77] Hosseinidoust Z., Mostaghaci B., Yasa O., Park B.-W., Singh A. V., and Sitti M. Bioengineered and biohybrid bacteria-based systems for drug delivery. Adv. Drug Delivery Rev. 2016; 106 27-44.
[78] Singh A. V. and Sitti M. Patterned and specific attachment of bacteria on biohybrid bacteria-driven microswimmers. Adv. Healthcare Mater. 2016; 5 (18): 2325-2331.
[79] Vikram Singh A. and Sitti M. Targeted drug delivery and imaging using mobile milli/microrobots: A promising future towards theranostic pharmaceutical design. Curr. Pharm. Design 2016; 22 (11): 1418-1428.
[80] Yasar H., Gordon S., Loretz B., Schulze K., Guzmán C. A., and Lehr C.-M. Follicular drug delivery: A root to success. The Medicine Maker 2016; (816):
[81] Lademann J., Knorr F., Richter H. *et al.* Hair follicles as a target structure for nanoparticles. J. Innov. Opt. Heal. Sci. 2015; 8 (4):
[82] Lademann J., Richter H., Knorr F. *et al.* Triggered release of model drug from aunp-doped bsa nanocarriers in hair follicles using ira radiation. Acta Biomater. 2016; 30 388-96.
[83] Tran N. B., Knorr F., Mak W. C. *et al.* Gradient-dependent release of the model drug tritc-dextran from fitc-labeled bsa hydrogel nanocarriers in the hair follicles of porcine ear skin. Eur. J. Pharm. Biopharm. 2016;
[84] Gulati G. K., Chen T., and Hinds B. J. Programmable carbon nanotube membrane-based transdermal nicotine delivery with microdialysis validation assay. Nanomedicine 2017; 13 (1): 1-9.
[85] Kim H., Kong W. H., Seong K. Y. *et al.* Hyaluronate-epidermal growth factor conjugate for skin wound healing and regeneration. Biomacromolecules 2016; 17 (11): 3694-3705.
[86] Kong W. H., Sung D. K., Kim H. *et al.* Self-adjuvanted hyaluronate--antigenic peptide conjugate for transdermal treatment of muscular dystrophy. Biomaterials 2016; 81 93-103.
[87] Qiu Y. Q., Guo L., Zhang S. H. *et al.* DNA-based vaccination against hepatitis b virus using dissolving microneedle arrays adjuvanted by cationic liposomes and cpg odn. Drug Deliv. 2016; 23 (7): 2391-2398.
[88] Guo L., Mao P., and Gao Y. Dissolving microneedle arrays for intradermal immunization of hepatitis b virus DNA vaccine. Procedia Vaccinol. 2015; 9 24-30.
[89] Mo R., Jiang T., Di J., Tai W., and Gu Z. Emerging micro- and nanotechnology based synthetic approaches for insulin delivery. Chem. Soc. Rev. 2014; 43 (10): 3595-629.
[90] Daddona P. E., Matriano J. A., Mandema J., and Maa Y. F. Parathyroid hormone (1-34)-coated microneedle patch system: Clinical pharmacokinetics and pharmacodynamics for treatment of osteoporosis. Pharm. Res. 2011; 28 (1): 159-65.
[91] Anselmo A. C. and Mitragotri S. An overview of clinical and commercial impact of drug delivery systems. J Control Release 2014; 190 15-28.