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Rzhevskiy, A., Thakur, R., Donnelly, R., & Anissimov, Y. (2018). Microneedles as the technique of drug delivery enhancement in diverse organs and tissues. *Journal of Controlled Release*, *270*, 184-202. https://doi.org/10.1016/j.jconrel.2017.11.048

Published in:
Journal of Controlled Release

Document Version:
Peer reviewed version

Microneedles as the technique of drug delivery enhancement in diverse organs and tissues

Alexey S Rzhevskiy[1], Thakur Raghu Raj Singh[2], Ryan F Donnelly[2], Yuri G Anissimov[3,*]

[1] Institute of Molecular Medicine, Sechenov First Moscow State Medical University, Trubetskaya 8, 119991 Moscow, Russia

[2] School of Pharmacy, Queen's University Belfast, Belfast BT97BL, UK

[3] School of Natural Sciences, Griffith University, Gold Coast, Queensland, 4222, Australia

[*] Correspondence email: y.anissimov@griffith.edu.au, phone: +61755528496

Running Head: Drug delivery with microneedles

Abstract

Microneedles is the technique of drug delivery enhancement, which was primarily designed for facilitating percutaneous drug delivery. Started from the development of simple solid microneedles, providing microporation of stratum corneum and therefore enhancement of topical drug delivery, for two decades the technique has progressed in various modifications such as hollow, coated, dissolving and hydrogel forming microneedles. In their turn, the modifications have resulted in new mechanisms of drug delivery enhancement and followed by the expansion of applicability range in terms of targeted tissues and organs. Thus, in addition to percutaneous drug delivery, microneedles have been considered as an efficient technique facilitating ocular, oral mucosal, gastrointestinal, ungual and vaginal drug administration. It is anticipated that the technique of microneedle-assisted drug delivery will soon become relevant for majority of organs and tissues.

Keywords: microneedles, transdermal drug delivery, ocular drug delivery, oral mucosal drug delivery, gastrointestinal drug delivery, trans-ungual drug delivery

1. Introduction

For the last two decades, microneedles have actively been investigated as a technique of physical enhancement of transdermal drug delivery. Originally, the purpose of this technique was to facilitate drugs in overcoming stratum corneum (SC), the outermost skin layer and a formidable barrier that is almost impermeable for large and hydrophilic molecules [1]. The investigation of microneedles provided an opportunity to deliver drugs with higher molecular weights and hydrophilicity within the skin or deeper to the underlying tissues, with a subsequent local accumulation and effect or further release into systemic circulation.

The technique of microneedles is minimally invasive, commercially feasible and simple in use. Being located on a supporting base, microneedle arrays are conventionally applied with a patch, roller, applicator, or with an injection system in case of the hollow type, manually or with electric force. To date, there are five known methods whereby percutaneous drug administration can be achieved according to the type of microneedles: solid removable, dissolving, hollow, coated and hydrogel-forming [2]. The mechanism of drug delivery enhancement via solid removable microneedles is based on the creation of pores/holes in SC prior to, or combined with, the application of a drug onto the skin surface. Such pores/holes increase the conductance of SC and improve flux of the delivered drug. Among the five types of microneedles, solid removable is the original type which became a platform for the development of dissolving, hollow, coated and hydrogel-forming microneedles. In case of the hollow type, following the insertion of mironeedles into the tissue, a drug is injected through bores in the central part of microneedles. Coated microneedles contain a drug which envelopes their surface and is released after the microneedles have been inserted into a tissue. Dissolving microneedles, made of biodegradable materials, are loaded with drugs which release from the applied microneedles due to their degradation. Finally, the most recently investigated type, hydrogel-forming microneedles are composed of non-dissolving crosslinked hydrogels, which are carried by a drug-loaded adhesive patch. After such patch is applied to the skin surface, the hydrogel-forming microneedles are swelled by an aqueous media of the skin creating hydrogel conduits which facilitate the flux of drug deeper into the skin [3].

Microneedles are made of numerous materials (silicon, ceramics, glass, sugars, biodegradable polymers, steel, etc.), and differ in length (25-2000 μm) and shape [4]. The listed variety of features and different types of mechanisms of drug delivery enhancement, provide the technique with a high range of applicability and make microneedles a focus of research in the field of transdermal drug delivery. However, in recent years researchers investigated the potential of the

technique to be extended to other organs and tissues such as oral cavity [5], gastrointestinal tract [6], nails [7] and eyes [8]. The essence of microneedle application is to mechanically break epithelial or fibrous biological barrier and therefore facilitate the delivered drug in overcoming the barrier and being further delivered to the targeted site of an organ or tissue (Figure 1). The aim of this review is to highlight the progress achieved in drug delivery with microneedles in diverse organs and tissues.

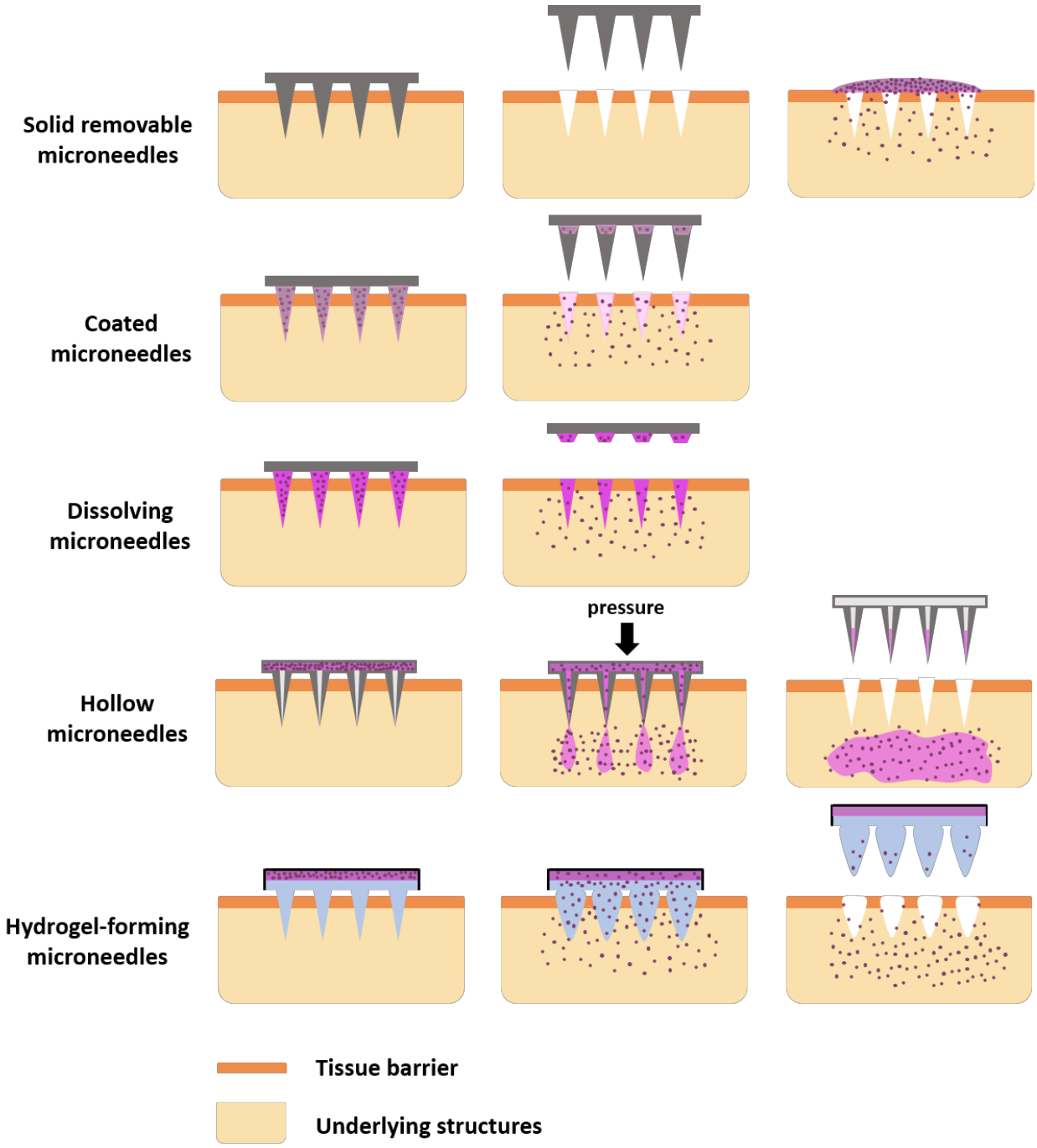


Figure 1. Types of microneedles, step-by-step process of their application, and corresponding mechanisms of drug delivery across a tissue barrier.

2. Percutaneous applications of microneedles

Skin is the most superficial organ which covers human body and primarily acts as a barrier, preventing the body from an excess water loss and protecting it from pathogenic agents coming from the environment. Also, due to its high barrier properties, intact skin significantly limits percutaneous permeation of topically applied drugs, and therefore is a serious obstacle for transdermal drug delivery as a route of drug administration. The properties of skin are defined by its anatomical and physiological organisation.

Skin is composed of three layers, taking the order from the most superficial to the deep: SC, epidermis and dermis [9]. The first two layers (SC and epidermis) mainly comprise the cellular component with a relatively low cellular interspacing, are not vascularised and do not contain nerve endings. In contrast, the structure of dermis is mainly presented by a framework of protein fibers (collagen and elastin) with an aqueous gel between them. Such composition of the dermis mechanically reinforces the skin, and at the same time keeps it elastic. Further, the metabolism and innervation of entire skin is served by blood vessels and nerves located in dermis.

Even though both SC and epidermis provide skin with the barrier functions [10], major contribution to the barrier properties of skin is presented by SC. The so-called brick and mortar structure of SC brings serious limitations to the transdermal and intradermal drug delivery [11]. Being composed of dead keratinocytes and intracellular matrix (often referred as bricks and mortar respectively), the latter being mainly cholesterol, triglycerides and ceramides, SC has a lipophilic and dense structure (1.4 g/cm^3) [12]. Due to the described structure, SC is almost impermeable for the drugs with molecular weight higher than 500 Da, and LogP out of the 1-3 range. Moreover, transdermal flux is often below the effective point for drugs which satisfy the criteria for successful percutaneous permeation but have a high therapeutically effective dose. The technique of microneedles enables these limitations to be overcome, and reveal a high potential of skin as a route of drug administration by providing an opportunity to effectively deliver drugs with differing features. Furthermore, due to their micro-size, microneedles provide an opportunity to overcome the SC barrier without causing pain or damaging blood vessels which are situated in the reticular dermis (Figure 2). Overall, microneedles have successfully been tested in transdermal delivery of drugs from diverse pharmacological groups, and there are currently three principal mainstreams of investigation of microneedle-enhanced transdermal drug delivery (TDD): microneedles in cosmetology, microneedles in percutaneous vaccination and microneedles in insulin delivery.

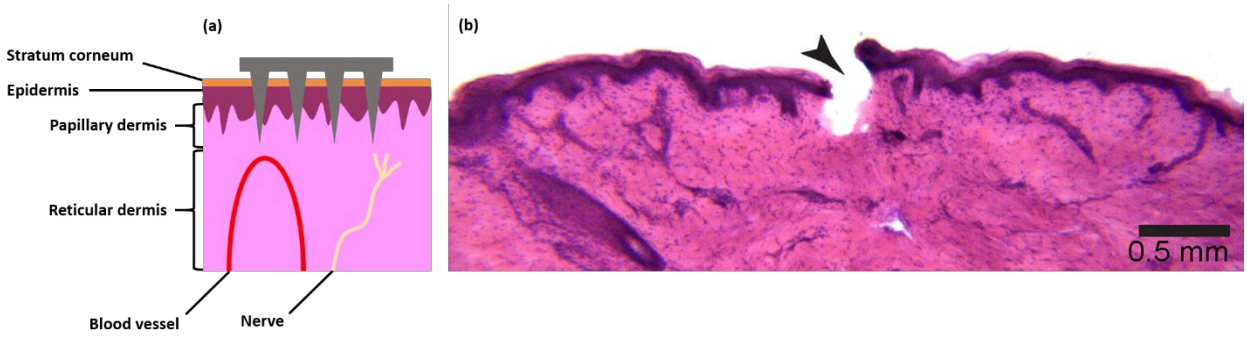


Figure 2. Schematic representation of the conventional application of microneedles. Thus, the microneedles overcome the skin barrier without reaching blood vessels and nerves in reticular dermis (a). The cross section of the skin sample stained with hematoxylin and eosin, and disruption of the skin barrier created with a 700 μm solid microneedle (b) (Adapted from [13]).

2.1. Microneedles in cosmetology

The application of microneedles is safe and painless [14]. Further, even though it decreases the barrier function of skin, normally it does not induce bacterial contamination and long-term irritation, and the skin is restored within 1-3 days after being treated [15, 16]. Such advantages allow the application of microneedles onto delicate skin areas, particularly face, making the technique attractive for being used in cosmetology.

The feature of microneedles to enhance transdermal drug delivery via overcoming the barrier of SC is widely investigated in cosmetology [17]. Cosmetically active ingredients are conventionally delivered intradermally by being applied onto the skin surface or injected with a syringe through a hypodermic needle. However, the technique of topical drug application usually does not provide a desirable effect due to a relatively low permeability of skin to majority of cosmeceuticals and injections with hypodermic needles are not the best option in terms of safety. Microneedles is an alternative which has the ability to effectively facilitate delivering drugs into the skin, being minimally invasive, painless and atraumatic.

One of the most attractive advantages of the technique for being applied in cosmetology is the ability to facilitate the transdermal delivery of peptides and proteins most of which are almost impermeable through intact skin. The most significant features which define low permeation rate of proteins and peptides across intact skin are the high molecular weight, from several hundred

daltons for peptides and thousands daltons for proteins, and hydrophilic nature. The recent *in vitro* study by Mohammad et al. demonstrated the efficacy of application of solid removable microneedles for the enhancement of lysine-threonine-threonine-lysine-serine (KTTKS), melanostatin and rigin, the peptides with a high cosmetic relevancy and potential to skin rejuvenation [13]. Thus, melanostatin decreases the production of melanin in the skin, rigin provides the anti-inflammatory effect and KTTKS contributes to the production of collagen. The results demonstrated a forceful increment in delivery and distribution of melanostatin and KTTS over the skin. In case of rigin, only a slight difference between the passive and microneedle-mediated intradermal delivery was observed.

In addition to facilitating transdermal permeation of peptides for skin rejuvenation, solid microneedles have recently been investigated as a technique to enhance transdermal delivery of cosmeceuticals regulating hair growth. Thus, Kumar *et al*. demonstrated the effectiveness of solid microneedles to enhance transdermal delivery of eflornithine hydrochloride *in vitro*. Further, *in vivo* experiments with the preliminary application of a microneedle roller (192 needles, 500 μm length) to the dorsal skin of mouse models, trimmed with an electric clipper, has significantly increased the effect of topically applied Vaniqa eflornithine hydrochloride (13.9%) cream in terms of hair growth depression [18]. The results were compared with hair re-growth after chemical depilation, plucking and trimming without further pretreatment with microneedles (Figure 3). Therefore, it was assumed that the technique of the enhancement of eflornithine delivery with the microneedle roller may effectively facilitate the treatment of facial hirsutism. Also, more recently, it was indicated that the converse effect can be achieved if the skin pretreatment with a microneedle roller is followed by the topical application of minoxidil, which resulted in hair growth stimulation in the clinical trials of androgenic alopecia treatment [19].

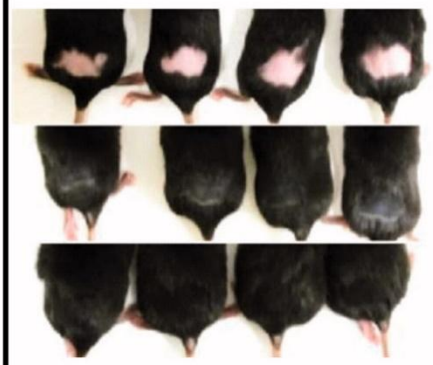
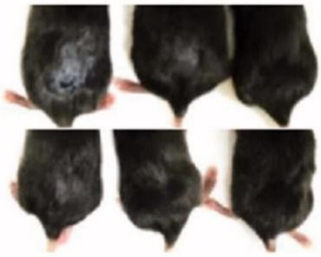
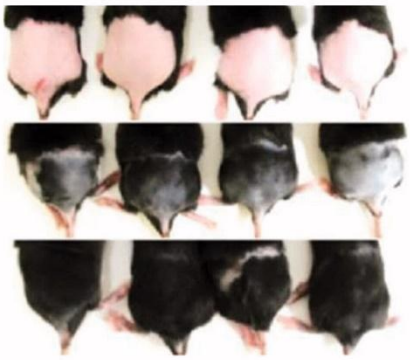
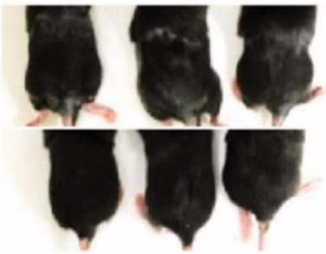
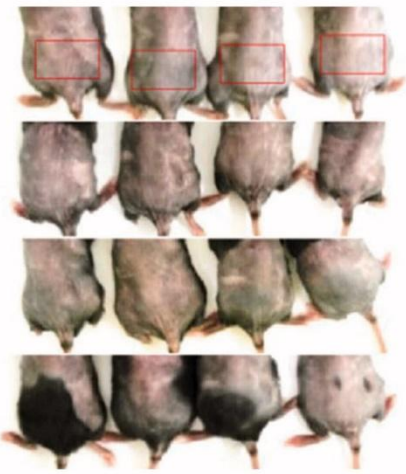
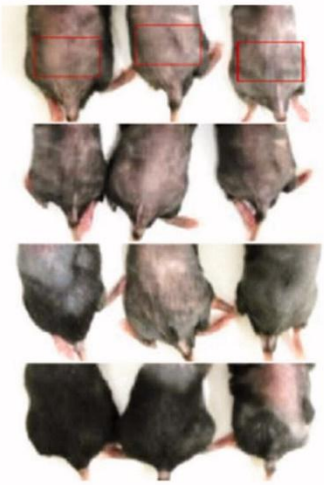
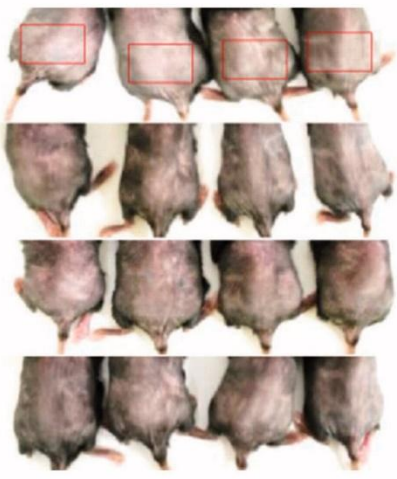
Procedure	Day	Eflornithine	No Eflornithine
Plucking with wax	0 12 17		
Chemical Depilation with Nair®	0 12 17		
Trimming with a clipper	12 17 28 36		
Pre-treatment with a microneedle roller	12 17 28 36		

Figure 3. The effectiveness of different techniques in terms of prevention of hair re-growth after its removal. The regimen of treatment with the eflornithine cream was: 2 times daily with the interval of 8 hours between two treatments, using 50 mg of the cream per treatment. Thus, among the tested techniques, maximal effectiveness in terms of hair re-growth prevention was demonstrated by microneedle pretreatment with further application of the eflornithine cream which highlights the effectiveness of skin pretreatment with solid removable microneedles in order to enhance transdermal drug delivery. (Retrieved with permission from [18]).

Currently, solid microneedles are widely used in cosmetology as rollers and pens (Figure 4). Despite the advantages of solid removable microneedles, application of this technique may sometimes be associated with such side effects as pain, skin irritation and inflammation, especially in the procedures of percutaneous collagen induction [20]. Furthermore, the combined application of solid microneedles with topical drug treatment is a multiple-step process, and most of the applied drug stays on the skin surface and does not reach deep skin layers. The listed disadvantages of solid removable microneedles may be avoided by using dissolving microneedles, which are a promising tool for cosmetology.

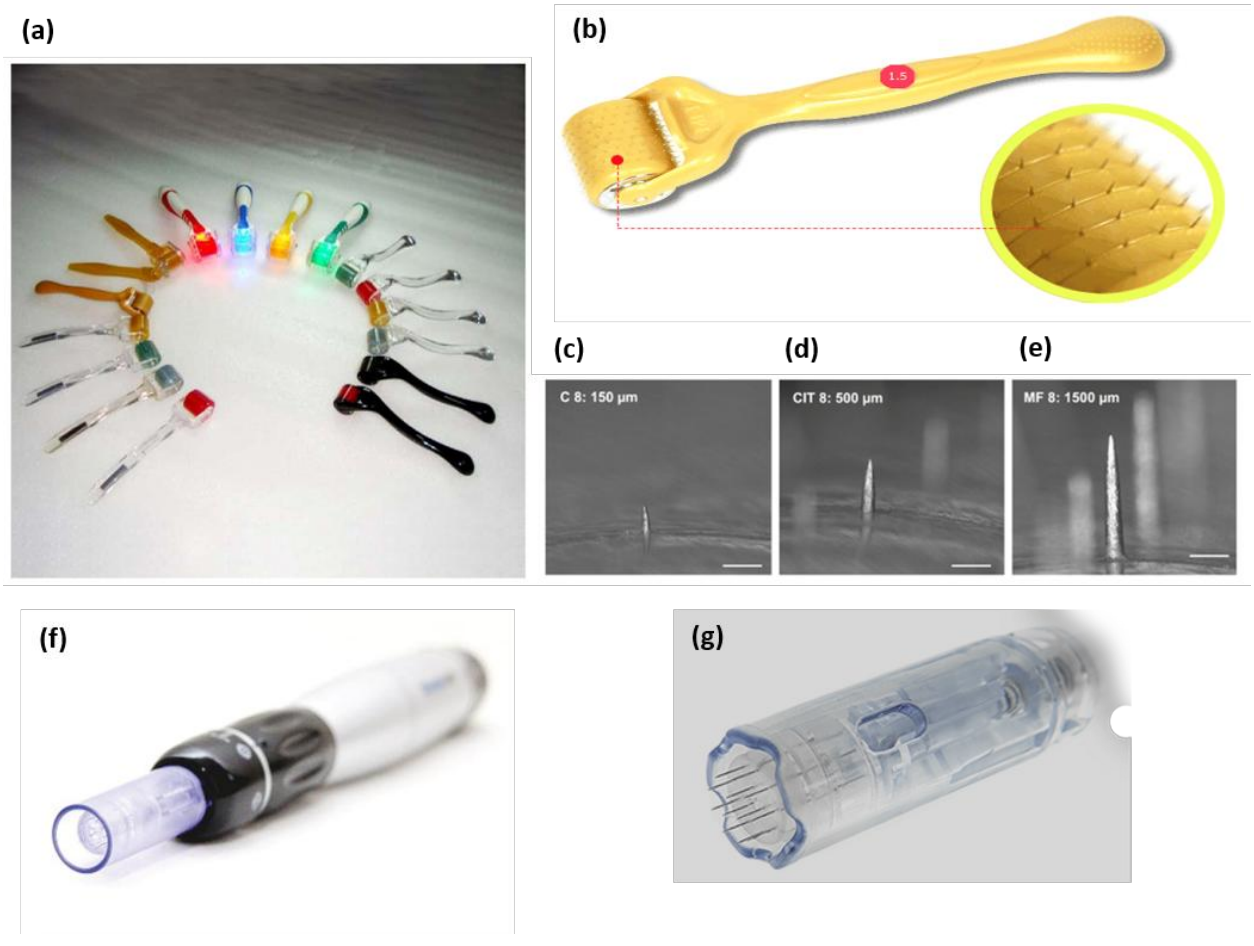


Figure 4. Solid microneedles used in cosmetology and devices, providing their application. Diverse types of dermarollers are available on the market including light emitting diode (LED) MicroNeedling Rollers (a) [21]; Dermaroller® MF8 (b) [22]; solid microneedles from diverse types of Dermaroller®: C8 (c), CIT8 (d), MF8 (e) (Retrieved with permission from [23]); Dermapen ® (f) (image taken from a marketing PDF obtained from Dermapen ®) and a Dermapen tip (g) [24].

In the *in vitro* study by Park et al. [25] adenosine, commonly used as an ingredient of anti-wrinkle products, was successfully delivered inside the skin with microneedles composed of hydrophilic biodegradable polymers: polyvinylpirrolidone (PVP) and PVP copolymerised with poly(ethylene glycol) dimethacrylate (PEGDMA) at 0.5 and 1 %. Being placed in PBS, such adenosie-loaded microneedles are dissolved in approximately five minutes. Further, the microneedles demonstrated a 150% higher rate of adenosine delivery inside the skin in comparison with the topical adenosine delivery through intact skin. Moreover, the non-copolymerised PVP microneedles and PVP-PEGDMA microneedles demonstrated different rates of mechanical strength and solubility. The PVP-PEGDMA microneedles with a concentration of PEGDMA at 1% demonstrated higher mechanical strength but lower dissolution in comparison with microneedles containing PEGDMA at 0.5 %, or PVP microneedles. Such investigation highlights the importance of a reasonable balance between the constituents of dissolving microneedles.

More recently, dissolving microneedles were successfully applied as patches for the intradermal delivery of ascorbic acid (AA) and retinyl retinoate (RR) [26] in a clinical study with the purpose of wrinkle improvement in a crow's feet area. Hyaluronic acid, dissolved at 15% in deionized water, was used as a biodegradable media of the microneedles. Patients were divided into two groups, and patients from each group received a 12-week treatment with one type of the patches: AA-loaded or RR-loaded. The patches were applied twice daily during the study. The results demonstrated a significant and similar rate of wrinkle improvement after treatment with both types of patches, and no adverse effects were observed during the study. The successful wrinkle reduction with AA-loaded hyaluronic microneedles was also demonstrated in the study by Lee *et al*. [27].

2.2. Microneedles in percutaneous vaccination

Skin is the most extensive organ of a human immune system. It is full of antigen-presenting cells such as dendritic cells, macrophages and Langerhans cells, which are responsible for the

facilitation to the development of active immune response [28]. For this reason, percutaneous vaccination is effectively applied due to increased immune response and reduced minimally effective vaccine dose in comparison with other routes available for vaccination [29]. Further, skin as a route of drug delivery is convenient for its superficial location, and therefore is used for the administration of most vaccines. However, the conventional percutaneous delivery of vaccines with hypodermic needle and syringe can cause needle phobias and, especially in mass vaccination campaigns, is associated with a huge amount of biohazardous waste and sharps, the need for a specifically trained staff and relatively high total cost [30-32]. Furthermore, such issues as the need for reconstitution of lyophilised vaccines with a diluent prior to injection, and high amounts of vaccine wastage in case of using multi-dose vials, bring additional disadvantages to conventional percutaneous vaccination. The microneedles provide an opportunity to overcome the listed issues, and so are widely studied by academics and are attractive for industries.

The first study of vaccination via microneedles was performed by Mikszta et al. in 2002 [33]. In this study silicon flat-tipped microneedles, arranged in arrays, were applied to gently scratch the skin of mice making microabrasions combined with the topical application of plasmid DNA. Thus, 35 μg of DNA encoding firefly luciferase was dissolved in 25 μl phosphate buffered saline (PBS) and applied onto 1 cm^2 of the shaved dorsal mouse skin, and then the treated area was scratched with microneedle arrays from 6 to 12 times to break SC barrier. As the comparison test, DNA PBS solution was applied onto the intact shaved mouse skin, and also injected intramuscularly and intradermally. The comparison between the results of intact skin gene treatment and gene delivery through microneedle-scratched skin demonstrated from 1000- to 2800-fold increment in luciferase activity, which was considered as a successful expression of delivered genes, depending on the number of scratches (6-12, respectively). The results of luciferase activity due to microneedle-treated gene delivery were also significantly higher in comparison with intradermal (750-fold increase) and intramuscular injections (460-fold increase). The further comparison of gene delivery effectiveness via the compromised skin scratched 6 times and intramuscular, and intradermal injections, was related to the administration of 100 μg plasmid DNA encoding hepatitis B surface antigen in a mouse model. It was observed that DNA delivery facilitated by skin scratching induced a significantly greater immune response in comparison with intramuscular and intradermal injection. Further, in case of the gene delivery enhancement with microneedle-scratching only two immunisations were required to achieve 100% seroconversion, while in case of intramuscular and intradermal injections the same amount of immunisations caused seroconversion only at 50% and 40%, respectively.

To date, different types of microneedles have been tested and have shown positive results in terms of the effectiveness in percutaneous vaccination. However, as vaccines are unstable when exposed to the environment, the technique of intradermal vaccine delivery through the pretreatment of skin with solid removable microneedles followed by vaccine application onto the skin surface does not provide an efficient vaccination. Moreover, it does not seem possible so far to reliably predict the amount of vaccine delivered to the skin compromised with removable microneedles. Therefore, the most promising types of microneedles for intradermal vaccination are coated and dissolving applied as patches, or hollow applied with injection systems [34], in which the amount of delivered drug is known [35]. Further, the significant feature of coated and dissolving microneedles is that they carry vaccines in a dried form which are released due to dissolution in dermis after the application of a microneedle patch. This reduces the need for vaccine reconstitution as one of the steps of conventional percutaneous vaccine administration. In case of vaccination via hollow microneedles, vaccines are administrated in a liquid form through the central bores of the microneedles. Also, vaccines stored in dried form within microneedle patches, which contain suitable constituents, are more thermostable. Such improved thermostability, indicated in the studies with microneedle patches containing influenza [36] and measles [37, 38] vaccines, provide an opportunity to reduce or even eliminate a cold chain conventionally required for transportation and storage of vaccines.

The *in vitro* and *in vivo* animal studies demonstrated that the vaccine administration via microneedle patches leads to dose sparing due to improved induction of immune response in comparison with conventional subcutaneous and intracutaneous injections [39, 40]. Further, as the microneedle technique is considered minimally invasive, its application is not as painful and distressful as the application of hypodermic needles. In addition, microneedles are much safer than hypodermic needles. Microneedle patches are designed for disposable use and are unlikely to be used repeatedly as can potentially happen with syringes and hypodermic needles, especially in developing countries [41]. Even in the case of accidental contact with contaminated utilised microneedle patch the chance of infection is negligible, as microneedles penetrate skin with the force commensurate to pressing by a thumb [42]. Consequently, vaccination via microneedle patches reduces the risk of sharps, and requires minimum of skills for being implemented independently after a brief training. To date, the issue of microneedle-associated vaccinaton has been investigated in the studies with influenza [43-51], measles [35, 38], poliovirus [52, 53], rotavirus [40], adenovirus [37, 54-56], BCG [57], botulism [58, 59], tetanus [60], anthrax [58, 61] hepatitis B [62, 63] and C [64], HIV-1 [65] and other vaccines. Table 1 collates the *in vivo* studies implemented with humans and nonhuman primates as a study objects, which are of

particular interest at the current stage of investigations in the field of microneedle-associated immunisation

***In vivo* subjects**	**Microneedles**	**Vaccine**	**Ref.**
Humans	Hollow	Influenza	[48, 49, 66-71]
		Poliovirus	[72, 73]
		Varicella-zoster	[74]
		Rabies	[75]
	Solid removable		
Nonhuman primates		Poliovirus	[53]
		Measles	[38]
		HIV-1	[65]
		Influenza	[76]
	Solid removable	Japanese encephalitis	[77]
	Hollow		
		Staphylococcus aureus	[58]
		Botulism	
		Anthrax	
		Plague	

Table 1. Studies with humans and nonhuman primates as subjects of microneedle-mediated vaccination.

Economic benefits of the technique are also significant, and are associated with both the low cost of manufacturing of microneedle patches and reduced cost of vaccination [78]. Microneedles are manufactured from low-cost polymers, metal and silicon, and other expendable materials which are used in small amounts. The average size (1 cm^3) and weight (1 g) of a single microneedle patch is significantly smaller than the sizes of a vaccine vial, needle and syringe required for the administration of one vaccine dose, which makes the transportation of vaccine-loaded (coated or dissolving) microneedle patches very advantageous. Further, each vaccine-loaded patch contains a single dose of vaccine. It reduces the wastage of vaccines, a serious disadvantage of multi-dose vials [79]. In addition to the above, the lack of need for vaccine reconstitution in microneedle-induced immunisation decreases the amount of required consumables, and therefore the price of procedure. Finally, the possibility of independent administration, or administration with the assistance of minimally trained staff results in significant reduction of expenses [42]. The listed advantages provide immunisation via microneedles with a high potential to reduce the overall cost of mass immunisation, and therefore make an official certification of the technique an important task.

2.3. Microneedles in insulin delivery

In current medical practice a control of type 1 diabetes, and particular cases of the type 2 is generally achieved by hypodermic/ subcutaneous insulin injections [80]. While this practice has been proven successful, many patients find injections painful and inconvenient [81] necessitating research into alternative delivery methods. A topical delivery would have been attractive, but a very large molecular weight of insulin (5808 Da) precludes meaningful penetration through the intact skin. Microneedle assisted transdermal insulin delivery provides an opportunity for painless overcoming of skin barrier, which is extremely warranted by diabetic patients as insulin administration is a daily procedure for them, and therefore received a significant attention of researchers.

As early as 1997 a skin perforating device was patented and suggested for facilitating transdermal delivery of insulin [82], but more consistent and fundamental research efforts in this direction started with the work of Martanto et al. [83]. Solid removable microneedles were investigate in this work for the delivery of insulin and it was demonstrated for the first time, using the hairless rat model in vivo, that the microneedles application allowed to increase skin permeability to insulin, which effectively reduced blood glucose, consistent with 0.05–0.5 U insulin injected subcutaneously. From the investigation of feasibility of simple removable microneedles, insulin delivery progressed to examination of dissolvable [84], hollow [80, 85], and most recently to hypoxia-sensitive vesicle-loading [86] microneedles.

Ito et al. investigated dissolvable dextrin microneedles loaded with various doses of insulin [84]. The microneedles were applied to mice in vivo and it was demonstrated that pharmacological availabilities were for all doses above 90%. Investigation of the stability of insulin in the microneedle preparations established that the remaining insulin after 1 month of the storage was more than 98%. Overall this work demonstrated the usefulness of self-dissolving microneedles for the transdermal delivery of insulin [84].

Davis et al. demonstrated the feasibility of arrays of hollow microneedles for the delivery of insulin [80]. Using hairless rat model it was proven that modified-LIGA process manufactured microneedles were sufficiently strong to pierce living skin without breaking. Insulin delivery through microneedles achieved blood glucose reduction to 47% of pretreatment values [80]. Blood glucose pharmacodynamics analysis of the experiments suggested that 50 mU of insulin was delivered by microneedles. It was therefore concluded that microneedles are an appropriate technique for continuous or possibly modulated administration over time [79]. This work was followed by investigation of hollow glass microneedles by Wang et al [85] where precise

insertion of needles was assisted with a rotary drilling device. Injections of fluorescently labeled insulin to hairless rat skin in vitro demonstrated weak fluorescence in the epidermis, suggesting that insulin penetrates slowly the dermal–epidermal junction and mostly remained within the dermis. It was concluded that regardless of the versatility of microneedles made from glass, clinical applications would be more practical from microneedles made from different materials and by different methods [85].

Dissolving microneedles were investigated by Fukushima et al as a tool for delivering insulin in dogs [87]. The insulin was loaded onto a patch with 100 chondroitin sulfate dissolvable microneedles and two to four patches were applied to shaved abdominal skin of dogs. It was established that the bioavailability of insulin from patches was greater than 70% on average. The insulin in patches demonstrated excellent stability with more than 99% recovered after 1 month [87]. It was concluded that the dissolvable microneedles are useful for immediate-acting insulin delivery which can be economically fabricated and used for minimally invasive delivery of insulin. The added usefulness of the work is that a larger animal models was used, providing insight into the scalability of the technique to human applications.

Insulin as a model drug was used in experimental [88] and computational [89] investigations. In the first work the effect of removable microneedles insertion force on insulin penetration through porcine ear skin in vitro was investigated and it was concluded that that insufficient force markedly reduces the insulin flux through the skin regardless of the geometry of the microneedles [88]. The computational work [89] investigated insulin penetration using detailed numerical modelling using COMSOL software to identify the most efficient geometry of coated microneedles. It was concluded that microneedle penetration depth was the most significant factor in determining the flux of insulin.

A very significant step in demonstrating the feasibility of hollow microneedles for the delivery of insulin in humans was the work of Gupta et al [90]. For the first time the insulin delivery with microneedles study was conducted in two adults with type 1 diabetes and compared insulin delivery with hollow microneedle to a catheter infusion. The study established that microneedles inserted to 1 mm provided the fastest insulin absorption and the best reduction of glucose levels. Importantly, the subjects reported no pain from microneedle insertion and there were no adverse reactions [90]. The same type of custom made glass hollow microneedles were used to investigate a pharmacokinetic and pharmacodynamic response to insulin injection in five type 1 diabetes subjects [91]. It was concluded that intradermal insulin infusion using microneedles resulted in quicker insulin peak time (Tmax), about half the time than that of subcutaneous catheters, and also led to better reduction in plasma glucose levels. A larger clinical study with

29 type 1 diabetes subjects investigated intradermal insulin delivery using a 34-gauge 1.5-mm steel microneedle and compared it to standard subcutaneous delivery [92]. It was demonstrated that pharmacodynamic response was similar or better for intradermal vs subcutaneous delivery for immediately premeal and 17 min premeal infusions respectively. More recently, commercially available microneedle device (MicronJet600) was used to inject insulin to seventeen patients with type 2 diabetes [93]. In this study, similarly to the previous studies, a significantly shorter Tmax compared to subcutaneous injections were observed. Thus, the use of hollow microneedles for the delivery of insulin is well advanced and is now being tested clinically.

An interesting approach of insulin delivery from positive nanovesicles driven by iontophoresis through the skin with microneedle-induced microchannels was investigated by Chen et al. [94]. Male guinea pig skin was used for the in vitro studies and male Sprague–Dawley rats were utilized for the in vivo investigation. Microchannels in the skin were created by applying slid microneedles and then the nanovesicles with positive zeta potential were driven through the microchannels with small electric current of 0.2mA/cm^2 with an on/off ration of 1:1 and frequency of 100 Hz. The significance of the work is that the current application has a potential to provide additional modulation of the rate of delivery of insulin that is easy and can be controlled in real time.

While removable and dissolving microneedles techniques were assessed for insulin delivery and found interesting, perhaps a more promising technique, capable of delivering meaningful amounts of insulin, with better control over the delivered amount and the rate of delivery, is using hollow or injectable microneedles. The latest significant progress in the microneedles techniques for insulin delivery is the use of patches loaded with hypoxia-sensitive vesicles that can provide fast automatic glucose-responsive insulin delivery [86]. This approach allowed glucose-responsive “closed-loop” insulin delivery system in which the local hypoxic microenvironment rapidly triggers the dissociation of vesicles and subsequent release of insulin. Another glucose-responsive system was based on a microneedle patch integrated with pancreatic β-cells in semi-permeable biomaterials combined with glucose signal amplifiers [95]. Application of the cells using microneedles allowed their isolation from the immune system, while still allowing the diffusion of nutrients and oxygen to the encapsulated cells. The system was tested in vitro and in vivo in STZ-induced type-1 diabetic mice and demonstrated the potency of the microneedle patches for glucose regulation for a prolonged period [95]. These body-modulated and minimally invasive systems for delivery of insulin, if developed to a viable

product, will constitute a “holy grail” of control of type 1 diabetes that mimics the function of pancreatic cells.

2.4. Microneedles in the transdermal delivery of a diverse range of high and low molecular weight drugs

Currently, a limited number of drugs have been approved by FDA for transdermal drug delivery [96] mainly due to formidable barrier properties of SC. Potentially, the technique of microneedles can be applied for the enhanced delivery of all the approved drugs, being at the same time prospective for transdermal delivery of any other relevant high and low molecular weight formulations such as non-cosmeceutical peptides, oligonucleotides, DNA, desmopressin, oxytocin, human growth hormone, acyclovir, lidocaine, carnitine, botox etc [97-100]. Thus, it is anticipated that microneedle-mediated transdermal drug delivery may substantially increase the range of transdermally delivered drugs, which previously had no chance for being effectively delivered through this route of drug administration, and promising clinical studies have been conducted with the number of them. Over the past decade, there has been a substantial increase in research of MN technologies. Indeed, the number of academic publications on the subject has more than tripled since 2007. While biological agents have been the main focus, water soluble drugs not currently suitable for passive transdermal delivery are also of great interest. A number of companies are investing heavily in development of MN-based transdermal delivery systems. These include 3M, Corium, Zosano Pharma, Vaxxas, Nemaura, Becton-Dickinson, LTS Lohmann and NanoPass Technologies [101-108].

Currently, Zosano Pharma is developing transdermal delivery products based on the Macroflux® technology originally designed at Alza. Having apparently moved away from its initial focus on delivery of parathyroid hormone for management of post-menopausal osteoporosis, Zosano has recently announced successful results of a double-blind placebo controlled clinical trial focused on a delivery of zolmitriptan for treatment of migraine [101]. Further, Vaxxas is a venture capital-funded technology start-up company developing the coated MN Nanopatch™ technology that originated from Mark Kendall's research group at the Australian Institute of Bioengineering & Nanotechnology at The University of Queensland [102]. In 2015, Vaxxas announced that it had secured equity funding of $20 million from new and existing investors. These funds represented the first closing of a Series B venture financing round, the proceeds from which were to be used to advance a series of clinical programs and develop a pipeline of new vaccine

products for major diseases using Vaxxas' Nanopatch™ platform. This round of financing brought the total capital raised by Vaxxas to $33 million.

NanoPass Technologies have shown their MicronJet™ device to be useful not only in the previously mentioned delivery of influenza vaccines (Table2), with the evidence of dose-sparing compared to conventional routes of immunisation, but also insulin [103]. However, it should be noted that this device is more similar to a small set of very short silicon needles attached to the barrel of a conventional syringe, rather than a true microneedle array. Meanwhile, Beckton-Dickinson's Soluvia™ device, consisting of a single 1.5 mm 30-gauge stainless steel needle on the end of a conventional syringe barrel, has been widely used for a number of years in Sanofi-Pasteur's market-approved intradermal influenza vaccine products Intanza® and Fluzone® [104]. 3M's microstructured transdermal systems (MTS), based on either hollow or coated solid MN, have been evaluated in a range of pre-clinical studies focussed on delivery of proteins, peptides and vaccines [105]. Nemaura Pharma, a specialist drug delivery firm based in the east Midlands in England, are developing MN systems for applications in cosmeceuticals, dermatology, analgesia, osteoporosis, immunology and oncology [106].

Whilst the above mentioned MN devices have been based upon solid or hollow MN systems, it is envisaged that devices based upon FDA-approved, biodegradable/dissolving polymeric MN formulations will, in the future, receive increased attention from pharma companies. This is due to the self-disabling nature of such systems. Once inserted into skin, these MN will either dissolve or swell, thus making insertion into another patient post-removal virtually impossible. This will, therefore, reduce transmission of infection by preventing needlestick injuries associated with conventional needles. Disposal issues will also be bypassed, since there is no "sharp" remaining. Ultimately, the impact on healthcare in the developing world in particular could be significant. Encouragingly, Mark Prausnitz recently reported the first successful human clinical trial of a dissolving MN vaccine patch (Table 1) [76].

Corium has stated that they are exploring several applications of dissolving MN with pharma partners, with a particular current interest in delivery of human parathyroid hormone (hPTH) and zolmitriptan, with the hPTH project, using MN manufactured at Corium, now in Phase 2 clinical trials [108]. Most notably, perhaps, LTS Lohmann (LTS), the world's largest transdermal patch manufacturer, have now entered into the MN field and are inviting partners to collaborate on development of new MN products based on such technology [107]. Given the manufacturing capabilities, expertise and customer base of LTS, it will be surprising if they do not claim a sizeable proportion of the developing MN market in the coming years. Indeed, LTS recently

announced that they now hold Europe's first manufacturing licence for MN patches. Fujifilm also appears to have considerable manufacturing capability for MN patches, but they have not made any significant announcements since 2012 [109],

It is notable that the companies publically engaged in MN development at present could all be reasonably categorised as "drug delivery" companies, or companies with a specialised drug delivery component. It is very unlikely that any of them will ever end up holding the product licence for a MN vaccine or drug product themselves. Their business models suggest that they would either sell their technology, or indeed the entire company, to a pharmaceutical company seeking to bring a MN product to the market or, alternatively, as may be the case with Corium, Fujifilm and LTS, the drug delivery company would act as the product manufacturer, with the product taken through clinical trials, regulatory scrutiny and ultimate product registration by a sponsoring pharma company. Rumours in the field abound about the involvement of big pharma companies in MN development. Indeed, GlaxoSmithKline hosted the 2016 Microneedles Conference in London. However, to date, involvement of major players has, perhaps understandably, been kept under wraps. It is possible that big pharma see MN as only a vaccine product suitable for the developing world from which they are unlikely to make money. Alternatively, they may not want to make their interest public for fear of "tipping off" competitors, or they may be unsure of the technology, but want to keep an interest by sponsoring work with academic groups or specialist MN firms, so as to avoid "missing the boat" if MN products were to be marketed by a competitor. Whatever the reasons, the financial muscle of a large company would certainly expedite the first MN-based drug or vaccine product's route to market. There may be an alternative, of course. The Bill & Melinda Gates Foundation have invested heavily in development of MN vaccines [110]. For example, $6 million has recently been awarded to Vaxess to develop inactivated polio and live attenuated measles rubella vaccines [110]. PATH (formerly Program for Appropriate Technologies in Health) are now developing a Centre of Excellence in this area to focus on commercial development of MN delivery systems with applications in developing countries. The question remains here as to who will be the product licence holder, however. Whatever the route ultimately taken is, once the first MN drug or vaccine product is finally approved for human use, one could reasonably expect numerous other products to quickly follow suit.

3. Ocular applications of microneedles

Eye is an organ responsible for perception of the visual world, which is associated with the reaction on light, color distinguishing and visual estimation of shape and distance. The perception of such vital information by the eye makes ocular impairments, most of which lead to blindness, highly devastating for a person [111]. Ocular diseases can be divided into two groups: diseases of an anterior segment of the eyeball which include the impairments of cornea, conjunctiva, ciliary body and lens, and diseases of the posterior segment which is comprised of the sclera, vitreous humor, retina, choroid and optic disc (optic nerve) [112].

Drug delivery to the eye can be achieved *via* a number of routes such as systemic administration (e.g. oral and parenteral routes), intravitreal injections, surgical implantation of drug vehicles for sustained drug release into the ocular or periocular tissues, and targeted topical administration *via* injections and conventional topical applications [113]. Even though each of the routes is effective in particular diseases scenario, these routes of delivery have serious disadvantages and limitations. For example, blood-ocular barriers [114] significantly decrease the permeation of systemically administered drugs, necessitating high doses of medications which can be toxic or cause severe side effects. The surgical implantation of sustained release drug vehicles, as with any surgical intervention, is highly invasive and is performed only in absence of the effective alternatives.

To achieve targeted drug delivery in the eye direct injections of medication is practiced using hypodermic needles – where injections are either given in the eyeball (e.g. intravitreal, intracorneal, subconjunctival and intrascleral injections) or into the tissues surrounding the eye (i.e. periocular injections). The injections provide more localised delivery of drugs to the targeted eye sites than systemic drug administration and are successfully used for a treatment of numerous ocular impairments of both anterior and posterior eye segments, especially such acute/chronic conditions as bacterial and fungal keratitis, uveitis, blepharitis, age-related macular degeneration, diabetic macular edema etc. However, ocular and periocular tissues are delicate, and it is preferable to avoid such highly invasive methods as injections with hypodermic needles which cause significant discomfort to a patient and damage to the tissues of the eye. Further, ocular injections with hypodermic needles have such limitations as the possibility of intraocular pressure increment, bacterial invasion, mechanical tissue damage, inflammation, retinal detachment and local hemorrhage. Furthermore, the method of intraocular injections is technically challenging and requires skilled medical staff. The conventional topical administration with a liquid drug forms (i.e. ointments, gels, solutions) is generally useful in treatment of the anterior segment of the eye, can be self-administered and thereby demonstrate a high patient compliance. However, it is often difficult to achieve a therapeutic effect with eye

drops or ointments due to such limitations as a lacrimal fluid which washes away and can bind the drug applied onto the eye surface, and the barrier properties of cornea. Furthermore, in the conventional topical treatment, some amount of applied drug may be released into the vessels of a highly vascularized conjunctiva, which decreases bioavailability of the drug even more and potentially leads to the undesired systemic effects of the drug.

Considering the listed features of the drug administration routes used in ocular treatment, the technique of microneedles has recently been proposed as a reasonable minimally invasive alternative [115]. The technique of microneedles has been investigated as the enhancing technique of treatment of both anterior and posterior eye segments [116]. The related studies have indicated microneedles of coated, dissolving and hollow types as promising in the field of ocular drug delivery. Thus, in ocular treatment microneedles are conventionally applied to the cornea or sclera, or to the suprachoridal space (SCS). Such application facilitates the delivered drugs to overcome the corneal or scleral barriers and then to be deposited inside the tissues, or to be released inside the anterior or posterior eye segment [117]. Being minimally invasive, the technique reduces pain, tissue damage and the possibility of bacterial contamination in comparison to the hypodermic injections. Further, the technique increases patient compliance by the possibility of self-administration, and decreases discomfort of the administration. Further, ocular treatment with microneedles is targeted, so its application overcomes physiological barriers of the eye and as a result is dose sparing and has higher bioavailability. However, even though the technique of microneedles is considered safe, it does not entirely satisfy the safety requirements of ocular interventions so far. Thus, the risk of microneedle fracture of non-dissolvable microneedles within the eye is a major concern. The other important factor which has to be investigated is the effect of microneedle application on intraocular pressure. Even though little research has been done, the technique of microneedles has already been considered as promising in the field of microneedle-associated ocular treatment. Table 2 collates current progress achieved in the field of ocular applications of microneedles.

Microneedles	**Chemicals, or Composites**	**Experiment / targeted site**	**Purpose**	**Outcome**	**Ref.**
Coated	Sulforhodamine B BSA[a] Plasmid DNA	*In vitro* / human sclera	The first attempt to apply microneedles in ocular treatment to estimate the potential effectiveness of microneedles for drug delivery.	Microneedles were inserted into the sclera for a short time of 20 seconds. Rapid dissolution and a subsequent forming of drug depot within the scleral tissue was indicated.	[115]

	Sodium fluorescein (SF) Pilocarpine	*In vivo* / rabbit cornea	The evaluation of the effectiveness of drug delivery via microneedles in the *in vivo* model. Moreover, safety examination in terms of post-applicative inflammation and tissue damage.	Microneedles were inserted into the cornea for 20 seconds in either cases with SF and pilocarpine. The drugs located on the microneedles' surface were primarily dissolved into the corneal tissue and then being gradually released into the anterior eye chamber. As a result, the delivery of drugs via microneedles demonstrated 70-fold and 45-fold increase in drug concentration within the anterior chamber of the rabbit eye in case with SF and pilocarpine, respectively, in comparison to the topical drug administration. Further, it was indicated that the microneedle-associated delivery of pilocarpine led to the rapid eye constriction. There was no inflammatory response indicated, and the created corneal abrasion disappeared after 3 hours, so the technique was considered safe to be used in ocular treatment.	
Hollow	Sulforhodamine B Fluorescent nanoparticles and microparticles	*In vitro* / human sclera	The first attempt to apply hollow microneedles for the intrascleral delivery of drug solutions, and nanoparticle and microparticle suspensions.	Overall, the results indicated hollow microneedles as appropriate to be used for the intrascleral delivery of drug solutes, nanoparticles and microparticles. There were two major investigations performed. First, the infusion of fluid was extremely low and in spite of various insertion depth or infusion pressure, only a partial retraction of a microneedle increased the flow rate. Second, differently from the delivery of nanoparticles, it was possible to deliver microparticles within the sclera only after it was exposed to hyaluronidase and collagenase which demolished the fibrous carcass of the tusssue. Therefore, only nano-sized particles	[118]

				were considered appropriate to be easily delivered within the sclera.	
	Sulforhodamine B Fluorescent nanoparticles and microparticles	*Ex vivo* / suprachoroidal (SCS) space of a human, rabbit and pig eyeballs	The first attempt to provide the delivery of drug solution, and nano- and micro-particles within the SCS of the eye.	The results indicated hollow microneedles as a suitable option for delivering drug solutions, and nano- and micron-sized particles within the SCS of the eye. Such delivery of nano- and micro-particles may potentially provide sustained drug release from SCS. Furthermore, the success in delivery of the particles within SCS was dependent on the spatial properties of microneedles and pressure applied to drive the particles through microneedle bores into the SCS. Thus, the most appropriate microneedle length was indicated at 1000 μm and pressure at 250–300 kPa.	[119]
	Sodium fluorescein Fluorescein isothiocyanate dextrans (40 – 250 kDa) Bevacizumab Particles (20 nm – 10 μm in diameter)	*In vivo* / SCS of the rabbit eye	To investigate a pharmacokinetics of the drugs and particles delivered within the SCS.	The drugs and particles were successfully delivered within the SCS with a 750 μm hollow microneedle. Thus, the drugs were cleared after one day, while the particles remained within the SCS for two months. Such technique revealed a potential of hollow microneedles to deliver drug solutions and particles of a diverse size within the SCS for the rapid (with drug solution) or prolonged (with particles) treatment of posterior segment diseases. No post-procedure adverse effects were indicated.	[120]
	Triamcinolone acetonide (TA)	*In vivo* / SCS of a porcine eye	To compare the effectiveness of anti-inflammatory therapy with TA between SCS microneedle injection and 27G needle intravitreal injection. Further, the evidence of procedural adverse effects and drug	According to the results, the microneedle-associated therapy of artificially-induced acute posterior uveitis model with 0.2 mg of the drug was the same affective as the intravitreal treatment of the model with 2 mg of the drug. There were no hemorrhage, drug toxicity, or	[121]

			toxicity, and intraocular pressure (IOP) were estimated.	significant increase of IOP indicated.	
	Nanoparticles (200 nm in diameter)	*Ex vivo* / suprachoroidal (SCS) space of a human, rabbit and pig eyeballs	First, to investigate the movement of injected nanoparticles within the SCS. Second, to determine barriers which hinder the circumferential flow of the particles in SCS and impact the ability to reach the targeted areas of SCS, as chorioretina is usually to evenly diseased.	In rabbit eyes, the long posterior ciliary artery prevented the particles which are injected in the superior or inferior hemisphere from crossing into the other hemisphere. In human eyes, the barrier formed by the short ciliary artery hindered circumferential spread toward the macula and optic nerve. These results suggest that the anatomical barriers could hinder even spread of the administered drug or formulation within the SCS. Thus, it is essential to make a judicious selection of the injection region.	[119]
	Bevacizumab	Clinical trial	To compare intrastromal delivery of bevacizumab via the microneedles with subconjunctival and conventional topical delivery with eye drops in terms of effective dose required to reduce corneal neovascularization.	The technique of drug administration via the hollow microneedles was considered dose sparing. Thus, the delivery of only 4.4 μg of the drug via the microneedles caused the same therapeutic effect as the delivery of 2500 μg and 52.500 μg with subconjunctival injections and eye drops, respectively.	[122]
	Fluorescein sodium	*In vitro* / rabbit sclera	The first attempt to use hollow microneedles (HMN) for the intrascleral delivery of *in situ* implant-forming gels	HMN devices of different heights (400, 500 and 600 μm) were fabricated from hypodermic needles (27, 29 and 30G) and investigated for depth of penetration into rabbit sclera. Upon HMN injection, the gel turned into a semisolid implant and formed an intrascleral implant. Sustained release of fluorescein sodium was observed over 24 h and varied with the	[123]

				depth of implant delivery in the sclera. The results demonstrate that HMN device can localize *in situ* forming implants in the scleral tissue and sustain drug delivery.	
	Fluorescently tagged nanoparticles and microparticles	*In vivo* / SCS of the rabbit eye	To achieve targeted particle delivery by controlling polymeric formulation properties.	Particles suspended in saline were distributed over 29-42% of the SCS upon injection. The addition of hyaluronic acid increased particle spread up to 100% of the SCS. Strongly non-Newtonian polymer solutions containing carboxymethyl cellulose or methyl cellulose immobilized particles at the site of injection for up to 2 months, which could enable targeted drug delivery to the ciliary body to treat glaucoma. This study demonstrates that targeted drug delivery via injection into SCS can be controlled by using different polymeric formulations.	[124]
Dissolving	Methotrexate	*In vivo* / rabbit eye scleral pocket	The investigation of pharmacokinetics of methotrexate released from surgically implanted biodegradable microneedles as a potential option to be used in vitreo-retinal lymphoma.	The results indicated successful sustained release of methotrexate from the implanted microneedles. No postoperative adverse effects, or drug toxicity were observed.	[125]
	Fluorescein sodium Fluorescein isothiocyanate–dextrans (70 k and 150 k Da)	*In vitro* / pig sclera and cornea	The first attempt to investigate the feasibility of using polyvinylpyrrolidone (PVP) to fabricate MN and the potential of using dissolving PVP MN arrays to overcome barriers in ocular tissues and enhance ocular drug delivery.	*In vitro* studies showed MNs penetrated into the ocular tissues could rapidly dissolve and form a depot within the tissues. These depots provided a sustained drug release into ocular tissues. Significant enhancement of macromolecular permeation across both the scleral and corneal tissues was observed when using MNs, in comparison to topical administration of aqueous solutions (Figure 5). The results from this study	[126]

				indicate that rapidly dissolving MNs could deliver macromolecules to the eye through the intrastromal or intrascleral route.	

Table 2. The significant investigations in the field of ocular treatment with microneedles

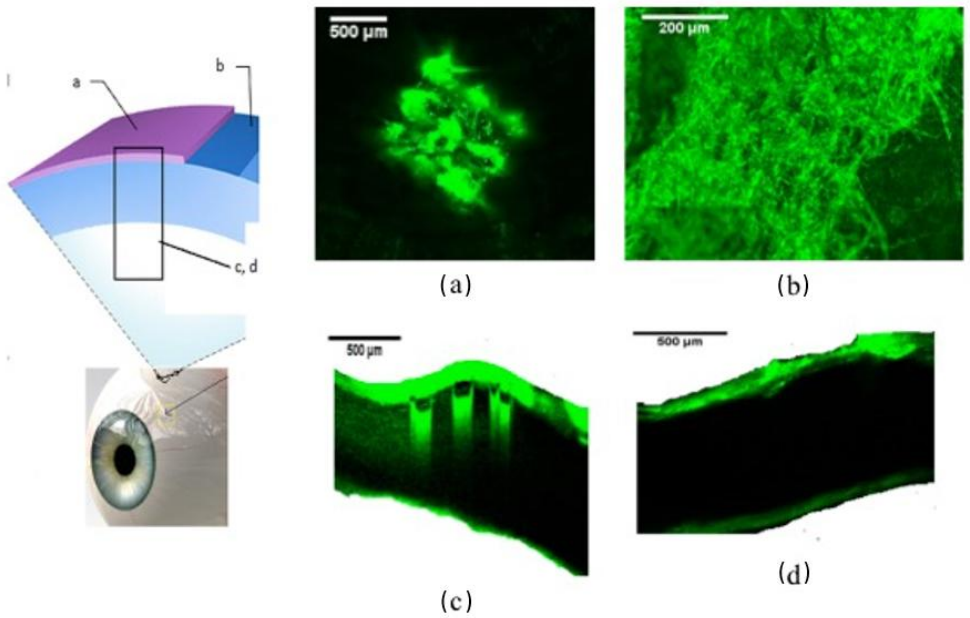


Figure 5. Schematic representation of administration of PVP-based dissolving microneedles (MNs) – the left hand side represents the collection and processing of confocal images of scleral tissues following application of MN arrays, where (a) topical image of tissue after 5 min following insertion of MN array, (b) cross section image of tissue after 5 min following MN array insertion, (c) topical image at a depth of 80 µm from surface of the tissue after 1 hr following MN insertion, and (d) cross section image of tissue 1 hr after applying an aqueous drug solution (Retrieved with permission from [126]).

4. Oral mucosal and gastrointestinal applications of microneedles

Oral cavity and gastrointestinal tract are the second, after the skin, parts of a human body most exposed to xenobiotics. Furthermore, these parts are in periodic contact with endogenic enzymes and digestive products. Thus, the highly-resistive oral and gastrointestinal barrier mainly consists of a complex mechanical, chemical, microbal and immune barrier which provide adequate protection [127]. Drug permeation across oral and gastrointestinal mucosa is limited by the barriers of superficial mucus and deeper epithelial tissue, with the additional barrier of luminally-secreted and membrane-bound enzymes, which cover the underlying lamina propria

full of capillaries, nerve endings and immune cells [128]. Mucus, bound to the external site of epithelium, has a structure of hydrogel of approximately 83% water containing diverse inorganic compounds, proteins, lipids, carbohydrates and proteins with mucin as the main component mainly produced by Goblet cells of the epithelium [129]. Mucin is represented by glycoproteins of diverse molecular weights ranging at 1-40 × 10^6 Da, and defines the 3D network of mucus determining its viscous properties [130]. The average thickness of mucus is at 70 and 80-200 μm for oral and intestinal mucosa, respectively [127]. The barrier of mucus does decreases permeation of macromolecular drugs across mucosa, and owing its complex chemical structure may contribute to degradation and alter concentration of the applied drugs. Further, mucosal epithelium, simple columnar in intestine and nonkeratinized stratified squamous in oral mucosa, acts as a physical barrier reducing passive diffusion of molecules with the radius over 15 Å. Barrier properties of the epithelium are principally based on a multi-layered structure with 40-50 cell layers in oral mucosa, and tight junctions between epithelial cells and lamina propria in intestinal mucosa.

Numerous drug delivery systems were previously developed for providing enhanced delivery across oral and gastrointestinal barriers, and shield orally administered drugs from enzymes presenting in oral cavity and gastrointestinal tract [131-136]. Among such enhancing techniques, microneedles is the youngest option suggested so far. Thus, microneedles have already been investigated in the studies of oral mucosal vaccination, oral cancer management and gastrointestinal drug delivery by a microneedle-equipped pill.

4.1. Microneedles in oral mucosal vaccine delivery

Microneedles were clearly designed originally to enhance delivery of therapeutic or prophylactic substances into or across the skin. However, the predominant use of microneedles as vaccine delivery devices has recently prompted several researchers to consider targeting vaccines to the oral mucosa, in addition to the skin. Mucosal sites are typically rich in professional antigen-presenting cells and vaccination at one mucosal site often provokes mucosal immune responses at distant sites, thus providing more complete immunity. This is especially important, since most pathogens enter the body through the mucosa. The mucosa in the oral cavity are easily accessible and have been used for many years as a site for delivery of systemic therapeutics, for example prochlorperazine for management of nausea and vomiting and nicotine for smoking cessation. Since vaccine antigens and adjuvants are typically large molecules, often with appreciable water solubility, they do not typically traverse the lipid barrier of the oral mucosa efficiently.

Reversible disruption of this barrier using microneedle devices represents one possible strategy for enabling oral mucosal vaccine delivery, facilitating transport of the vaccine to the antigen-presenting cells of the viable tissue.

Wang *et al*. [137] aimed to develop an effective, convenient and stable mucosal vaccine against hepatitis B virus (HBV). Mannose-PEG-cholesterol/lipid A-liposomes (MLLs) loaded with HBsAg were prepared by emulsification-lyophilisation, filled into microneedle moulds and dried to form proHBsAg-MLLs microneedle arrays (proHMAs). These proHMAs were stable, even at 40 °C for up to 3 days and possessed sufficient mechanical strength to pierce porcine skin. Upon hydration, the microneedles rapidly dissolved, recovering the HBsAg-MLLs without obvious changes in size and antigen association efficiency. Immunisation of mice by a single application to the oral mucosa robust systemic and widespread mucosal immune responses, as evidenced by high levels of HBsAg-specific immunoglobulin G (IgG) in the sera and immunoglobulin A (IgA) in the salivary, intestinal and vaginal secretions. In addition, a strong cellular immunity against HBV was established through a mixed Th1/Th2 response, as confirmed by a significant increase in CD8(+) T cells, as well as enhanced levels of IgG2a and IFN-γ in the treated mice. The authors concluded that this novel microneedle system could be used to induce a multi-modal immune defence against HBV infection and may, in due course, be shown to enhance storage stability at elevated temperatures, thus obviating the expensive and inconvenient cold chain.

Further, almost identical, work by the same Group (Zhen *et al*. [138]) prepared mannose-PEG-cholesterol (MPC)/lipid A-liposomes (MLLs) entrapping model antigen bovine serum albumin (BSA), again using emulsification-lyophilisation. ProMLL-filled microneedle arrays were inserted into the oral mucosa of mice and elicited robust systemic and mucosal immune responses against the loaded antigen, as evidenced by high levels of BSA-specific IgG in the sera and IgA in the salivary, intestinal and vaginal secretions of mice. Enhanced levels of IgG2a and IFN-γ in treated mice revealed that proMMAs induced a mixed Th1/Th2 response. Moreover, a significant increase in CD8(+) T cells confirmed that strong cellular immunity had also been established.

Ma et al. [139] sought to evaluate the feasibility of using coated microneedles to deliver vaccines into the oral cavity to induce systemic and mucosal immune responses. Microneedles were coated with sulforhodamine (Figure 6), ovalbumin and two HIV antigens. Coated microneedles were inserted into the inner lower lip and dorsal surface of the tongue of rabbits (Figure 7). Histology was used to confirm microneedle insertion, and systemic and mucosal immune responses were characterized by measuring antigen-specific IgG in serum and IgA in saliva, respectively. Histological evaluation of tissues showed that coated microneedles could penetrate

the lip and tongue to deliver coatings. Using ovalbumin as a model antigen, it was found that the lip and the tongue were equally immunogenic sites for vaccination. Importantly, both sites also induced a significant secretory IgA in saliva, compared to pre-immune saliva. Microneedle-based oral cavity vaccination was also compared to the intramuscular route using two HIV antigens, a virus-like particle and a DNA vaccine. Microneedle-based delivery to the oral cavity and the intramuscular route exhibited similar yet considerable levels of antigen-specific IgG in serum. However, only the microneedle-based oral cavity vaccination group stimulated a significantly higher antigen-specific IgA response in saliva.

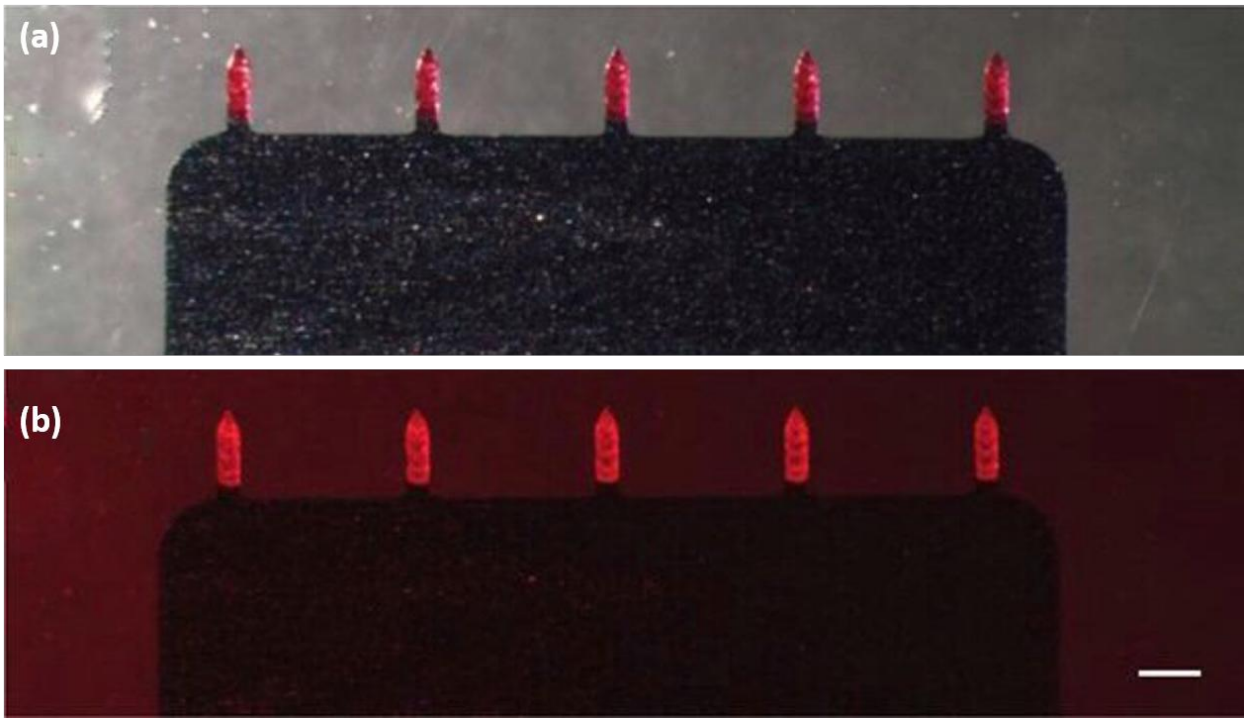


Figure 6: Sulforhodamine-coated 1D microneedle arrays. Array visualized using (a) brightfield microscopy, and (b) fluorescence microscopy. Scale bar indicates 500 μm (Adapted from [139]).

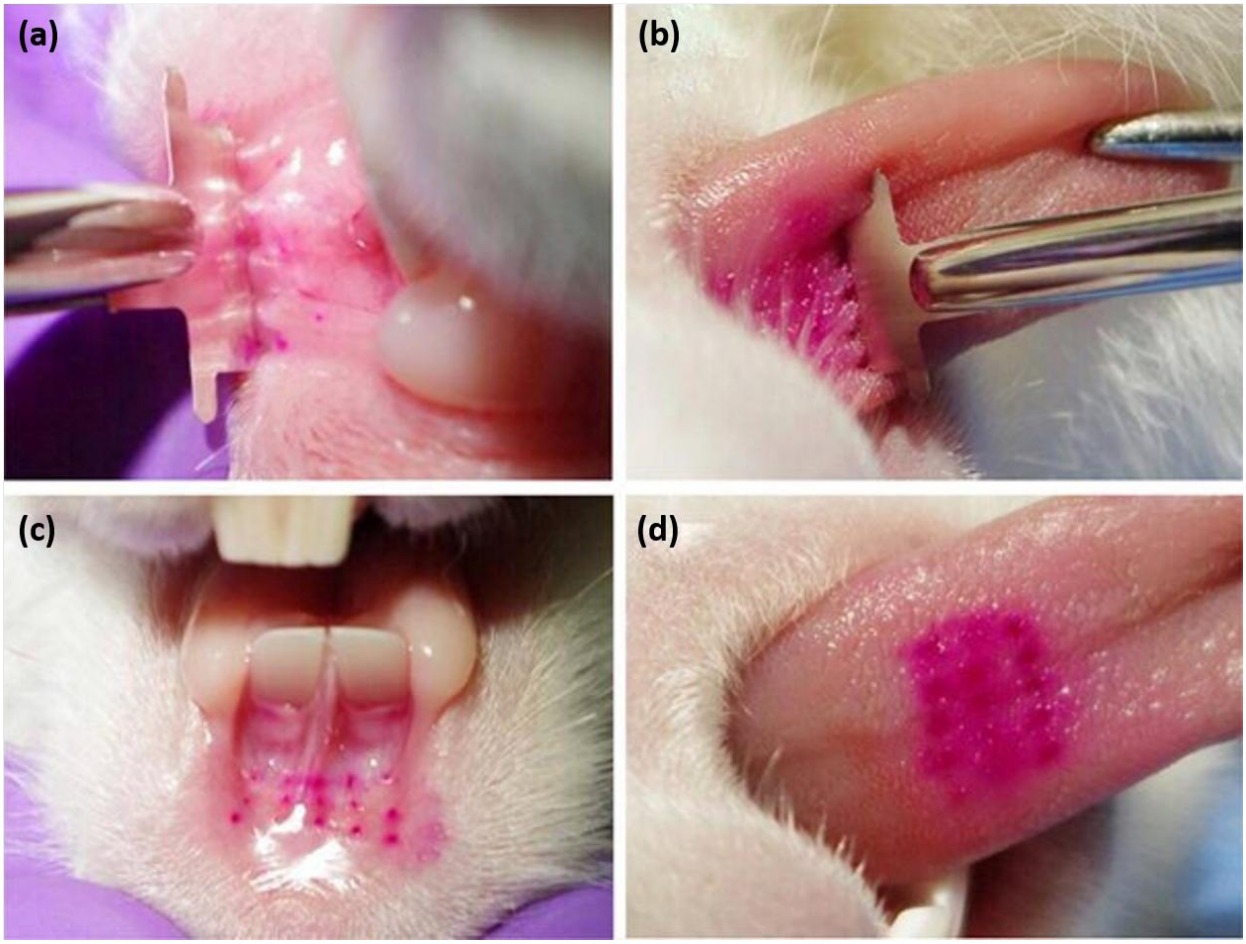


Figure 7: Insertion of sulforhodamine-coated 1D microneedle arrays in rabbit oral cavity tissues. 1D array held in a Kelly locking forcep inserted into (A) stretched lower lip and (B) tongue. Regular array of dots formed by insertion of coated microneedles into rabbit (C) lip and (D) tongue (Adapted from [139]).

4.2. Microneedles in management of oral cancer

Oral cancers and precancerous dysplasias are sometimes diagnosed in a non-invasive fashion using optical coherence tomography (OCT), which can basically be described as the optical analogue of ultrasound imaging. Contrast in OCT images can be enhanced by utilising surface plasmon resonant gold nanoparticles (Au NP). To improve the poor *in vivo* transport of gold nanoparticles through biological barriers, an efficient delivery strategy is needed. Kim *et al*. [140] showed improved penetration and distribution of gold nanoparticles in induced dysplasias of the oral mucosa of hamsters *in vivo* following combination treatment with microneedles and ultrasound. CR3 roller microneedles (MTS dermaroller with miniscule holes of 70-μm diameter and 300-μm depth; Clinical Resolution Laboratory, Inc., Beverly Hills, CA) were rolled on both the DMBA-untreated and DMBA-treated sides of the hamster cheek pouches three times at three different angles (i.e., 0 deg, 45 deg, and 90 deg). A aliquot (200 μL) of anti-EGFR antibody-conjugated PEGylated Au NP suspension (1.78×10^{10} particles/mL) was applied to the hamsters'

cheek pouch for 10 min by dropping it directly into the 1-cm-diameter aperture of a ring-shaped clamp. After the Au NP topical administration, 0.3 W/cm^2 of 1-MHz ultrasonic force was applied to the cheek pouch using the Dynatron 125 ultrasonicator (Dynatronics Corporation, Salt Lake City, UT) for 1 min. It was demonstrated that this multimodal delivery of antibody-conjugated PEGylated gold nanoparticles enhanced the contrast in *in vivo* OCT images of oral dysplasia in a hamster model (Figure 8).

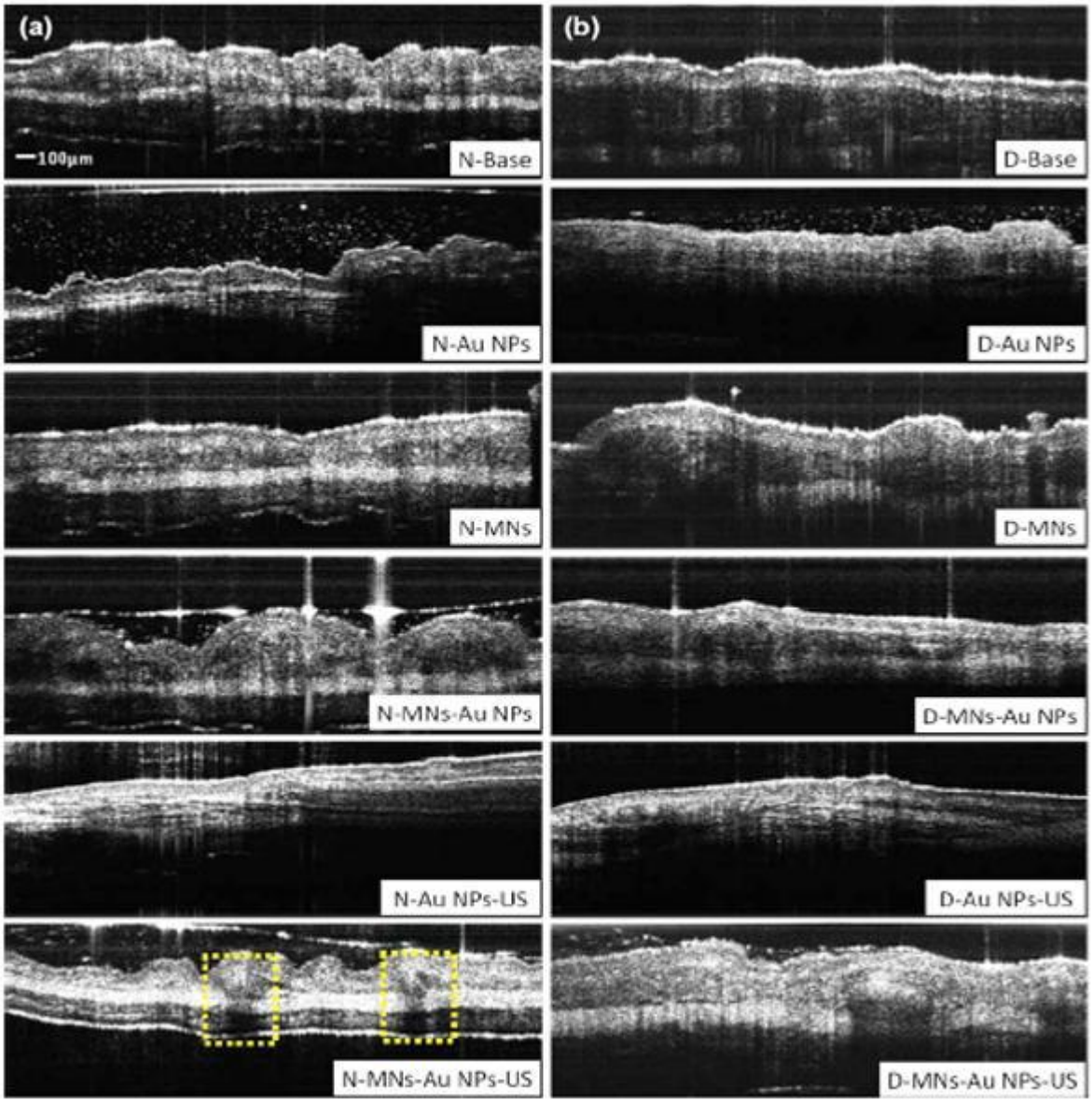


Figure 8. *In vivo* SD-OCT images of (a) normal, and (b) dysplastic hamster cheek pouches. MN = microneedle treated; Au NPs = gold nanoparticles administered; US = ultrasound applied (Retrieved with permission from [140]).

A reasonable question would be whether, in buccal tissues, after insertion and removal of coated microneedles, the presence of saliva over the insertion site can lead to loss of the deposited drug or vaccine and if saliva can influence permeation across the tissue. Serpe *et al*. [5] coated

microneedles with the model drug sulforhodamine (SRD) and inserted them into porcine buccal mucosa in vitro. Fluorescence microscopy was used to study microneedle coating quality and the diffusion of SRD through the mucosa. Permeation experiments were conducted for simulated dynamic or static salivary flow by adding 100 μL/h or 100, 200 or 300 μL of phosphate buffered saline (PBS) in the donor compartment of Franz diffusion cells, into which buccal tissue after insertion of SRD-coated microneedles was placed. Microscopy showed that microneedles were uniformly coated with SRD and that SRD was successfully delivered into the mucosa. Some SRD remained in the tissue even after 24h, despite presence of PBS on top of the coated microneedle insertion site. It was found that salivary washout can possibly result in loss of drug/vaccine that has been deposited in the oral cavity mucosal tissues using coated microneedles and presence of fluid over the coated microneedle insertion site can increase flux across the tissue. Thus, it is advisable to include salivary flow during developmental *in vitro* studies related to the use of coated microneedles for drug delivery to the oral cavity in order to not obtain misleading results.

The small number of studies carried out to date suggest that microneedles may well be a viable delivery system for delivery of vaccines and possibly also drug substances across the mucosal barrier in the oral cavity. It would be interesting, however, to see how such systems perform in human volunteers. Pain may possibly be an issue, given the sensitive nature of the mucosa. It will be crucial that microneedles are not exposed to significant amounts of saliva prior to insertion, since this will initiate drug release before it is required and may also compromise the ability of polymeric microneedles to subsequently penetrate the mucosal barrier. If sustained drug delivery is required, the microneedle baseplate will need to exhibit mucoadhesive properties to keep the needles in place, with the reverse of the device coated with a moisture-impermeable backing layer to resist early dissolution. Safety is another consideration, since the mucosal cavity is replete with microorganisms. Passage of such across a compromised mucosal barrier would be undesirable, due to the possible risk of local or systemic infection. As more studies are published and the technology evolves, industry may well become interested. This will be essential for progression of the technology to commercial return on investment and, importantly, patient benefit.

4.3. Microneedles in gastrointestinal delivery

It is well known that both patients and healthcare professionals generally prefer the oral route of drug delivery. The gastrointestinal (GI) tract, however, limits the bioavailability of certain therapeutics because of its protease and bacteria-rich environment, as well as general pH

variability from pH 1 to 7. These extreme environments make oral delivery particularly challenging for the biologic class of therapeutics, the absorption of which is further compromised by their high molecular weights. The Langer Group [6] demonstrated proof-of-concept experiments in pigs that microneedle-based delivery (Figure 9) has the capacity for improved bioavailability of a biologically-active macromolecule, namely insulin. The authors showed that microneedle-based devices can be passed and excreted from the GI tract safely. The basic theory is that the contraction of the smooth muscle of the small intestine causes the hollow metal microneedles to penetrate the mucosa, releasing the insulin from a central protective reservoir thought the lumen of the microneedles and into the viable tissue for systemic absorption.

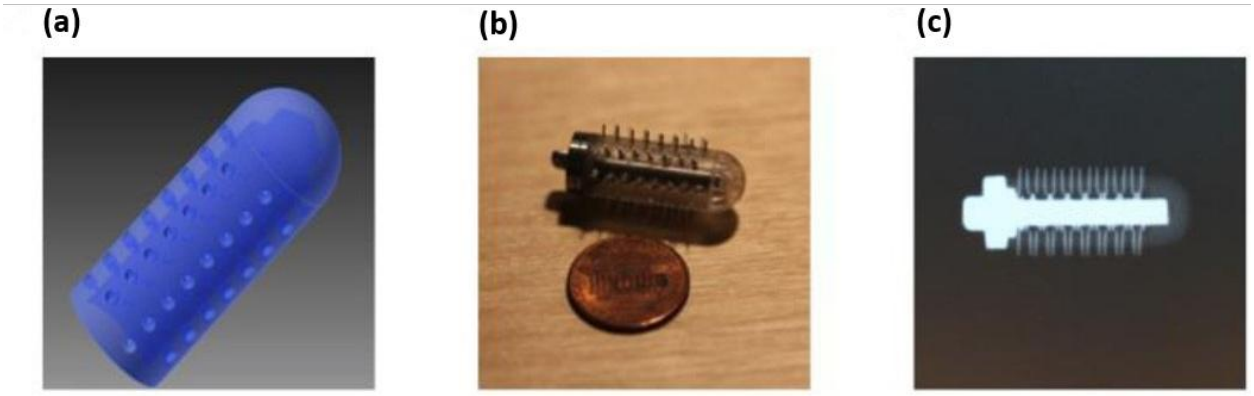


Figure 9. A cylindrical microneedle "pill" for the oral administration of biologic drugs. (A) Computer-aided design of the radial prototype housing used for in vivo safety evaluation. (B) Finished prototype used for in vivo safety showing the metal endcap and pin. (C) Radiography of the prototype in (B). Pill length 2cm, diameter 1cm, needle gauge – 25G (Adapted from [6]).

While it may at first seem that this technology will immediately revolutionise delivery of biological therapeutics, taking the needle out of the equation, caution must be exercised. The system relies on pressure within the lumen of the small intestine for insertion. This will clearly vary from one patient to another. Presence of food in the gastrointestinal tract will also lead to variation in penetration, especially if it is viscous or particulate (e.g. nuts) in nature. For a molecule such as insulin, which requires tightly-titrated dosing, such a delivery system could cause more problems than it solves. Should a patient have to take such "pills" regularly, variable absorption, and hence therapeutic response. is almost inevitable. Considerable further evaluation and development is clearly required before confidence in the safety, efficacy and reliability of this technology will be widespread.

5. Nail applications of microneedles

Due to its convenience, opportunity to avoid systemic side effects, improved patient compliance and dose sparing, topical therapy has been a preferable technique in treating various nail diseases, particularly onychomycosis and nail psoriasis [141]. However, the effectiveness of topical drug applications onto nails is restricted due to low permeability of the applied drugs across nail plate, defined by the plate's compact keratinized structure. Further, hindered drug permeation demands a prolonged presence of an applied drug formulation onto the nail plate, which often leads to the evaporation of a drug vehicle with the subsequent immobilisation of the drug. Thus, there is a need for facilitating techniques to achieve more efficient topical treatment of nail ailments [142].

As an example of such technique, facilitating to ungual drug permeation, Chiu et. al proposed an original method of microneedles application [7]. Thus, a commercially available dermaroller, with microneedles at 250 μm long, was applied to fingernail clippings by being rolled down and forth for 5 times. The procedure was followed by topical application of Nile Red (NR) loaded within 5,10,15,20-tetrakis-(4-aminophenyl)-porphyrin (PCL) nanoparticles, or (TAPP) - labelled PCL nanoparticles loaded with methoxycinnamate (OMC). OMC was used as a model for terbinafine, a conventional remedy for treatment of onychomycosis [143]. The NR-loaded nanoparticles were also topically applied to intact nails and the results obtained from the cases with microneedle-treated and intact nails were compared.

The depth of NR permeation from the PCL NPs through the nails was registered with laser scanning confocal microscopy (LSCM) and presented as a function of time (Figure 10). Thus, the permeation of NR across the nails treated with the dermaroller had a sbustantially higher rate in comparison with the intact nails. Further, a high intensity of NR fluorescence signal after a short-time from the start of the experiment (Figure 10 (b)), in the microneedle-treated nails, signifies a quick disposition of the NPs within the created micro-cavities. It was indicated that after 7 days of the experiment, NR released from the nanoparticles permeated through the nails treated with the dermaroller down to the depths of 70-90 μm (approximately 1/3 of nail's thickness), while the uptake of NR in the experiments with intact nails was negligible. The experiments with TAPP-PCL nanoparticles loaded with OMC demonstrated similar results to those described above for the NR-loaded nanoparticles. Thus, the nanoparticles were primarily deposited within the created micropores, with a subsequent release of OMC and its diffusion within the nail. At the same time, no permeation of the nanoparticles through the nail was observed. Consequently, the application of solid removable microneedles to nail created micro-cavities which acted as reservoirs for topically applied drug-loaded nanoparticles, providing a sustained release of the loaded drug during several days. These results indicate that the described

technique of nail poration, with a subsequent application of controlled drug delivery formulation, is a promising approach for further investigation.

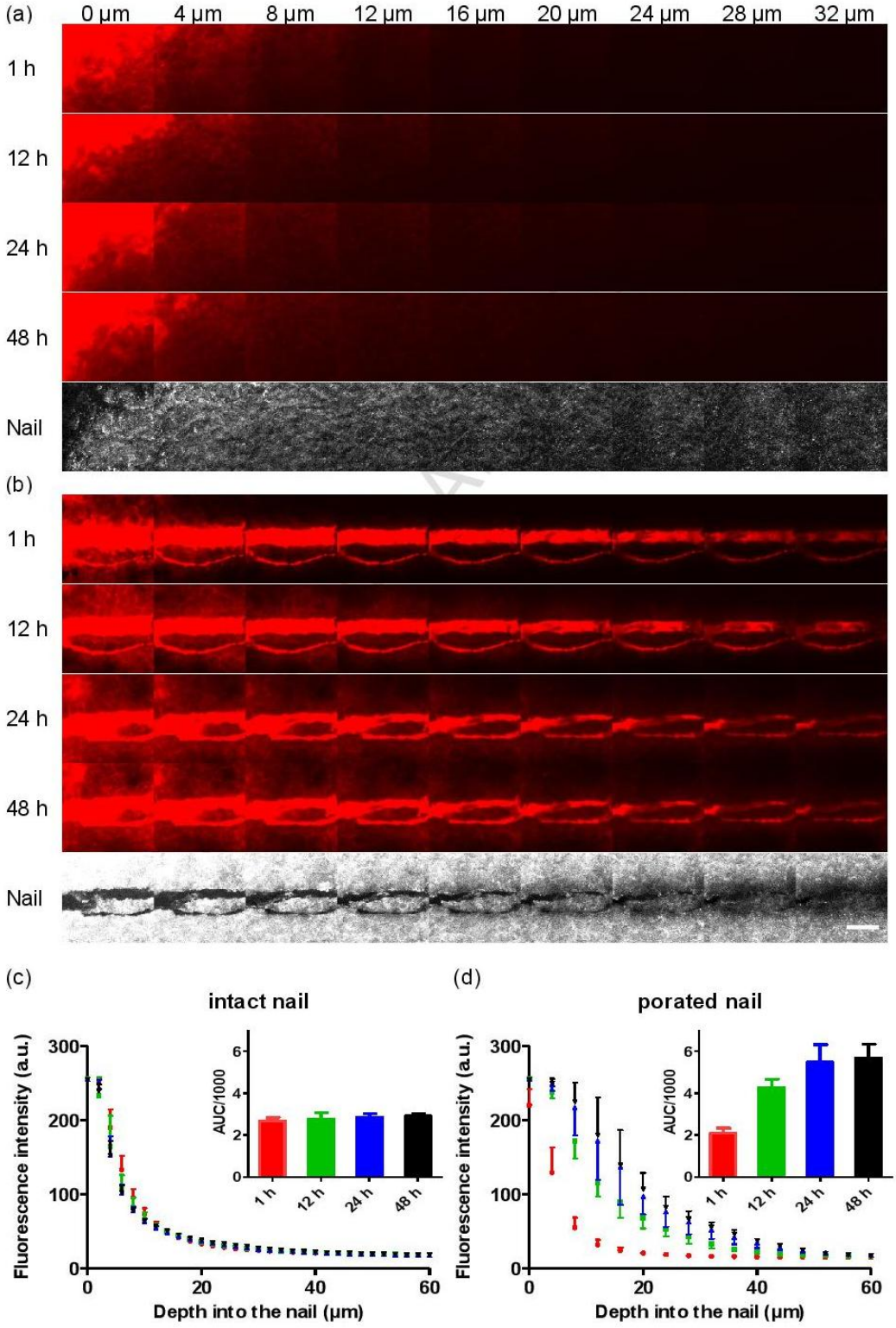


Figure 10. LSCM visualization of NR permeation across the nail, presented as the functions of time for intact (a) and microneedle-treated nails (b). Scale bar at 50 µm. Intensity of fluorescence versus nail depth for intact (c) and microneedle-treated (d) nails, presented as the functions of time (Retrieved with permission from [7]).

6. Summary and outlook

In 20 years, drug delivery via microneedles has grown from a novel fancy idea [144] into a wide and rapidly developing research field. Being first successfully applied to the skin, microneedles have been further proposed for ocular, oral mucosal, gastrointestinal and ungual drug delivery enhancement, and as the most recent advancement – vaginal [145, 146]. It is anticipated that the range of various microneedle-associated applications will be gradually growing, involving other tissues and organs. At the current stage, the focus is on commercialisation of microneedle devices and integration of the existing microneedle-associated drug delivery methods into a clinical practice for skin applications, and demonstration of effectiveness of the drug delivery within other organs and tissues.

To date, among the variety of diverse microneedle types and subsequent techniques of drug delivery, and variability of applications, only intradermal vaccine and drug delivery with the devices based on hollow microneedles such as MicronJet ® 600 (NanoPass Technologies Ltd, Ness Ziona, Israel) [147] and Soluvia ® (Becton Dickinson, NJ, USA) [148] has been approved by official regulatory authorities including FDA. In the field of percutaneous applications, there are several issues which determine the success of integration of microneedle-associated drug delivery, and the major of them are: patient/healthcare provider acceptability, patient safety, manufacturing and regulatory considerations, and clear evidence of consistent pharmacokinetics. Thus, the acceptability and safety of microneedle-mediated drug delivery primarily relies on such factors as reduced pain, stress and risk of transmitting infections and needle stick injuries, lower chance of misuse, feasibility for self-administration and improved acceptance for use in children. In the study by Birchall et al. [149], it was demonstrated that 100% of the public participants and 74% of the health care professionals considered the technique of microneedle-mediated percutaneous dug delivery prospective. Also, a high level of acceptability was determined for microneedle applications in children [150]. Further, Norman et al. revealed the possibility of consistent self-administration once appropriate instructions are provided [42]. However, despite high patient compliance, a variety of issues still have to be addressed to enable microneedle-based delivery systems to move closer to commercialisation. Thus, for more successful and convenient self-administration, the systems should be provided with indicators of a successful skin penetration and administration of an appropriate amount of the delivered drug, and with informative labelling containing the information about usage and disposal. Further, the applications of microneedles in pediatric patients are recommended to avoid any reference to "needles".

From a regulatory point of view, even though microneedles are considered minimally invasive and relatively safe, many safety aspects still should be addressed. Thus, it is known that microneedle applications result in disruption of skin barrier, by creating micro-pores in SC and micro-conduits in deeper skin layers, which recovers within few hours after microneedle removal [97, 151] preventing microbial penetration within the skin [152]. However, there is no information on the effect of multiple microneedle applications on skin barrier, which is especially relevant for the cases when microneedle devices of a multiple microneedle arrays are used to provide local drug delivery and therefore can be applied only to a limited skin area. The investigations on barrier disruption and restoration are necessary in the fields of microneedle applications to other organs and tissues, as due to lower regenerative abilities of the related barriers the results are anticipated to be less positive in comparison with skin. It will be important if all types of microneedles are considered as injections rather than any other kind of delivery systems, since this will determine whether the final product will need to be sterilised and prepared under aseptic conditions. Possible allergic reactions related to microneedle applications are also of a great interest for the industry and researchers. Thus, there is a significant concern on possible side effects of polymers which reside within the skin, particularly for the scenario with dissolving microneedles as it leads to deposition of microneedle matrix within the tissue and its accumulation in case of multiple applications. Further, few cases on transdermal drug delivery enhanced by the application of solid removable microneedles were reported [153]. In the reported cases, topical application of cosmeceuticals onto the skin treated with solid removable microneedles resulted in hypersensitivity reactions, which to our opinion were caused by such factors as: the lack of medical supervision, unsterile nature of the applied formulations and the tremendous rate of enhancement of transdermal delivery of multiple excipients presenting in the formulations native for the scenario with solid removable microneedles (Figure 1) [154, 155].

A lack of understanding of pharmacokinetics of the delivered drugs, and therefore impossibility to predict their desirable and side effects, is another significant cause of a relatively slow integration of microneedles as the technique of drug delivery enhancement. Even though an efficient microneedle-mediated percutaneous drug delivery was demonstrated in large number of *in vitro*, *in vivo* and clinical studies, there is still not enough highly reliable evidence for massive integration of the technique into a practical use. Thus, there is a strong demand for objective mathematical models which would provide an understanding of how the delivered drugs act, being administered with that or another type of microneedles in diverse tissues and organs. An

important progress towards such understanding was recently achieved by Rzhevskiy et. *al* [156] with the development of straightforward and convenient model, and subsequent equation, describing transdermal delivery of topically applied drug through the skin treated with solid removable microneedles. Such model may be adopted for the cases, which satisfy the scenario for solid removable microneedles, with tissues other than skin.

Another important issue which demands deeper investigation, to improve the range of practical usability of microneedles for drug delivery, is related to the optimisation of microneedles' design [157, 158]. Currently, most of the studies in this field are again related to skin applications. Such studies can be divided into two groups: the group of fundamental investigations relevant for microneedles of different types, and the group of investigations specific for certain type of microneedles. The group of fundamental investigations address the problem of most successful skin piercing when microneedles are applied with an adequate force [88] and are made of an appropriate material [159]. Thus, it was found that the depth of microneedle insertion depends on such interrelated parameters as microneedle density [98, 160] and their distribution over a supporting base [161], length, tip radius, tip angle and base radius [162, 163]. A successful insertion of microneedles determines the amount of drug released from biodegradable [164] or coated microneedles, successful microporation of a tissue barrier for the scenario with solid removable microneedles, and prevention of leaking when the delivered drug is administered via injection through hollow microneedles. One more significant issue is the estimation of mechanical strength of microneedles as there is a possibility of needle fracture after being inserted into a tissue [165], which is especially important for the cases with microneedles of non-dissolving materials as their failure within the tissue in in vivo or clinical applications may lead to a serious harm. For the scenarios with coated [89] and dissolving microneedles [166, 167], spatial properties are primarily investigated in terms of their effect on sustained drug release. It's worth mentioning a recent study by Romgens et al. [168] where an optimal design for the patch with vaccine-loaded dissolving microneedles, providing the most effective induction of Langerhans and dendritic cells within the skin and therefore successful immunization, was modelled.

Despite the substantial success achieved in understanding of the effect of microneedles' design on drug delivery enhancement across the skin barrier, there are numerous challenges which have to be addressed for applications to diverse organs and tissues. For skin applications, such challenges are mainly related to the optimization of design for hollow and hydrogel-forming microneedles. Thus, even though it was demonstrated that hollow design provides a microneedle additional weakness and may lead to tissue occlusion of a bore opening, there is still not enough

of published evidence regarding the most optimal design for hollow microneedles [85]. At the same time, little is known about the influence of design on drug delivery enhancement for the case with the newest – hydrogel-forming type of microneedles. For applications to other organs and tissues, the investigation of relations between microneedle design and character of drug delivery enhancement is at its very beginning. It is obvious that the experience and knowledge obtained from the studies related to application of microneedles to skin should be taken into account for progressive development of the new trends in microneedle-mediated drug delivery, as the challenges which have to be addressed are in general similar to those for skin. At the same time, investigations of the drug delivery to a particular tissue have to be considered in a context with its specific anatomical and physiological properties.

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