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REVIEW ARTICLE

Non-dermal applications of microneedle drug delivery systems

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Abstract
Microneedles (MNs) are micron-scaled needles measuring 100 to 1000 μm that were initially explored for delivery of therapeutic agents across the skin. Considering the success in transcutaneous drug delivery, the application of microneedles has been extended to different tissues and organs. The review captures the application of microneedles to the oral mucosa, the eye, vagina, gastric mucosa, nail, scalp, and vascular tissues for delivery of vaccines, biologics, drugs, and diagnostic agents. The technology has created easy access to the poorly accessible segments of eye to facilitate delivery of monoclonal antibodies and therapeutic agents in management of neovascular disease. Microporation has been reported to drastically improve the drug delivery through the poorly permeable nail plate. Curved microneedles and spatially designed microneedle cuffs have been found to be capable of delivering stem cells and therapeutic macromolecules directly to the cardiac tissue and the vascular smooth muscle cells, respectively. Besides being minimally invasive and patient compliant, the technology has the potential to offer viable solutions to deliver drugs through impermeable barriers owing to the ability to penetrate several biological barriers. The technology has been successful to overcome the delivery hurdles and enable direct delivery of drug to the target sites, thus maximizing the efficacy thereby reducing the required dose. This review is an attempt to capture the non-dermatological applications of microneedles being explored and provides an insight on the future trends in the field of microneedle technology.

Keywords Microneedles · Transmucosal · Ocular · Transungual · Vascular

Abbreviations

MN	Microneedles
BSA	Bovine serum albumin
OCT	Optical coherence tomography
VADDS	Vaginal mucosal adjuvant dual delivery system
HIV	Human immunodeficiency virus
HSV	Herpes simplex virus
GI	Gastrointestinal
PLGA	Poly lactic co glycolic acid

Introduction

After oral or parenteral administration, drug would typically get distributed in the plasma that constitutes the central compartment. The drug in the plasma that constitutes the central compartment would subsequently get distributed to other tissues or organs that comprise the peripheral compartment at a specific rate until equilibrium is achieved. Generally, the rate of drug distribution to most peripheral tissues or organs is limited by poor regional blood flow and low tissue perfusion rates. In addition, the extent of drug distribution or the regional bioavailability is constrained by drug's tendency to bind to tissue or plasma protein binding, its lipophilicity, and degree of ionization (pKa) [1]. Owing to these hurdles, drug distribution to peripheral organs or tissues following systemic administration is substantially hampered. At the same time, drug distribution to the peripheral tissues is considerably hindered as the biological membranes generally act a formidable barrier to delivery of topically applied therapeutic agents and macromolecules. Thus, systemic delivery involving oral or parenteral route invariably needs a

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high dose to be administered in order to achieve and maintain effective local drug concentrations that usually is associated with off-target systemic adverse effects. Considering the need, attempts are made to breach the epithelial or the endothelial barriers using microneedle technology to directly deliver the drug to the distant target tissue or organs.

Microneedles (MNs) are micron-scaled needles that were initially explored for delivery of therapeutic agents across skin. Microneedle patches generally contain arrays of needles that are typically 100–1000 μm in length. These patches have been successful as a physical enhancement strategy to improve the transdermal drug delivery [2]. Microneedles offer various advantages with respect to hypodermic needles as shown in Table 1. The technique was known to facilitate the delivery of macromolecules and hydrophilic therapeutic entities across the stratum corneum that forms a formidable barrier to permeation of such drugs [2, 3] skin drug delivery is the stratum corneum which forms the outermost layer of the skin that measures about 10–20 μm in thickness. Microneedles were found to offer viable solution for permeation of such therapeutic agents across the stratum corneum enabling local accumulation and eventually systemic delivery. Microneedle represents a minimally invasive, commercially feasible, and easy-to-use technique to deliver therapeutic agents as a potential attractive alternative for invasive parenteral administration. Microneedle patches were found to penetrate the outermost layer of the skin, making micron size pores in the stratum corneum, thus enabling the entry of therapeutic macromolecules and other drugs into body. These micron size pores created during application of MNs are small enough to be painless and at the same time avoid any possible irritation [3, 4]. There are five different types of microneedles available: solid microneedles, dissolving microneedles, hollow microneedles, coated microneedles, and hydrogel-forming microneedles as shown in Fig. 1. Solid microneedles are used to create pores in the stratum corneum, which would be subsequently followed by application of drug [5, 6]. These pores created by the solid microneedles improve the permeation flux of the drug. Solid microneedles have served as a rising platform for the advent of other types of microneedles available today. On the other hand, hollow microneedles on insertion into the tissue deliver the drug through the central channels present in the microneedles. In contrary, coated microneedles, as the name suggest, comprise drug present in the surface which is released once the microneedle is inserted into the tissue. Finally, dissolving microneedles are made of up of soluble materials, that on insertion into the skin dissolve releasing the entrapped drug. Most recently, hydrogel-forming needles are being extensively explored for several other applications [7, 8]. These MNs comprise crosslinked drug-loaded hydrogels, mounted on an adhesive patch. On application of these microneedles onto the skin, they tend to swell in contact with the aqueous media of skin, creating a hydrogel that facilitates the influx of drug into the tissues [9, 10]. MNs can be made up of different materials such as water insoluble materials like metals, ceramic, or water soluble materials such as saccharides and polymers. In recent years, extensive investigation has been carried out by researchers to exploit the potential of microneedle technology to deliver drugs to other organs and tissues such as the oral cavity, nails, eyes, scalp, and muscular delivery that otherwise would require the use of injections as shown in Fig. 2. Based on the biopharmaceutical property of the drug, a portion of drug may tend to bind to the target tissue while a fraction of the same is prone to pre-systemic metabolism. The rest of the drug is likely to enter the systemic circulation. This review is an attempt to summarize the progress that has been achieved by microneedles in drug delivery to the other tissues and organs apart from the skin (Table 2) [11, 12].

Microneedles for oral mucosal delivery

The oral cavity that is lined by oral mucosa basically comprises stratified squamous epithelium that forms the upper one fourth to one third of the oral mucosa. Oral epithelium is a complex microbial, chemical and immune barrier that ensures protection to the underlying tissues. The thickness of the oral mucosa varies depending on the site of the epithelium. Underlying the epithelium are the basement membrane and lamina propria and submucosa that is rich in blood vessels and nerve endings. The thickness of the oral mucosa varies from one site to another. It is about 500–600 μm thick in the buccal region whereas it reduces to a maximum of 200 μm thick in the sublingual region. Microneedles have been also explored for vaccination, therapeutics, and for diagnostic purpose.

Table 1 Advantages of microneedle technology in drug delivery

	Hypodermic needles	Microneedles
Pain	Painful	Painless; faster healing at site of application compared to hypodermic needles
Patient compliance	Poor	Highly compliant
Administration	Requires trained personnel for administration	Could be self-administered

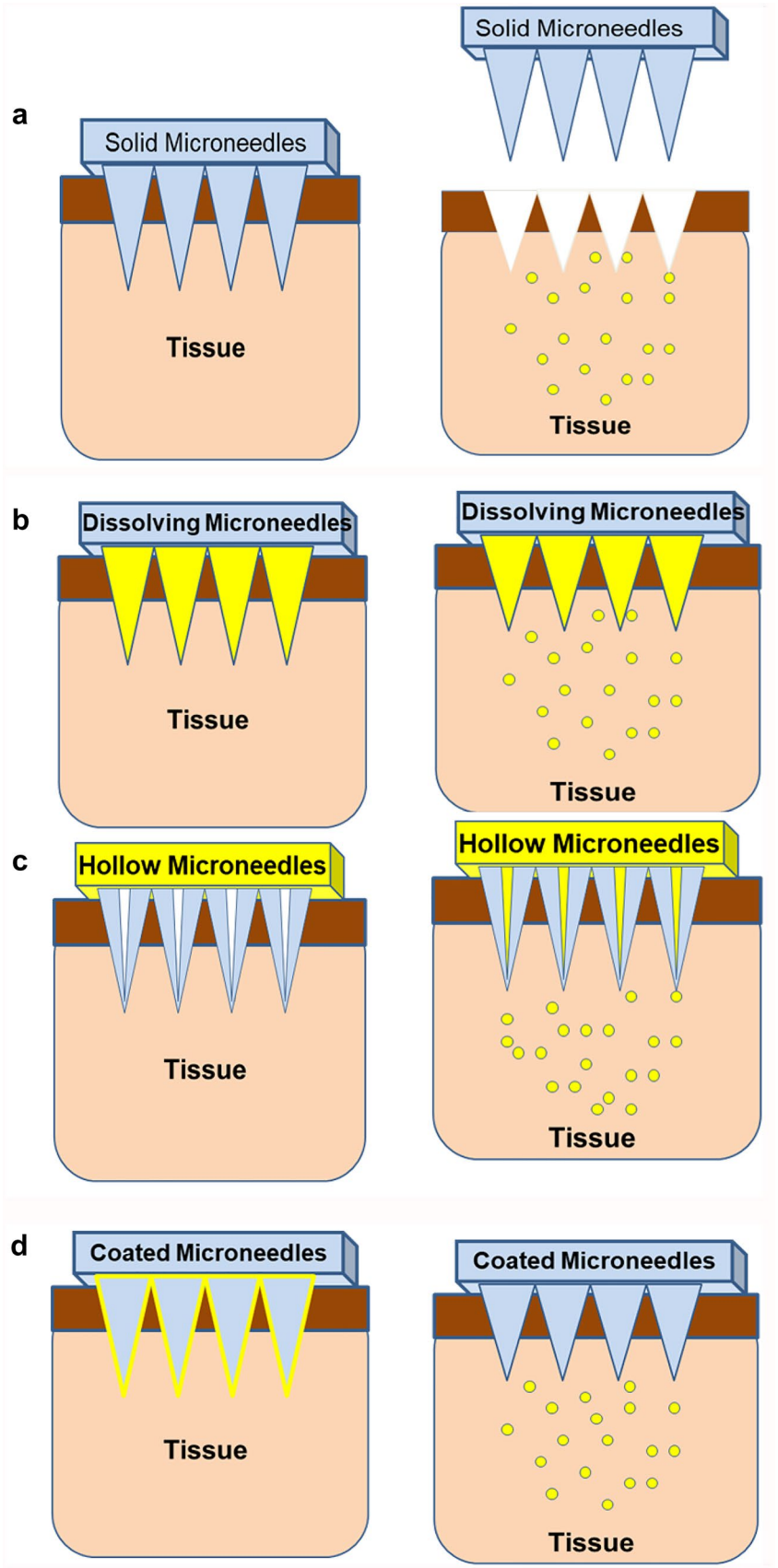

Fig. 1 Types of microneedles. **a** Solid microneedles are generally made of metals or insoluble polymer to create pores in the skin. **b** Dissolving microneedles, where the polymeric microneedles would dissolve in the tissue fluid and release the drug. **c** Hollow microneedles are meant for injection of formulation. **d** Coated microneedles have the drug coated on the surface of the polymeric or metal microneedles which get released in the tissue fluid

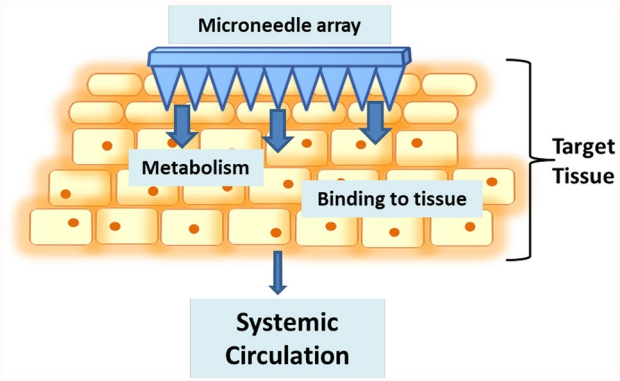

Fig. 2 Schematic representing the fate of drug post microneedle application

Microneedles for vaccination

Oral mucosal surface is the primary site that is prone to microbial attack as it gets directly exposed to external environment. Vaccine delivery to the oral mucosa would be highly favorable as most of the pathogens enter the body through the mucosa. Moreover, delivery of vaccine to the buccal or sublingual mucosa would elicit a strong immune response at distal sites such as urogenital, respiratory, and intestine. However, aqueous formulations of the vaccine can only be taken up by the Langerhans cells present in the superficial layers of oral submucosa thereby eliciting a poor immune response. On the contrary, microneedles have proved to be a promising tool for oral vaccination as they can easily access the dendritic cells and Langerhans cells residing in the upper few hundred micrometers in order to generate a strong immune response. Moreover, microneedles are known to improve the residence time and permeation through the epithelium thereby facilitating efficient delivery of vaccine. In addition, microneedles are known to ensure precise depth of penetration and dosing. Coated microneedles measuring 700 μm were used to deliver HIV antigens to the oral mucosa [13]. The systemic and mucosal immune response was assessed by measuring the IgG and IgA levels in serum and saliva, respectively, after inserting the microneedles into the inner lower lip and dorsal surface of the tongue in rabbits. The microneedles were able to elicit comparable antigen-specific IgG levels in serum although significantly higher antigen-specific IgA response was noted in saliva [13]. Microneedles containing stabilized liposomal formulation were developed for oral mucosal delivery of hepatitis B virus by Wang et al. High levels of specific immunoglobulin G were observed in serum, saliva, intestinal, and vaginal sections after a single application of the microneedles to oral mucosa in mice. It was observed that the microneedle systems could be used to induce multi-modal immunity against hepatitis B infection on a single application in mice. On similar lines, bovine serum albumin (BSA) antigen was entrapped into a stabilized liposomal formulation that was incorporated into microneedle system. On insertion into the oral mucosa of mice, robust systemic and mucosal immune responses were elicited against the BSA [13]. Microprojection array measuring 110 μm with dry-coated influenza vaccine was designed to immunize at the mucosal surface of the mouse model [14]. The projections when inserted on a buccal mucosa were easily able to access the antigen presenting cells to release the vaccine in the proximity. The resultant systemic immune response elicited was similar to the systemic immunization but superior to conventional oral immunization. Microneedles constituted by multifunctional liposomes loaded with model bovine serum albumin as a

Table 2 Application of microneedles to diverse tissues and organs

Sl. No	Type of microneedles	Target tissue	Application
1	Coated	Oral mucosa	Vaccination [13, 14]
	Coated		Systemic chemotherapy [17]
	Microneedle pretreatment		Delivery of diagnostic agent[18]
2	Coated	Anterior segment of eye	Delivery of marker by intrascleral route to anterior segment of eye [23]
	Hollow	Posterior segment of eye	Delivery to suprachoroidal space [29]
	Biodegradable		Drug delivery [32]
3	Dissolving	Vaginal mucosa	Delivery of antigen [35] and antiretrovirals [36]
4	Hollow	Gastrointestinal tract	Delivery of biological [38]
	Solid		Delivery of biological [38]
5	Biocompatible	Cardiac	Delivery of stem cells
6	Microneedle pretreatment	Scalp	Drug delivery [45–47]
7	Microneedle pretreatment	Nail	Drug delivery [51]
8	Biodegradable	Vascular	Drug delivery [55, 56]

model antigen were applied to oral mucosal route in mice model. The microneedles measuring 660 μm in height were found to elicit a robust immune response as noted by high levels of immunoglobulin A (IgA) in systemic, salivary, intestinal, and vaginal secretions [15]. The studies indicated that the microneedles were found to exhibit superior immunization efficiency owing to the minimal loss and ability to access the deep seated antigen presenting cells. Microneedles consisting of multifunctional liposomes constituting stable mucosal vaccine were designed against hepatitis B. Application of microneedles to oral mucosa was found to induce robust systemic and wide spread immune response in mice model as noted with high Ig A levels in saliva, vaginal, and intestinal secretion [16].

Microneedles for drug delivery and diagnosis

Microneedles have evolved as an attractive option to systemic chemotherapy as they would ensure delivery of the anticancerous drugs to the target sites. Microneedles thus enable dose reduction of antineoplastic agents that are invariably associated with several side effects. The microneedle technology would minimize the systemic exposure and the associated systemic and off-target side effects of chemotherapeutic agents. Ma et al. also evaluated doxorubicin-coated microneedles as a minimally invasive treatment strategy for treatment of oral carcinomas. The drug was initially encapsulated into poly (lactic-co-glycolic) acid (PLGA) nanoparticles before being coated on microneedles. The in vitro evaluations suggested that these coated microneedles measuring 3 mm in height were able to deliver 0.6 μg of drug which was proven toxic enough for cancer cells within a 1-cm^3 area [17]. These studies carried out demonstrated the potential of microneedles for oral vaccine delivery and management of oral cancers.

Microneedles have been employed as diagnostic aids in the management of oral cancers. Optical coherence tomography (OCT) is used as a valuable noninvasive tool to diagnose oral cancer. However, gold nanoparticles, used as contrast agent in OCT imaging, fail to penetrate through the oral mucosal barrier affecting the efficiency of the imaging technique. Dual treatment with microneedles and ultrasound was found to improve the penetration and distribution of gold nanoparticles in induced dysplasia of the oral mucosa of hamsters [18]. It was observed that microneedle pretreatment followed by application of ultrasound on topically applied nanoparticle formulation demonstrated a multi-modal delivery of antibody conjugated PEGylated gold nanoparticles that enhanced the contrast of OCT images of the oral cancer. The combination strategy was found to clearly identify pathological structures of early stages of cancer. But the possible risks of systemic infections and drug dissipation in saliva present in the oral mucosa need to be further explored for such delivery systems [19, 20]. The studies provided vital leads to further explore the possibility of the microneedle technology to get translated into commercial use.

Microneedles for ocular delivery

Ocular diseases generally involve ailment in the anterior segment and/or posterior chamber of the eye. Diseases of the anterior segment such as corneal neovascularization, glaucoma, bacterial or fungal infection blepharitis, and herpes simplex keratitis can cause serious impairment of vision. Ocular diseases affecting the posterior segment of the eye like age-related muscular degeneration, diabetic muscular edema, chorioretinal diseases, diabetic retinopathy, or cytomegalovirus retinitis usually can cause permanent loss of vision if left untreated. The ailments that affect the posterior chamber of the eye are more sight threatening compared with those affecting the anterior chamber.

Microneedles for drug delivery to anterior segment of eye

The lacrimal turnover rate (1 μl/min) and nasolacrimal drainage is the primary limiting barrier to delivery of the drugs to the anterior segment. Moreover, cornea that is the outmost layer of the eyeball acts as a physiological barrier to drug delivery. In addition, absorption of the drug by conjunctiva hinders the drug delivery to the anterior segment of the eye. [21]. Several products have been introduced into the market that can prolong the retention of the drug in the precorneal space and improve the ocular bioavailability. Lipophilic esters and transporter targeted prodrug approach have been employed to enhance the ocular absorption. Abundance of peptide transporters on the corneal epithelium has enhanced the scope to develop peptidomimetic prodrugs that can improve the ocular bioavailability. Prodrugs like valacyclovir and valganicyclovir have revolutionized the era of peptide prodrug design targeting oligopeptide transporters. Formulation approaches such as incorporation of viscosity imparting mucoadhesive pseudoplastic polymers in ophthalmic products are found to be practically feasible to improve the residence time of the therapeutic agents in the precorneal space in view to increase the ocular absorption. Attempts have been made to develop novel drug delivery systems such as ocular inserts, nanosuspensions, nanoemulsions, liposomes, dendrimers, and drug polymer conjugates with the aim to enhance the drug retention time in the precorneal space. These systems offer the advantages of sustained drug release, evade the drug efflux, enhance the precorneal drug retention time, and thereby reduce the dosing frequency [22]. An attempt to deliver the marker sodium fluorescein into the anterior chamber was made using individual stainless steel microneedles. Coated microneedles

measuring 500–750 μm were evaluated for delivery into the anterior chamber of the eye administered either by intrascleral route [23]. The coated microneedles were inserted halfway into the cornea of rabbit eye and left for 2 min before retrieval. The microneedle coat was primarily dissolved first into the anterior eye chamber followed by a gradual release into the anterior eye chamber. A sharp increase in the concentration of the marker was noted in 1 min that peaked in 3 min and reached the baseline values in 24 h. The study indicated that the microneedles were able to increase the concentration of the marker by nearly 60-fold compared with topical application. In the same study, it was noted that pilocarpine-coated microneedles were able to increase the drug concentration by nearly 45-folds when compared with topical application.

Microneedles for drug delivery to posterior segment of eye

Delivery of the drug to the posterior segment of the eye continues to be a challenge in treating diseases associated with the posterior segment of the eye owing to the poor accessibility, delicate nature of the eye, and barrier properties of the ocular tissue. The standard treatment for management of diseases involving posterior segment would be an intravitreal injection that involves direct administration of medication into the space in the vitreous cavity using hypodermic needle [24, 25] However, hypodermic injections are invasive causing discomfort, pain, and damage to the tissues present in the eye. Additionally, intraocular injections are challenging as they warrant sterile clinical settings and need skilled clinicians to avoid infections. Generally, surgical implantation of controlled release preparations is invasive and is considered only in absence of effective alternative procedures. As a promising option to deliver the drugs to the back of the eye, microneedles have been proposed in recent years as a potential alternative to the current invasive techniques [26]. MNs were demonstrated to be the practically feasible approach to the posterior segments of the eye. Being minimally invasive, microneedles reduce the pain sensation, damage to the eye tissues, and possibility of any bacterial infections. Also, MNs enable self-administration leading to improved patient compliance by decreasing discomfort during and post application.

An attempt was done to employ hollow microneedles measuring 200–300 μm for delivery of sulforhodamine B solution or particles into sclera of the human cadaver eye. Histological observations indicated that on insertion of dye-loaded microneedles into the human cadaver scleral tissue for 20 s, a rapid dissolution of dye followed by a depot formation within the scleral tissue was observed [27]. The studies indicated that hollow microneedles have been useful in the intrascleral delivery of drugs. Attempts were also done in the same study to deliver nanoparticles and microparticles into the sclera using hollow microneedles. A 33G hollow microneedle measuring 850 μm was used to deliver triamcinolone acetonide to the suprachoroidal space. A dose ranging from 0.2 to 2 mg was as effective as high-dose intravitreal injection in reducing the inflammation in the posterior segment of the eye. Moreover, the microneedle administration was found to be safe and devoid of any adverse effects and toxicity [28]. Patel et al. used hollow microneedles as a suitable option for delivery of particulate system into the suprachoroidal space of the eye. Drug delivery into the suprachoroidal space of excised rabbit, pig, and human eyes was achieved by hollow microneedles having a length of 750 μm ex vivo [29]. The studies indicated the possibility of delivering solution or particulates to the back of the eye. A preclinical trial involving bevacizumab-coated stainless steel microneedles measuring 400 μm in rabbits was carried out to compare the microneedle delivery with subconjunctival injection and topical application. The aim of the study was to determine the effective dose of bevacizumab required to treat corneal neovascularization using microneedles [30]. It was noted that 4.4 μg of the drug delivered through microneedles had the same therapeutic effect as that obtained with 2.5 mg subconjunctival injections and 52.5 mg of eye drops. The study clearly indicated that microneedles have the potential to substantially reduce the dose if directly applied to the eye using hollow microneedles.

A pilot study in rabbit has undertaken the toxicity profile of biodegradable microneedles of methotrexate [31]. Methotrexate microneedles measuring 2 mm in length were designed using poly (D, L-lactide) by solvent casting technique. The microneedles designed were evaluated for their degradation and toxicity profiles in rabbit model. The histopathological studies indicated that the biodegradable microneedles proved to be a viable option for vitreoretinal lymphoma. No adverse evidence of ocular inflammation or infection was noted on insertion into to the eye demonstrating the safety of the microneedles. The feasibility of using polyvinylpyrrolidone to fabricate dissolving microneedles was determined by observing the microneedle penetration into the ocular tissues. A significant increase in permeation across the scleral and corneal tissues was observed compared with topical administration of eye drop solutions. An eye patch equipped with detachable microneedles displayed the ability to penetrate the ocular surface tissue and deliver the drug in a controlled fashion [32]. The microneedle patch measuring 500 μm was loaded with anti-angiogenic monoclonal antibody that was designed by micromolding technique using sodium hyaluronate as polymer. The microneedles were found to display biphasic release kinetics. Delivery of the antibody was found to reduce the neovascular area by almost 90% in a neovascular disease-induced mice model. Hollow microneedle was

proposed for targeted delivery of antiglaucoma drugs into the supraciliary space [33]. A 33-gauge hollow microneedle measuring about 700–800 μm was used to infuse the drugs into the supraciliary space in rabbit model. The drugs sulprostone and brimonidine were delivered using the microneedles and topical application, and the intraocular pressure was monitored by rebound tonometry. Supraciliary delivery of the drugs was able to reduce the intraocular pressure by 3 mm Hg with reference to topical application enabling dose reduction by nearly 100-fold. However, like other ophthalmic preparations, the microneedles would warrant terminal sterilization prior to application to the eye to ensure the safety of the technology.

Microneedles for vaginal delivery

Vaginal mucosa is found to be suitable site for vaccination as it can prevent sexually transmitted pathogens. The other reasons for its suitability would be its large surface area, easy accessibility, and less safety concerns compared with other mucosal sites [34]. Vaginal mucosa is lined by stratified squamous epithelium that forms a tough barrier owing to tight junctions. The tightly packed cells are likely to substantially hinder the topically applied vaccines to come in contact with the antigen-presenting cells that are responsible to elicit an immune response. Moreover, the vaginal mucosa is covered by a layer of mucus that gets constantly renewed that would further prevent the access of the topical vaccines to the deep seated antigen-presenting cells. In addition, the mucus comprises lysosomal enzymes, proteins, and glycoprotein that would deactivate the topically applied vaccines.

Microneedles for vaccination

Mucosal vaccination is known to stimulate the body's immune system to produce not only sufficient pathogen-neutralizing antibodies but also sufficient pathogen-specific T lymphocytes. The mucosal-associated lymphoid tissue (MALT) comprises on interlinked mucosal compartments that are rich in antigen-presenting cells like the dendritic cells and macrophages. One of the most recent advancement in the field of drug delivery through microneedles has been through the vaginal route of drug administration. The vaginal tissues can be exploited as a portal for vaccination to prevent sexually transmitted diseases like human immune deficiency virus (HIV) [34]. Microneedles were used as a platform to deliver multifunctional liposomes loaded with a model antigen into the vaginal cavity. The microneedles when applied to the vaginal mucosa in mice were found to elicit a robust antigen-specific immune response in the reproductive and gastrointestinal mucosa. The liposomes were likely to be taken up by the vaginal mucosal dendritic cells or transferred directly to the draining lymph nodes and get eventually picked up by the macrophages. The studies indicated that microneedles would be effective vaginal mucosa adjuvant dual delivery system (VADDS) which could be helpful in clinical use for protection against infectious pathogens [35].

Microneedles for intravaginal drug delivery

Dissolving microneedle patch constituted of long acting nanosuspension of rilpivirine was developed. The designed microneedles were found to be discrete and mechanically strong [36]. The in vivo studies performed by the same research group in rats indicated displayed a mean plasma concentration (116.5 ng/ml) at the 56-day endpoint when inserted in the vagina that was comparable with intramuscular injection (118.9 ng/ml). Rilpivirine was detectable systemically, in the lymph and in the vagina indicating the potential of the microneedle patch to deliver the therapeutic levels of drug to the primary sites of viral replication. These observations indicate the potential of the microneedles as a possible platform for pre-exposure prophylaxis against human immunodeficiency virus (HIV) [37]. Microneedle patch has the potential to improve the patient acceptance and adherence to the treatment regimen as they can avoid the instances of needle-stick injuries and blood-borne diseases. Application of microneedles for vaginal drug delivery is still being explored at this stage with insights still needed on mucosal irritation and toxicity along with user feasibility studies that would enhance this microneedle application furthermore.

Microneedles for gastrointestinal tract delivery

Oral route for drug administration has been a preferred route for both patients as well as health care professionals. However, the oral bioavailability of several biologicals would be limited due to poor absorption across gastrointestinal tract. The poor bioavailability of these biological agents can be attributed to the variation in the pH prevalent in the gastrointestinal tract. Moreover, presence to protease and bacteria-rich environment of the tract curtails the oral bioavailability. Considering these constraints, attempts are made to administer biologicals using microneedles. Microneedles act as a platform for the oral delivery of broad range of drugs by penetrating the endothelial barrier of the GI tract in a swine model. Traverso et al. were the first to demonstrate a proof of concept using microneedles for delivering biologicals through the gastrointestinal tract [38]. The acrylic microneedle device that had 25 G needles projecting 5 mm from the surface was fitted into a pill that was 2 cm long and 1 cm in diameter contained. A radio opaque central metallic core was embedded to enable

radiographic detection of the microneedle device. The pill containing hollow or solid microneedle device was carefully positioned in the stomach of the animals under endoscopic radiographic visualization. The retention time of the microneedle pill was determined till it was no longer visible on radiographs for a period of 48–72 h. In case of hollow microneedles, the drug reservoir was compressed owing to peristaltic movement of the gastrointestinal tract releasing the drug. On the other hand, in case of solid microneedles, the device broke down into the constituent needles that in turn were found to penetrate the gastrointestinal tissue to release the drug in a controlled fashion. The study clearly demonstrated the ability of microneedles to improve the bioavailability of orally administered drug. Although using microneedles for drug delivery through the gastrointestinal tract has potential, many aspects need to be investigated before it makes its way into the clinic. Exploratory novel formulations including polymer-based systems will be essential in maximizing active pharmaceutical ingredient profile in terms of stability and functionality. The needle geometry could play a pivotal role in determining the residence time inside the gastrointestinal tract and in providing an extended release profile. Moreover, the potential tissue damage that could likely be caused during the transit through the gastrointestinal tract on chronic administration warrants thorough elucidation prior to clinical investigation.

Microneedles for cardiac delivery

No approved therapies are available till date to reduce the size of heart scar developed during coronary heart disease like myocardial infarction. Stem cell therapy has evolved as one of the attractive therapeutic options to treat myocardial infarctions. Randomized phase 1 clinical trials have indicated that cardiac stem cells are likely to offer benefit in mild to moderate myocardial infarction [39]. The stem cell therapy is said to regenerate the viable myocardial tissue and decrease the scar size [40]. However, one of the major limitations of the cardiac stem cell therapy is extremely low cell retention rate post delivery. Nearly 90% of the injected cells are known to get cleared off within 24 h, irrespective of the route of administration that may be in the form of intracoronary or intramyocardial injection [41]. In this context, polymeric microneedle patch integrated with cardiac stromal cells for therapeutic heart regeneration after acute myocardial infarction [42]. The microneedle patch was designed using biocompatible poly (vinyl alcohol) by micromolding technique. The 144-mm^2 microneedle patch was fabricated to have a 400 microneedle array. The microneedles integrated with the stromal cells were found to be biocompatible and non toxic to cardiomyocytes in rats. The microneedles had a base diameter of 300 μm and a height of 600 μm. The microneedle displayed a good mechanical strength of ~ 2 N per needle that enabled sufficient penetration without breaking. When implanted surgically onto the heart, the microneedles were found to provide a portal of entry to enable the regenerative factors to easily diffuse from the cardiac stem cells to repair the infracted heart. Surgical transplantation of stem cell-loaded microneedles to rats with acute myocardial infarction was found to promote angiomyogenesis, reduce the scar size, and thereby augment cardiac functioning. Application of microneedle patch by open heart surgery following acute MI in porcine was found to preserve the cardiac function without inducing any toxicity. However, since the method employed in the study was invasive, the possibility of using minimally invasive approaches needs further exploration to deploy the microneedle patch on the surface of the heart.

Microneedling for delivery to the scalp

Microneedling was primarily investigated for as a viable and practical option to treat hair loss by creation of micron-sized holed using a device called dermaroller. Dermaroller was the first microneedle device developed by Dr Desmond Fernandes (Dermaroller Deutschland GmbH, Wolfenbüttel, Germany) primarily used for application to the skin. A scalp roller on the contrary is known to utilize titanium needles unlike the stainless steel needles used in dermaroller [43]. Unlike the conventional dermarollers, the optimal length of microneedles to facilitate drug delivery through the scalp is 1.2 to 1.5 mm [44]. The device is made available in variety of designs for vivid applications in drug delivery. The technique is basically known to activate the stem cells in the hair bulge area of the scalp by platelet activation and skin wound generation that eventually releases the hair growth-related gene vascular endothelial growth factor and therefore the hair growth. Microneedling is hypothesized to enhance the growth factors by enhancing the collagen and elastin synthesis. A pilot clinical study has indicated that microneedling was found to restore the scalp hair in subjects with male alopecia. The mechanism underlying hair re-growth by application of scalp roller involved stimulation of the follicular stem cells and activation of growth factor in dermal papillae [45].

Microneedling of the scalp can create pores in the stratum corneum of the scalp thereby improving the permeation flux of applied therapeutic agents. However, few papers indicate that combination of microneedling with topical application works well compared with topical application alone. A 12-week randomized blinded study was conducted to assess the effectiveness of microneedle treatment in alopecia areata [45]. About 50 patients each were allocated into two groups consisting of microneedling group and minoxidil group. A scalp roller of 1.5 mm sized needles was rolled on the affected scalp in vertical, horizontal, and diagonal directions

till mild erythema was observed. The patients were in the group who were instructed to apply 1 ml of minoxidil lotion (5%) twice daily 24 h after the procedure. The minoxidil group involved the application of minoxidil lotion (5%) without scalp roller treatment. The study proved that scalp roller along with minoxidil treatment was found to be statistically significant compared with minoxidil treatment alone in men with alopecia areata. The study concluded that microneedling was a safe and promising technique in stimulation of hair re-growth. Microneedling was more effective in men with androgenetic alopecia who failed to respond to conventional therapy (finasteride and minoxidil). More often, treatment of alopecia is hampered by poor drug delivery to the scalp. It was concluded that microneedling could be quite effective in patients who failed to respond to triamcinolone acetonide injections, topical corticosteroids, and minoxidil (5%). A pilot study involving 15 patients with alopecia areata indicated that the combination therapy of microneedling and topical application of triamcinolone acetonide resulted in better therapeutic outcomes [46]. About 1 ml of triamcinolone acetonide was applied dropwise to the affected area. A scalp roller having 192 needles measuring 500 microns each was used to create 250 holes per square cm when rolled over the area for about 15 times. The microneedling procedure was found to be painless and did not require the use of any local anesthesia. The procedure was repeated for six sessions with an interval of 3 weeks between each. The patients were instructed to apply minoxidil (5%) after 24 h of treatment with scalp roller. Following six sessions of treatment nearly 12 out of 15 patients experienced clinically significant therapeutic outcomes. However, the big limitation of the report was that there was no control group involved in the study.

The effect of topical application of growth factor solution followed by microneedle treatment was assessed in a pilot study involving 11 women with female pattern hair loss [47]. Each subject received weekly treatment for a total span of 5 weeks. The device comprised nine 33 gauge needles that moved vertically to a depth of 0.5 mm. A significant difference in the hair shaft density was observed in 2nd, 4th, and 5th week compared with the control that involved treatment with normal saline followed by microneedle treatment. Microneedle treatment was likely to increase the permeation by creating of pores in the stratum corneum. However, no adverse reactions were observed following the microneedle treatment in the study. The study revealed a new therapeutic option to enhance the hair density in female pattern hair loss.

Microneedles for delivery to the nail

Topical nail formulations are usually used for treatment of nail diseases such as onychomycosis and nail psoriasis. However, the efficacies of the conventional topical nail formulations are found to be poor due to low permeability of the therapeutic agents though the dense keratinized structure of the nail plate [48]. Microporation involves drilling or creating micron-sized holes in the nail plate without affecting the nail bed. The technique has the ability to overcome the barrier properties of the nail plate. Microporation has the ability to drastically improve the drug delivery through the poorly permeable nail [49]. Micropores of the nail plate are normally induced by mechanical procedures using dermaroller or using a device called PathFormer (Path Scientific, Carlisle, USA) or application of low-frequency ultrasound or laser ablation. The effect of microporation of diseased toenail trephinated with FDA-approved "mesoscission" prior to treatment efficacy of terbinafine cream (1%) was assessed. An enhanced permeation of terbinafine across nail plate was observed on microporation. Standardized drilling technique was used in a pilot clinical study that involved 28 patients with proven subungual toenail onychomycosis. Follow-up clinical evaluation at 4, 8, 16, 24, 36, and 48 weeks assessed the treatment efficacy. The study established a clinical evidence that microporation could be well-tolerated and effective procedure to enhance the transungual delivery. Polymeric biodegradable nanoparticles were proposed as drug reservoirs for sustained topical delivery of microporated nail plate [50]. Microporation of the nail clippings was initially performed using commercial dermaroller equipped with 250-μm-long titanium needles. The dermaroller was rolled five times back and forth on dorsal surface of the hydrated nail plate to create the micropores prior to application of the nanodispersion. The nanodispersion that comprised octyl methoxycinnamate-loaded poly-(caprolactone) nanoparticle was labeled with Nile red. The depth of Nile red permeation was observed using laser scanning confocal microscopy. The nanoparticles are basically particulate drug delivery systems ranging from 1 to 100 nm in size. Nanoparticles of poly (ε-caprolactone) loaded with Nile red were prepared by solvent displacement method. The particles displaying an average particle size of 152 ± 3 nm were found to be biodegradable as they degraded over a period of time releasing the dye in a controlled manner. The nano dispersion applied on the nail plate was found to have a concentration of 206 μg/ml. Owing to the small size, the nanoparticles were found to be easily filled into the micropores created in the nail plate. Once filled into the micropores, the nanoparticles were found to act as immobile micro reservoirs from which the dye was released that diffused laterally into the nail plate over prolonged period of time. The transungual permeation rate of Nile red was considerably higher for the microporated nail compared with the untreated nails. It was observed that Nile red released from the nanoformulation permeated to a depth of 70–90 m after 7 days. The study demonstrated that nanoparticles deposited in the micropores act as reservoir for prolonged and local release of therapeutic agents lasting for several days. A strategy involving combination of nanoformulation and microporation approach was investigated to enhance the tioconazole delivery into the nail

plate [51]. Nanoparticles of poly (ε-caprolactone) containing tioconazole were prepared by interfacial deposition of polymer. The nail lacquer formulations having a concentration of 1 mg/ml were found to display a particle size of less than 200 nm. A series of nanoformulations of tioconazole were assessed for in vitro permeation across microporated human nail clippings. Microporation of the human nail clippings was performed adapting the same procedure using the dermaroller. Microporation was found to successfully increase tioconazole delivery in single-dose experiments. The nanoparticles get filled into the micropores created and function as reservoirs for hydrophobic drugs and prolong their local release over several days. The study proved the nail poration and nanoformulation approach could be employed in combination to improve the ungual drug delivery to eradicate nail diseases.

Nail poration by laser ablation can also be induced prior to application of topical formulation [52]. The technique could improve the efficacy of the topical nail formulation used to treatment of different nail diseases. However, the technique could be clinically applied only if the depth of nail poration could be better controlled and the thermal damage encountered during treatment minimized. In addition, creation of smaller diameter pores is highly warranted to ensure the patient comfort.

Microneedles for vascular delivery

In patients with atherosclerosis, insertion of stent is a common clinical practice. When occlusion happens in patients with previously inserted stents, bypass grafting is the clinical procedure employed. However, some of the patients who undergo the bypass grafting surgery suffer from neointimal hyperplasia at the site of grafting. Neointimal hyperplasia involves proliferation and migration of vascular smooth muscle cells present in tunica intima eventually leading to thickening of the artery walls and narrowing of the arterial lumen. Therapeutic compounds like proteins, oligonucleotides, and anti-proliferating agents are commonly used to prevention of neointimal hyperplasia [53]. Even though drug-eluting stents and eluting balloons have been developed to deliver drugs to treat neointimal hyperplasia, the devices have proved to be less efficient due to drug loss to the blood stream. Microneedle cuffs were developed as perivascular drug devices with the aim to achieve higher to higher drug delivery efficiency to inner layers of the blood vessels. Microneedle cuffs are found to be advantageous as they can be easily applied around the affected blood vessel and at the same time do not cause endothelial injury. The cuffs are known to specially benefit the delivery of therapeutic macromolecules that have issues permeating the biological membranes owing to their high molecular weight.

Biodegradable microneedles made of poly (lactic co-glycolic acid) [PLGA] with ultra-fine tips were fabricated using spatial thermal drawing method for vascular drug delivery [54].The mechanical strength of the microneedles was found to depend on the shape of the microneedles. The bullet-shaped microneedles were found to have a superior mechanical strength compared with the slender microneedles. Ex vivo studies with canine aorta indicated that microneedles with bullet-shaped tips were found to penetrate deeper into tunica media. In vitro release of the drug-coated microneedles displayed a sustained release for a span of 1 week. Curved microneedles composed of PLGA were designed using the same procedure. Ex vivo studies with canine and rabbit aorta indicated that the microneedle cuffs easily penetrated the tunica adventitia layer and touched the tunica media layer [55]. In vivo studies in canines demonstrated the feasibility of the cuff to penetrate through the adventitia layer and reach the tunica media. An attempt was made to fabricate biodegradable microneedle cuffs of PLGA and surgically implant the same for delivering paclitaxel to the inner vascular layers. In vivo studies in rabbits indicated the possibility of drug delivery to tunica media. The higher drug concentration in the layer was ascribed to the affinity of the drug to the smooth muscle cells. The studies also demonstrated a significant decrease in neointimal hyperplasia in 2 to 4 weeks [56]. Despite some success reported, slow diffusion of the therapeutic entities through the different vascular layers continues to pose as a challenge in perivascular drug delivery.

Conclusion

In the last few years, microneedle technology has grown from mere idea that has been successfully scaled up a pilot and eventually a commercial scale. Although the extensive studies are carried out on the skin application of microneedles, investigations are still in progress to deploy the technology for delivering drug to other tissues and organs. With the success of technology in drug delivery to the skin, applications of microneedles have been explored for oral mucosa, ocular, muscular, cardiac, nail, scalp, and vascular delivery. The advent of technology into other domains involving various tissues and organs is likely to further evolve in times to come. At present, the main objectives are to commercialize the microneedle devices and explore the already developed microneedles in clinical settings [5]. Till now, only intradermal vaccine and drug delivery based on hollow microneedles such as the Microjet® 600 by NanoPass Technologies, Israel and Soluvia® (Becton Dickson, NJ, USA) have been approved by the FDA. Even though microneedles are a minimally invasive technique and safe, there are many safety concerns that need to be thoroughly investigated [57, 58]. However, there is dearth of information available on the barrier disruption and the restoration of the live tissues and organs. In this context, it is necessary to have a thorough understanding of the barrier disruption and its subsequent restoration when microneedles are directly applied to other tissues and organs. Other issues that need to be dwelled upon

include the possibility of an allergic reaction upon microneedle application on repetitive or chronic applications. At this stage, usage of microneedles from a drug delivery perspective into other tissues and organs is still at very early stages. Considering the viability of living tissues and organs, we anticipate stringent regulatory measures in place in order to commercialize the technology. In case the safety concerns of the microneedle technology are duly addressed, drug delivery to the remote inaccessible affected organs and tissues may become a true reality in future. However, in such cases, surgical intervention by a clinician/clinical practitioner becomes inevitable.

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Sarasija Suresh: Manuscript preparation and writing.

Shiva Kumar H. N: Manuscript organization, guidance, and revision.

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Declarations

Consent for publication All authors agree to the material for publication.

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References

1. Rowland M, Tozer T. Clinical pharmacokinetics and pharmacodynamics: concepts and applications. 4th ed. Philadelphia: Lippincott Williams & Wilkins; 2010.
2. Prausnitz MR, Langer R. Transdermal drug delivery. Nat Biotechnol. 2008;26(11):1261.
3. Quinn HL, Kearney MC, Courtenay AJ, McCrudden MT, Donnelly RF. The role of microneedles for drug and vaccine delivery. Expert Opin Drug Deliv. 2014;11(11):1769–80.
4. Prausnitz MR. Engineering microneedle patches for vaccination and drug delivery to skin. Annu Rev Chem Biomol Eng. 2017;8:177–200.
5. Henry S, McAllister DV, Allen MG, Prausnitz MR. Microfabricated microneedles: a novel approach to transdermal drug delivery. J Pharm Sci. 1998;87(8):922–5.
6. Matriano JA, Cormier M, Johnson J, Young WA, Buttery M, Nyam K, Daddona PE. Macroflux® microprojection array patch technology: a new and efficient approach for intracutaneous immunization. Pharm Res. 2002;19(1):63–70.
7. Verbaan FJ, Bal SM, van den Berg DJ, Dijksman JA, van Hecke M, Verpoorten H, van den Berg A, Luttge R, Bouwstra JA. Improved piercing of microneedle arrays in dermatomed human skin by an impact insertion method. J Control Release. 2008;128:80–8.
8. Wang Q, Yao G, Dong P, Gong Z, Li G, Zhang K, Wu C. Investigation on fabrication process of dissolving microneedle arrays to improve effective needle drug distribution. Eur J Pharm Sci. 2015;66:148–56.
9. Cai B, Xia W, Bredenberg S, Li H, Engqvist H. Bioceramic microneedles with flexible and self-swelling substrate. Eur J Pharm Biopharm. 2015;94:404–10.
10. Park JH, Allen MG, Prausnitz MR. Biodegradable polymer microneedles: fabrication, mechanics and transdermal drug delivery. J Control Release. 2005;104(1):51–66.
11. Lee JW, Prausnitz MR. Drug delivery using microneedle patches: not just for skin. Expert Opin Drug Deliv. 2018;15(6):541–3.
12. Rzhevskiy AS, Singh TRR, Donnelly RF, Anissimov YG. Microneedles as the technique of drug delivery enhancement in diverse organs and tissues. J Control Release. 2018;270:184–202.
13. Ma Y, Tao W, Krebs SJ, Sutton WF, Haigwood NL, Gill HS. Vaccine delivery to the oral cavity using coated microneedles induces systemic and mucosal immunity. Pharm Res. 2014;31(9):2393–403.
14. McNeilly CL, Crichton ML, Primiero CA, Frazer IH, Roberts MS, Kendall MAF. Microprojection arrays to immunise at mucosal surfaces. J Control Release. 2014;196:252–60.
15. Zhen Y, Wang N, Gao Z, Ma X, Wei B, Deng Y, Wang T. Multifunctional liposomes constituting microneedles induced robust systemic and mucosal immunoresponses against the loaded antigens via oral mucosal vaccination. Vaccine. 2015;33(35):4330–40. https://doi.org/10.1016/j.vaccine.2015.03.081.
16. Wang T, Zhen Y, Ma X, Wei B, Li S, Wang N. Mannosylated and lipid A-incorporating cationic liposomes constituting microneedle arrays as an effective oral mucosal HBVvaccine applicable in the controlled temperature chain. Colloids Surf B: Biointerfaces. 2015;126:520–30.
17. Ma Y, Boese SE, Luo Z, Nitin N, Gill HS. Drug coated microneedles for minimally-invasive treatment of oral carcinomas: development and in vitro evaluation. Biomed Microdevice. 2015;17(2):1–14. https://doi.org/10.1007/s10544-015-9944-y.
18. Kim CS, Wilder-Smith PB, Ahn YC, Liaw LHL, Chen Z, Kwon YJ. Enhanced detection of early-stage oral cancer in vivo by optical coherence tomography using multimodal delivery of gold nanoparticles. Journal Biomed Optics. 2009;14(3):034008.
19. Kim Y, Yoo D, Compans RW, Kang S, Prausnitz MR. Cross-protection by co-immunization with influenza hemagglutinin DNA and inactivated virus vaccine using coated microneedles. J Control Release. 2013;172(2):579–88. https://doi.org/10.1016/j.jconrel.2013.04.016.
20. Edens C, Collins ML, Ayers J, Rota PA, Prausnitz MR. Measles vaccination using a microneedle patch. Vaccine. 2013;31(34):3403–9. https://doi.org/10.1016/j.vaccine.2012.09.062.
21. Cholkar K, Patel SP, Vadlapudi AD, Mitra AK. Novel strategies for anterior segment ocular drug delivery. J Ocul Pharmacol Ther. 2013;29(2):106–23.
22. Cholkar K, Patel A, Vadlapudi AD, Mitra AK. Novel Nanomicellar formulation approaches for anterior and posterior segment ocular drug delivery. Recent Pat Nanomed. 2012;2:82–95.
23. Jiang J, Gill HS, Ghate D, McCarey BE, Patel SR, Edelhauser HF, Prausnitz MR. Coated microneedles for drug delivery to the eye. Invest Ophthalmol Vis Sci. 2007;48(9):4038–43. https://doi.org/10.1167/iovs.07-0066.
24. Thakur RRS, Tekko IA, Al-Shammari F, Ali AA, McCarthy H, Donnelly RF. Rapidly dissolving polymeric microneedles for minimally invasive intraocular drug delivery. Drug Deliv Transl Res. 2016;6(6):800–15.
25. Thakur RRS, Tekko IA, McAvoy K, Donnelly RF, McMillian H, Jones D. Minimally invasive microneedles for ocular delivery. Expert Opin Drug Deliv. 2017;14(4):525–37.
26. Kim YC, Oh KH, Edelhauser HF, Prausnitz MR. Formulation to target delivery to the ciliary body and choroid via the

suprachoroidal space of the eye using microneedles. Eur J Pharm Biopharm. 2015;95:398–406.
27. Jiang J, Moore JS, Edelhauser HF, Prausnitz MR. Intrascleral drug delivery to the eye using hollow microneedles. Pharm Res. 2009;26(2):395–403.
28. Gilger BC, Abarca EM, Salmon JH, Patel S. Treatment of acute posterior uveitis in a porcine model by injection of triamcinolone acetonide into the suprachoroidal space using microneedles. Invest Ophthalmol Vis Sci. 2013;54(4):2483–92.
29. Patel SR, Lin AS, Edelhauser HF, Prausnitz MR. Suprachoroidal drug delivery to the back of the eye using hollow microneedles. Pharm Res. 2011;28(1):166–76.
30. Kim YC, Grossniklaus HE, Edelhauser HF, Prausnitz MR. Intrastromal delivery of bevacizumab using microneedles to treat corneal neovascularization. Invest Ophthalmol Vis Sci. 2014;55(11):7376–86.
31. Palakurthi NK, Correa ZM, Augsburger JJ, Banerjee RK. Toxicity of a biodegradable microneedle implant loaded with methotrexate as a sustained release device in normal rabbit eye: a pilot study. J Ocul Pharmacol Ther. 2011;27(2):151–6.
32. Than A, Liu C, Chang H, Duong PK, Cheung CMG, Xu C, Wang X, Chen P. Self-implantable double-layered micro-drug reservoirs for efficient and controlled ocular drug delivery. Nat Commun. 2018;9:4433. https://doi.org/10.1038/s41467-018-06981-w.
33. Kim YC, Edelhauser HF, Prausnitz MR. Targeted delivery of antiglaucoma drugs to supraciliary space using microneedles. Investig Ophthalmol Vis Sci. 2014;55(11):7387–97.
34. Neutra MR, Kozlowski PA. Mucosal vaccines: the promise and the challenge. Nat Rev Immunol. 2006;6:148–58.
35. Wang X, Wang N, Wang T, Zhen Y, Jin Y, Li N, Jiang S. Combining different types of multifunctional liposomes loaded with ammonium bicarbonate to fabricate microneedle arrays as a vaginal mucosal vaccine adjuvant dual delivery system (VADDS). J Control Release. 2017;246:12–29. https://doi.org/10.1016/j.jconrel.2016.12.009.
36. Mc Crudden MTC, Larrañeta E, Clark A, Jarrahian C, Rein-Weston A, Creelman B, Mayo Y, Lachau-Durand S, Niemeijer N, Williams P, McCarthy H, Zehrung D, Donnelly RF. Design, formulation and evaluation of novel dissolving microarray patches containing rilpivirine for intravaginal delivery. Adv Healthcare Mater. 2019;8:119–29. https://doi.org/10.1016/j.jconrel.2018.11.002.
37. Mc Crudden MTC, Larrañeta E, Clark A, Jarrahian C, Rein-Weston A, Lachau-Durand S, Donnelly RF. Design, formulation and evaluation of novel dissolving microarray patches containing a long-acting rilpivirine nanosuspension. J Control Release. 2018;292:119–29. https://doi.org/10.1016/j.jconrel.2018.11.002.
38. Traverso G, Schoellhammer CM, Schroeder A, Maa R, Lauwers GY, Polat BE, Langer R. Microneedles for drug delivery via the gastrointestinal tract. J Pharm Sci. 2015;104(2):362–7. https://doi.org/10.1002/jps.24182.
39. Makkar RR, Smith RR, Cheng K, Malliaras K, Thomson LEJ, Berman D, Czer LSC, Marban L, Mendizabal A, Johnston PV, Russell SD, Schuleri KH, Lardo AC, Gerstenblith G, Marban E. Intracoronary cardiosphere-derived cells for heart regeneration after myocardial infarction (CADUCEUS): a prospective, randomised phase 1 trial. Lancet. 2012;379:895–904.
40. Boyle AJ, Schulman JM. Stem cell therapy for cardiac repair: ready for the next step. Circulation. 2006;114:339–52.
41. Nguyen PK, Neofytou E, Rhee JW, Wu JC. Potential strategies to address the major clinical hurdles facing in stem cell regenerative therapy in cardiovascular disease: a review. JAMA Cardiol. 2016;1:953–62.
42. Tang J, Wang J, Huang K, Ye Y, Sue T, Qiao L, Hensley MT, Caranasos TG, Zhang J, Gu Z, Cheng K. Cardiac cell–integrated microneedle patch for treating myocardial infarction. Sci Adv. 2018;38:1–12.
43. Deepak SH, Shwetha S. Scalp roller therapy in resistant Alopecia Areata. J Cutan Aesthet Surg. 2014;7(1):61–2.
44. Alsalhi WA, Alalola AS, Randolph MJ, Gwillim EC, Tosti A. Novel drug delivery approaches for management of hair loss. Exp Opinion Drug Del. 2020;17(3):287–95.
45. Dhurat R, Sukesh MS, Avhad G, Dandale A, Pal A, Pund P. A randomized evaluator blinded study of effect of microneedling in androgenetic alopecia: a pilot study. Int J Trichol. 2013;5:6–11.
46. Chandrashekar BS, Sandeep MA, Vani Vasanth JP, Rajashekar ML. Triamcinolone acetonide mesotherapy in the treatment of recalcitrant patches of alopecia areata—a pilot study. J Clin Dermatol Ther. 2015;2:1–4.
47. Lee YB, Eun YS, Lee JH, Cheon MS, Park YG, Cho BK, Park HJ. Effects of topical application of growth factors followed by microneedle therapy in women with female pattern hair loss: a pilot study. J Dermatol. 2013;40(1):81–3.
48. Shivakumar HN, Juluri A, Desai BG, Murthy SN. Ungual and transungual drug delivery. Drug Dev Ind Pharm. 2012;38(8):901–11.
49. Boker A, Cioccon D, Kimball A. A randomized, double-blind, placebo-controlled, pilot study of 1% terbinafine cream applied twice daily and delivered via nail plate microporation for the treatment of subungual toenail onychomycosis. J Am Acad Dermatol. 2007;56(2):AB114.
50. Chiu WS, Belsey NA, Garrett NL, Moger J, Price GJ, Delgado-Charro MB, Guy RH. Drug delivery into microneedle-porated nails from nanoparticle reservoirs. J Control Release. 2015;220:98–106.
51. Flores FC, Chiu WS, Beck RCR, da Silva CB, Delgado-Charro MB. Enhancement of tioconazole ungual delivery: combining nanocapsule formulation and nail poration approaches. Int J Pharm. 2018;535:237–44.
52. Vanstone S, Stone JM, Gordeev SN, Guy RH. Mechanism of human nail poration by high-repetition-rate, femtosecond laser ablation. Drug Deliv Transl Res. 2019;9(5):956–67.
53. Lee KJ, Park SH, Joo JY, Jang EH, Youn YN, Ryu WH. Perivascular biodegradable microneedle cuff for reduction of neointima formation after vascular injury. J Control Release. 2014;192:174–81.
54. Kim HW, Jang EH, Lee KJ, Lee JY, Park SH, Seo IH, Lee KW, Lee SH, Ryu WH, Youn YN. Polymers. 2017;9(56):1–13.
55. Choi CK, Kim JB, Jang EH, Youn EH, Ryu WH. Curved microneedles for vascular delivery. Small. 2012;8(16):2483–8.
56. Choi CK, Kim JB, Youn YN, Jang EH, Kim W, Min B-Y, Ryu WH. Spatially discrete thermal drawing of biodegradable microneedles for vascular drug delivery. Eur J Pharm Biopharm. 2013;83:224–33.
57. Marshall S, Sahm LJ, Moore AC. Microneedle technology for immunisation: perception, acceptability and suitability for paediatric use. Vaccine. 2016;34(6):723–34.
58. Birchall JC, Clemo R, Anstey A, John DN. Microneedles in clinical practice–an exploratory study into the opinions of healthcare professionals and the public. Pharm Res. 2011;28(1):95–106.