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Microneedle array systems for long-acting drug delivery

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Abstract:

The development of microneedles (MNs) assisted drug delivery technologies have been highly active for more than two decades. The minimally invasive and self-administered MN technology bypasses many challenges associated with injectable drug delivery systems, by delivering the therapeutic materials directly into the dermal and ocular space and allowing the release of the active ingredient in a sustained or controlled manner. Different types of MNs (biodegradable solid/dissolving MNs and nanoparticle loaded/coated polymeric MNs or delivery by hollow MNs) have been envisioned for long-acting sustained delivery of therapeutic payloads, with the aim of reducing the side effects and administration frequency to improve the patient compliance. In this review, we covered the different types of MNs loaded with different nano/biotherapeutics for long-acting delivery for a wide range of potential clinical applications. We also outlined the future development scenario of such long-acting MN delivery systems for different disease conditions to achieve improved clinical benefit. Finally, we discussed the challenges lie ahead to realize the full potential of sustained-release long-acting MNs in the clinic.

Keywords: Microneedles; Long-acting therapy; sustained-release, controlled release, implantable delivery; nano-microparticles, intradermal delivery, site-specific delivery, transdermal delivery, Ocular delivery

55 1. Introduction

56 In the field of drug delivery, the drug and device-based combined approach have improved the
57 success rate in product development and clinical benefits for patients. Drug delivery through
58 the skin, also called the transdermal route, is one of the most highly explored areas with this
59 combined approach in research, due to several benefits over conventional oral and injectable
60 drug delivery routes. Indeed, the transdermal drug delivery system (TDDS) has become an
61 evolving area for systemic and local delivery of drugs, macromolecules and
62 immunotherapeutics [1,2]. However, the poor permeability of therapeutics through the *stratum*
63 *corneum* (SC) limits its application to only a selected few potent and highly lipophilic small
64 molecules (log p-value between 1 and 3, relatively low molecular weight <600Da). Passive
65 permeation in this manner typically favours drug molecules with specific physicochemical
66 characteristics. To deliver the wide range of low to high molecular weight drug and
67 macromolecules, the SC layer should be broken or punctured temporally by either physical or
68 chemical method. One of such pioneering approaches is microneedles (MNs), expanding the
69 possibilities of delivery to a wider range of drug molecules and biotherapeutics. Also, MN-
70 assisted drug delivery technologies have shown great potential to circumvent the invasive
71 nature of conventional injections for greater patient acceptability and convenience.
72 Furthermore, the recent research associated with MNs further extended the range of its use with
73 a diversity of applications [2,3].

74 MNs typically consist of multiple microscopic projections of less than 2000 μm in height,
75 positioned on a single side of a supporting base or patch. MNs, also called microneedle (MN)
76 arrays or microarrays patch (MAP) are used interchangeably in the literature. Upon application
77 to the skin, the microprojections penetrate the epidermal layer and deliver or implant the
78 compounds within the dermis below, wherein lies a high density of vascular and lymphatic
79 beds available to absorb the drug systemically [4–6]. Besides the application of MNs for
80 intradermal drug delivery, they have also been used for ocular drug delivery, as well as
81 administrations of vaccines and DNA delivery [7–9]. MNs have been vastly exploited to
82 enhance penetration of small and macromolecules through the skin or the ocular tissues in a
83 pain-free manner with the potential capability of self-application. As our understanding of the
84 biological skin/ocular barrier and MNs formulation aspects have improved over time, the
85 design of controlled-release MNs based drug delivery systems have evolved from classical fast

release mechanisms to the utilization of slow-releasing polymers or nano/micro-particles, further exploiting local biochemical changes to trigger and activate drug release [10,11]. Consequently, nanoparticle-loaded or slow-releasing biodegradable MN patches with prolonged therapeutic effect have been under investigation for long-acting self-administration potential for chronic conditions [12].

The general aim and scope of this review are to provide an informative account of long-acting sustained drug delivery possibilities with MNs that can be utilised for further development and clinical application. Initially, we introduced a general background on long-acting drug delivery systems and technologies. Then, we provided a detailed overview of MNs technology and the most utilized types of MNs employed in drug delivery. Following this, we offered a retrospective account of the up-to-date progression made in utilising the MNs in delivering long-acting formulations or sustaining the drug release as an alternative to the conventional hypodermic needles and, how ground-breaking work in this field has evolved to produce the current application of MNs-assisted delivery to long-acting formulations. In this review article, we discussed present progress and potential future use of MNs assisted long-acting drug technologies. Finally, we provided an expert opinion on the best selection of MNs technology for efficient delivery of long-acting drug formulations and discussed the future challenges and the translatable clinical potential of long-acting MN from bench to bedside.

2. Long-acting drug delivery and technologies

The prevalence of chronic diseases (e.g. type 2 diabetes, cardiovascular disease) is projected to worsen in the coming years. Chronic disorders require long-term medicinal therapy that affects the patient's day to day activities and substantially increases associated healthcare cost [13]. Conventional immediate-release drug delivery systems are subject to various limitations including poor patient compliance, dose missing or skipping, high levels of plasma fluctuations, multiple-dose drug therapy augments the risk of toxicity affecting patient compliance and adherence to the therapy and, the overall efficacy and cost of the treatment.

Long-acting drug delivery technologies based on sustained and controlled release mechanisms can be used to maintain constant plasma drug concentrations for extended periods as highlighted in the associated illustration (Figure 1). These have the potential to improve patient compliance compared to daily pills or injections [14] or may be employed to provide site-specific drug delivery where the drug is most required and, thus, avoid higher systemic toxic exposure [15,16].

Indeed, long-acting dosage forms are mostly made of biodegradable and biodurable materials, thus, offer the potential to tailor drug release from hours to weeks or even to months for a wide variety of chronic disease conditions or prophylactic measures. Consequently, the well-established issues associated with conventional methods of delivery, such as those previously mentioned, can be easily overcome [17]. Furthermore, long-acting formulations could be particularly beneficial for classes of patients that are unable to adhere to treatment regimens, such as those suffering from psychiatric disorders [18].

Despite the substantial scientific output in this field, to date, no consensus in the terminology has been reached. A plethora of delivery technologies for long-acting formulations have been developed over the past few decades, however, inconsistent terms to describe the concept of long-term release are routinely employed: “long-acting injectable”, “controlled release”, “sustained release”, “extended release” or, “depot” formulations [14]. The United States Pharmacopeia (USP) describes “extended release”, a synonym for “prolonged” and, “sustained release”, as “*a deliberate modification to protract the release rate of an active pharmaceutical ingredient (API) in comparison to an immediate release dosage form*” [19]. To date, it is still unclear whether even prolongation of a few hours could be considered as a sustained release or if only a protraction expressed in days is noteworthy. Although a handful of examples of long-acting formulations able to extend release only for few hours are mentioned in the present review, the majority of technologies we considered provide a sustained release of the API in a timespan of days at least.

Figure 1. Representative pharmacokinetics of an ideal long-acting release formulation compared to the multiple-dose formulation.

In the literature, an abundance of technologies has been used to produce long-acting formulations including, implantable devices, polymeric micro/nanoparticles, liposomes, nanocrystals and, thermo-responsive hydrogels [14,15,20–23]. A good example for implantable devices is the implantable pumps with glucose monitoring features used to deliver insulin in the management of diabetes [20] or, the intrathecal pumps to directly administer a drug to the spine for the maintenance of chronic pain [24]. These pumps are typically programmable “active” devices, which necessitate regular resupply of drug *via* an access port. Subcutaneous implants that are made of biodegradable polymers, provide long time, “passive” release without the need for replenishment is a further example. This kind of thin and flexible rod shape implant is particularly useful for highly potent drug delivery such as hormones [25].

Polymeric micro/nanoparticle-based injectable systems are another formulation strategy for long-lasting sustained-release. Microspheres (1 μm to 300 μm) and nanoparticles (< 1000 nm in size) are the first of such particle engineering-based approaches for sustained release injectables. Categories of such polymeric micro/nanoparticles may include polymeric micro/nanospheres and micro/nanocapsules. The former consists of polymeric particle matrices, whilst the latter consists of a matrix (generally oily) covered with polymers. Both types of particles can be produced using various biodegradable synthetic polymers such as polylactic acid (PLA) and poly(lactide-co-glycolide) acid (PLGA). in a broad range of sizes [23]. These vehicles have been used extensively as drug delivery systems in a wide variety of different locations in the body [23]. The first marketed microsphere-based product (Lupron[®] depot) was developed in 1985 by Takeda, for the palliative treatment of advanced prostatic cancer [26]. At present, 11 FDA-approved sustained-release microsphere injections are available on the market. These marketed microsphere products are mainly made from PLGA and PLA polymers. Microsphere products have additionally shown great advantages in comparison with conventional dosage forms. For example, Sandostatin Lar[®] Depot resolved the short biological half-life of peptide drug (~2 h), extending the dosing period to 4 weeks and, therefore, improving patient adherence [27]. Similarly, the bi-weekly injection of Risperdal Consta[®] (Risperidone) resulted in increased efficacy and fewer antipsychotic side effects than that of its conventional counterpart. Additionally, this conferred a further benefit due to the long-acting microspheres formulation, as patients experiencing psychosis no longer had to worry about remembering to take their medication every day [28].

Liposomes are another success story for sustained and targeted parenteral drug delivery. A liposome is an artificial membrane, which primarily consists of phospholipids and cholesterol

[29,30]. The drug is then encapsulated within this phospholipid vesicle, in which the liposome then gradually degrades to release the drugs slowly. The liposome is the most versatile nanocarrier with superior biocompatibility and large-scale manufacturing possibility compared to other types of nanoparticles. The first FDA-approved liposome product, Doxil[®] (doxorubicin (DOX) hydrochloride), reached market 1995 and is used to treat certain cancers. Following the success of Doxil[®], five more liposomal products for different disease conditions have emerged. Since the 1990s, drug nanocrystals or nanosuspension systems have attracted widespread attention, mainly because of the potentially sustained-release profile *via* the injectable route they afford. Abraxane[®], an albumin-bound nanoparticle formulation of paclitaxel, achieved great commercial success for the treatment of recurrent breast cancer [31]. The formulation of paclitaxel within Abraxane[®] has significant advantages over pure paclitaxel. At present, a total of 13 suspension and nanoparticle/nanocrystals injection products are now available in the market.

One of the key disadvantages of the above mentioned long-acting drug formulations, is that they typically necessitate hypodermic needle for delivery or perhaps surgical implantation of macroscopic devices within the body. In addition to being painful and causing stress, these invasive procedures generally are limited to use in hospitals or clinics and, thus, are not suitable for patient self-administration [32]. Subsequently, further development of a minimally invasive delivery system is now required that allows self-administration without causing pain to the patient. Rather than using a hypodermic needle to introduce advanced sustained-release formulations into the body, recent advances in MNs technology have given the functionality for both MNs and the long-acting formulations for controlled/sustained release of a wide range of drug molecules and vaccines.

3. Microneedles

MNs are becoming a more promising tool to bypass the physical barriers in the human body to deliver the therapeutic agents in a non-invasive manner. MN technology was first conceptualized for drug delivery to overcome the disadvantages and, preserve the benefits of hypodermic needles and traditional transdermal drug-delivery systems. The first compound delivered using MN was the low-molecular-weight dye, calcein [33]. Numerous studies quickly followed, leading to the present ever-growing body of proof for the significant drug delivery competencies of MNs [34]. Numerous MNs designs and types have been developed with the

focus of achieving a pain-free, self-administrable device to replace the injectable hypodermic needles as described in the following section.

3.1 Type of MN formulations

MNs have been studied as an assisted drug diffusion device to deliver various types of therapeutics through the SC to overcome the limitations of the conventional TDD approach [1]. To make the skin accessible to deliver or deposit wide range of drug and nanoparticles, various type of MN formulations (Figure 2) studied by manipulating MN characteristics (a type of MN, geometry, numbers of arrays per patch, drug formulation etc). These different MN characteristics can affect delivery mechanisms (bolus or sustained) in the dermis when applied [42]. A MN patch is made by assembling hundreds of MNs on a tiny baseplate to deliver enough drug to produce the required therapeutic effect. The amount of the drug that can be delivered is dependent on the properties of MNs including mechanical integrity, insertion capability and the loaded dose. Such properties are crucially affected by the geometry of the MN and, the number of MNs per patch. The therapeutic load delivery depends on the type of MN that directly placed in the epidermis or upper dermis layer which then reaches into the systemic circulation and shows a therapeutic response on reaching the site of action [100]. The mechanism of drug delivery through MNs is depicted in Figure 3. MN drug delivery technologies can be divided into several categories based upon their exact mechanism of delivery or, formulation: solid MNs for skin pre-treatment to increase the skin permeability, MNs coated with drugs, polymeric MNs that encapsulate therapeutic agents or particulate drug carriers and completely or partially dissolve in the skin, and hollow MN that permit drug infusion into the skin [60]. Recently, more categories of MN type considered such as separable MN and bioinspired MN.

3.1.1 Solid MNs (Poke and Patch)

Solid MNs are generally employed in the so-called ‘poke with patch’ approach to perforate the skin’s SC layer, creating transient aqueous microchannels that reach the deepest layers of the epidermis (Figure 2a) [42]. Following initial skin barrier disruption by MNs, a conventional transdermal patch, drug solution or gel is applied to the skin as an external drug reservoir for passive diffusion through these microchannels. The primary restriction of this methodology is the requirement for a two-step application process, which may lead to practicality issues for patients. Additionally, the micropores generated by MNs are quickly closed, resulting in reduced drug permeation. The water-insoluble and mechanically robust materials used to

produce solid MN are classically silicon, metals, and polymers [43]. Disconcertingly, silicon and metals based MNs may remain solid after removal from a patient's skin, which may impose hazards of inappropriate re-use and, further lead to associated problems such as biofouling [44].

3.1.2. Dissolving MNs (Poke and Release)

The majority of recently published MN research focuses on dissolving MNs. This is owing to significant improvements in the drug loading in the polymeric MNs tips. Furthermore, dissolving MN arrays can now be produced from a wide range of biocompatible and biodegradable water-soluble materials, which is often achieved by relatively simple and inexpensive fabrication processes at ambient temperature, meaning they can be used to deliver a wide range of biotherapeutics. In contrast to coated MN, dissolving MNs have been created to entirely vanish in the skin without leaving any biohazardous sharps waste after use [35]. In this "poke and release" approach, the drugs can be loaded and released over time from the dissolving MN matrix (slow or rapid depending on the polymer composition) after skin insertion [36]. Dissolving MNs were designed to incorporate drugs or particles inside a water-soluble polymer matrix, and become fully dissolved once the MNs are implanted into the skin, with a dissolution time ranging from minutes to hours [37]. Dissolving MN have been previously fabricated to deposit the drugs into the skin for bolus and sustained release, thus allowing for the self-administration of therapeutics without the requirement of a hypodermic needle and syringe [35]. One of the most important features of dissolving MN is that the backing baseplate to which MN arrays are connected as a drug reservoir for sustained drug delivery throughout the application. This approach may increase total doses to milligrams the patch backing layer that swelled by imbibing interstitial fluid from the skin through channels created by MN arrays [35].

3.1.3 Particle loaded MN (Poke, dissolve, and deposit)

Particle integrated polymeric MN have been an emerging research topic, majorly due to advances in nanotechnology, thus potentially providing a wider therapeutic benefit [38,39]. Here, hydrophobic drug particles or slow-releasing drug-loaded particles (lipidic/polymeric) are localised in the MN matrix and, after the MN array is inserted into the skin, the MNs dissolve, thereby releasing the particles intradermally (Poke, dissolve and deposit) [15,39–41]. This particle-loaded polymeric MN approach has been shown to efficiently deliver both hydrophobic and hydrophilic drugs and expand the ranges of therapeutic agents [32, 35]. In recent days, this approach has become increasingly used in the deposition of nanoparticles

within the skin, [42] as previously nanoparticles did not penetrate the skin's surface [44]. This approach allows the deposition of particles within the skin by one-step application with controlled/sustained release possibilities.

3.1.4. Separable MN (*Poke and deposit*)

Recently, in order to shorten the application times of the biodegradable or non-degradable MN, fast separable tips from the baseplate have been designed (Poke and leave) (Figure 2d) [39,45]. This type of MN was fabricated by bilayer or multilayer techniques where a baseplate made of fast dissolving materials or, a baseplate and MN tips separated by bubble structure or, fast-dissolving layers (example, effervescent layer, freeze-dried porous layer) at the base of the cone that rapidly separates the needles from the patch [45–48]. These are the most promising tactic to implant the long-acting biodegradable MNs into intradermal or ocular space [49].

Figure 2. Different type of MNs. A schematic representation of six different MN types used to facilitate intradermal drug delivery (a) Solid MNs (b) Dissolvable MNs (c) Nano/microparticles loaded dissolvable MNs (d) Bilayer separable MN (e) Drug/ nanoparticles coated solid MN (g) Hydrogel-forming MNs h) Bioinspired MN

3.1.5 Coated MN (Coat and Poke)

This type of MN is first prepared by coating the solid MN with potent drug or vaccine formulation. This “coat and poke” approach allows the formulation to dissolve and release the coated drug or therapeutic into the subcutaneous tissue after skin insertion. Coated MNs have been mainly explored for the rapid intradermal delivery of potent molecules, particularly, biotherapeutics including parathyroid hormone (PTH) [50], desmopressin [51], salmon calcitonin [52] and DNA/RNA [53] to the skin [54].

3.1.6. Hollow MN (poke and flow)

With the help of advances engineering techniques, hollow MNs were designed to deliver the drugs intradermally through a controlled pressure-driven flow [55]. This “poke and flow” hollow MN approach was devised to introduce a drug solution or particles into the skin or eye by micron-size needles in a pain-free manner (Figure 2f). This type of MN permits continuous delivery of drugs across the skin or ocular space through the MN bore using different techniques, namely, pressure or diffusion or electrically driven flow. This MN system is possibly capable of delivering larger amounts of drug substances in comparison to solid, coated, and dissolving MNs [27]. However, a major constraint to their use is the potential blockage of the needle openings during the dermal tissue insertion and flow resistance, thus, reducing drug release [56]. Therefore, various geometries (in-plane, out-plane, side-opened, tip-opened) of MN designs were investigated to achieve an optimum good liquid flow pattern without clogging the needles following skin insertion. Hollow MNs are made from a range of materials, including silicon and metal, glass, polymers and, ceramic [55].

3.1.7 Hydrogel-forming MNs (Poke, swell and release)

Hydrogel-forming MNs are a new developed type of MNs, prepared from drug-free swellable hydrogel-forming polymers (Figure 2g). They were first described and patented by Donnelly *et al.* in 2007 and, have now progressed to the forefront of MNs research in recent years [8,36]. Unlike dissolving MNs, the hydrogel-forming MN system is integrated with a drug reservoir (in the form of a film or tablet) on top of the MNs baseplate, and drug delivery is not limited to that loaded in the MNs projections. Therefore, milligrams of the drug can be delivered from even a relatively small patch size [57]. After application of the integrated drug reservoir-MNs patch to the skin, the inserted needle tips rapidly imbibe interstitial fluid from the intradermal space and, then allow the drug to diffuse through the swollen microprojections. Furthermore,

the interstitial fluid absorption rate and release kinetics of the drug from hydrogel-forming MNs can be controlled by varying the polymeric composition and swelling properties of the hydrogel-forming MNs. The striking feature of this type of MNs is that they are removed intact from the skin, leaving no measurable polymer residue behind. However, they are sufficiently softened to preclude reinsertion, thus further reducing the risk of transmission of infection. These systems are manufactured using aqueous blends of various polymeric materials including Gantrez[®] AN-139 (methyl vinyl ether and maleic anhydride), Gantrez[®] S-97 (methyl vinyl ether and maleic acid) and poly (vinyl alcohol) (PVA). Gantrez[®]- hydrogel-forming MNs are prepared by crosslinking (*via* esterification reaction) with other polyol polymers/surfactants such poly(ethylene glycol) [58], pectin, [59] Tween 85 [60] and, hyaluronic acid [61]. PVA has been also used to prepare hydrogel-forming MNs by physical crosslinking (multiple freeze-thaw cycles). Demir *et al.* cast a formulation (gelatin and PVA) into moulds and the system was then frozen at -20 °C for 12 h, followed by thawing at 25 °C for 12 h to form hydrogel-forming MNs [62]. The primary limitation of this methodology is that it may be time-consuming and tedious. Recently, Tekko *et al.* developed a novel hydrogel-forming MN using an aqueous blend of PVA and PVP crosslinked with citric acid (esterification reaction) at 130 °C in a short time (45 -180 minute) [63]. The new hydrogel-forming MNs exhibited sufficient mechanical properties to be inserted in the skin and moderate swelling in the range of 250-600%. Furthermore, they possessed the ability to control the delivery rate (without affecting the drug delivery efficiency) by adjusting the polymeric composition and crosslinking time. This type of MN has been used to deliver a wide range of small hydrophilic and hydrophobic drugs and macromolecules in a sustained manner [63–66].

3.1.8 Bioinspired MN (*Easy to poke and hook*)

As the name suggests, this MN design is inspired by natural phenomena, where the different shape of sharp needle objects in insects or plants, used to suck the food or protect against the harm. The lateral faces of this biomimetic MN designs were ornamented with bug-inspired microstructures [67]. Extensive work is being carried out regarding an insight into female mosquitos' proboscis (mouthparts) insertion into the skin [68,69]. For the first time, Oka *et al.* manufactured a hollow silicon MNs with a jagged shape inspired by mosquito proboscis [70]. This type of biomimetic needle can significantly reduce the required insertion force (*Easy to Poke*). Additionally, MNs with swellable tips inspired by the proboscis of an endoparasite (*Pomphorhynchus laevis*) were proven to be efficient in the adhesion to and interlocking with the tissues (*Hook*) [71]. This biomimetic MN platform governed by shape change-mediated

mechanically interlocking MNs. These bioinspired multifunctional MNs inherited their advantages such as easy insertion, strong adhesion, tissue interlocking and, thus, are postulated to permit broad applications to various conditions.

3.2. Manufacturing of MN

The first patent on MNs drug delivery device as ‘puncturing projections’ was filed in the US in the year 1971 by Gerstel and Place [72]. However, it took 20 years from initial conception to produce the first true MN-mediated delivery; silicon MNs that demonstrated the successful delivery of calcein across human skin. Since then, advancement in the microelectronics and microfabrication technologies has led to faster development of the different forms of MN from various non-biodegradable and degradable materials. MNs have been demonstrated to be efficient in the delivery of many therapeutic agents across biological barriers, including skin, sclera, and mucosal tissue. It has also been reported recently, that MN arrays can be utilised to not only transport small molecules but, can be used to transport larger molecules and even nano/microparticles [33,54,73].

Dissolving/polymeric MNs have also been reported and, are known to be made from many biocompatible materials such as sugars and polymers. The most commonly employed polymers that are used in the fabrication of MNs are; PLA, polyglycolic acid (PGA), PLGA, poly(vinyl alcohol) (PVA), poly(vinylpyrrolidone) (PVP) and carboxymethyl cellulose (CMC) [32,35,38]. Polymeric MN has gained research interest recently, because of their simple micromoulding fabrication technique, in which MNs are prepared using a polydimethylsiloxane (PDMS) mould. PDMS moulds are created as precise inverse-replicates of the master structures. These master structures normally produced from metallic, polymeric or silicone materials that are obtained through techniques such as 3D-printing [74], lithography [75], etching [76], thermal drawing [77] and, laser micromachining [78]. Anisotropic etching of silicon wafer in KOH is a commonly used method for obtaining a sharp pyramidal structure that can be replicated by pouring elastomer, such as PDMS, which may then be further used as a production mould for obtaining a large number of MN patches [79]. Nowadays, simple and flexible manufacturing technique, two-photon polymerisation based 3D printing was employed to fabricate various size and shape of MN templates with high precision and low cost in a short time [74]. PDMS is a hydrophobic flexible material, which can be used to accurately reproduce the master structure as a negative template [36,80]. PDMS moulds are typically fabricated by pouring PDMS solution over the master structure and allowing the

polymer to cure at 60-80°C for less than 6 h or overnight at room temperature, depending on the specific protocol provided by PDMS supplier [74,81]. Another advantage of using PDMS moulds is that the master template can be re-used for MN fabrications after thorough cleaning of the mould's cavities. The principal benefit is the low cost of polymeric materials and their relatively simple fabrication by micro-moulding processes at ambient temperatures, which should allow for straightforward scale-up to industrial mass production [36].

Another type, Coated MN, has been relatively well documented in the literature or the delivery of potent compounds and vaccines [82]. Various fabrication techniques have been utilized to efficiently coat the individual MN for the fabrication of metal or polymer-based coated MNs including dip coating [83] spraying [84], gas-jet drying [85], and inkjet printing [86,87] and electrohydrodynamic atomization (EHDA) [88]. However, inconsistency arises in the uniformity and accuracy of the dosage coating of MNs, which may overcome by controlling coating formulations such as solution viscosity and contact angle [89,90]. Layer-by-layer coating techniques have also used for temperature-sensitive molecules such as DNA and proteins. This is achieved by coating the negatively charged proteins or DNA molecules onto the metal or polymer base mould and, the cationic charged polymer solution was, in turn, dipped to form a polyelectrolyte multilayer [91].

The manufacturing methods for hollow MNs utilised the wide range of fabrication technique include MEMS [92], lithography methods [93], laser cutting [94], laser ablation, metal electroplating[95] isotropic and anisotropic etching [96], injection moulding, surface/bulk micromachining, polysilicon micromolding, and additive manufacturing (AM) technologies [97], that more commonly known as 3D printing such as stereolithography (SLA) [98] and two-photon polymerisation [74].

Figure 3. Drug release mechanisms from eight different MN types that used to facilitate intradermal drug delivery (a) Solid MNs are used to puncture the skin and are then removed, followed by application of a drug/ nanoparticles formulation. (b) Dissolvable MNs for rapid or controlled release of the drug incorporated within the MNs. (c) Nano/microparticles loaded dissolvable MNs; for intradermal implantation of drug-loaded nano/microparticles (d) Bilayer separable MN designs; drug-containing biodegradable needles are separated from the backing layer and deposited/implanted in the skin for slow drug release. (e) Drug/ nanoparticles coated solid MNs are used to puncture and skin and are placed therein. (f) Hollow MNs used to puncture the skin and enable the release of a liquid drug following active infusion or diffusion of the formulation through the needle bores. (g) Hydrogel-forming MNs take up interstitial fluids from the tissue, inducing diffusion of the drug located in a patch through the swollen microprojections. h) Bioinspired MN; MNs with backwards-facing barbs that are mechanically interlocked with the skin/ocular tissue following their insertion.

4. MNs-assisted delivery of long-acting formulations

MNs have received a great deal of attention as a minimally invasive delivery system to deliver long-acting formulations into the biological tissues including the skin, the ocular and vaginal tissues. While pre-clinical and clinical development of MNs formulations mainly focuses on bolus drug delivery, recently MNs explored for sustained delivery to achieve long-acting treatment [99]. Particularly, polymeric MN shifted focus to controlled intradermal drug delivery because of tuneable properties of biodegradable polymers. MNs prepared from such biocompatible polymers are mechanically strong enough to penetrate the skin layers to deliver the contents intradermally where it can enable therapeutic dosing by manipulating the release

kinetics of pre-loaded drugs. Sustained release from delivered MNs depot can be achieved by modifying the degradation and swelling profiles of the polymer composition and the diffusion profiles of the encapsulated drugs [100].

MNs-assisted delivery of long-acting drug formulation has several potential clinical benefits as documented in many references [1,15,32,35,101], these include:

(1) Unlike, conventional long-acting parenteral therapeutic delivery often necessitates surgical implantation or hypodermic needle injection, MN-assisted delivery of sustained-release formulations offer the potential of self-administration and as such allows the patients to apply the MNs at home without the need for trained clinical personnel.

(2) It does not require sophisticated set-up or electrical supply which further lowers cost and complexity compared to other minimally invasive transdermal delivery methods.

(3) MNs-assisted sustained drug delivery can minimise drug administration frequency and therefore, decrease cost. Minimising the administration frequency, thus it enhances patient compliance through improved convenience and reducing forgotten doses and lessens the risk of infection at the site of administration, especially when compared to intravenous (IV) or intramuscular (IM) injections.

(4) It helps eliminate the well-established limitations associated with hypodermic needles such as improper needle disposal and inappropriate re-use, which are of particular concern in the developing world.

(5) MNs-based sustained release systems allow using less amount of total drug to treat a diseased condition. Additionally, by reducing the total amount of drug, it can decrease in systemic or local side effects as well as the overall cost of treatment.

(6) Sustained-release MN makes it an excellent option for localised skin or ocular disease conditions because the drug is administered locally, right to the target site, only a small amount of the drug is needed.

4.1. MNs design considerations for high drug loading and long-acting delivery

In general, the main purpose of MNs is to penetrate the skin or, any other biological tissue without breaking or bending [102]. The optimal MNs design should provide a low insertion force and high fracture force. For hydrophilic drugs, MNs that can only pierce the outermost layer of the skin would be sufficient to enhance transdermal drug delivery and drug-loaded in the MNs baseplate can be delivered by diffusing through the microchannels created by the MNs [103]. However, this is not the case for hydrophobic and long-acting formulations. For such drugs, the MNs should be able to inoculate the drug inside the skin. Consequently, any drug particles other than those loaded in the inserted part of the MNs projections will be wasted. Therefore, an appropriate and suitable MN system requires careful consideration of the MN geometry that further allows, sufficient mechanical properties, maximum insertion capability and, high drug loading per unit area to maximise the amount of drug which could be delivered per unit area. Moreover, MNs should be layered so that the drug should be loaded in the MNs projection, specifically in the inserted part of the projection, to minimise drug wastage and maximise the MNs delivery efficiency [104,105]. Since the dissolving MNs are the most used in delivering the long-acting formulations, therefore, this will be the focus in this section.

MNs mechanical properties, insertion capability and, drug loading capacity are governed by their geometry, including MN tip` radius, microneedle height (H), base width (W), aspect ratio (AR), interspacing (microneedle side- to side at the base) (IS) and shape [106]. Additionally, the materials used in the fabrication of the MNs and the skin properties further play a remarkable effect [106]. The inherent elasticity of the skin is, obviously, still a major challenge to the reproducibility of MN penetration. Some studies showed that, depending on the MNs' length, the skin could fold around them, leading to the partial or incomplete piercing of the SC [107]. In the literature, the most common shape used for microneedles design is either conical or pyramidal, with heights between 50 μm and 2000 μm , base width ranging from 50 μm to 500 μm and an interspacing of 50–600 μm [106]. A careful selection of these parameters is essential to produce functional MNs with the highest possible drug loading, maximum insertion capacity and acceptable mechanical strength, potentiating drug delivery efficiency.

In terms of the MNs mechanical strength, Lee *et al.* found that polymeric MNs with pyramidal shapes exhibited better mechanical strength than those with conical shapes, likely due to their larger cross-sectional area at the same base width/diameter [35]. With the same MN shapes, the mechanical strength of MNs could be further improved by increasing the base width/diameter (i.e. decreasing the aspect ratio). Park *et al.* found that the failure force increased with shortening of the tip length and widening of the base width/diameter [108]. Later, Chen *et al.* showed that pyramidal chitosan MNs with the smallest aspect ratio exhibited the highest mechanical strength and the deepest insertion depth [109]. Gittard *et al.* also indicated that lower failure forces were required with increasing aspect ratios. Besides, Chen's group showed that there is no significant difference in mechanical strength for MN with the same aspect ratio but different dimensions [110]. This suggested that both shape and aspect ratio of MN may be the most crucial parameters in influencing their mechanical properties.

Regarding drug loading per unit area, this can be increased by either increasing the MNs density per MNs unit area, by reducing the interspacing [111,112] or increasing the length and width of the needle [105]. However, it should be noted that the undue widening of needle bases to decrease the aspect ratio or microneedles density per MNs unit area would increase the difficulties in achieving efficient skin insertion [57,105]. In one study, Larrañeta *et al.* showed that MN arrays with 361 (19x19) pyramidal MNs (600 x300 x 50 μm) (LxWxIS), have much-reduced insertion capability in a skin-simulant membrane- Parafilm M[®] and a neonatal porcine skin (around 50% of the height of the MNs) in comparison with another MN array with 121 (11x11) conical MNs (600 x300 x 300 μm) (LxWx IS), (insertion >65% of the height of the microneedles) [112]. Also, an undue increasing needle length to increase the aspect ratio >2 would reduce the mechanical strength of the MNs [106].

Concerning MNs insertion capability and drug delivery efficiency, this can be enhanced by decreasing the needle tips radius, increasing the needle length or the interspacing, [106]. In the case of the needle tip radius, it is reported that the skin can be punctured more easily when the MN tip sharpness is increased. Chen *et al.* showed that chitosan MNs with a tip radius of 5 μm were capable of achieving an insertion depth twice those with a tip radius of 10 μm . The authors reported that polymeric MNs with a pyramidal shape, smaller aspect ratio and, sharper tips exhibit good skin insertion [109]. Overall, it has concluded that the most desirable MN width and interspacing to attain good microneedles insertion and reasonable drug loading per unit area is 300 μm and the ideal aspect ratio for good mechanical strength is 2 [106,110,113].

To improve MNs insertion capability, drug loading and drug delivery efficiency, and maintaining good mechanical strength, the Donnelly Research Group developed new MNs designs. In one study, Tekko *et al.* developed MNs design consisting of 256 (16x16) pedestal MNs/ 0.5 cm² array with interspacing of 100 μ m. Each MN measured 850 μ m in height and composed of a cuboidal base (Lx W: 250 x 300 μ m) and pyramidal tips (Lx W: 600 x 300 μ m) (Figure 4B) [104]. This design was used to prepare bilayer MNs with high drug loading (3 mg/array) of a hydrophobic commercially available cabotegravir nanosuspension loaded in the pyramidal tips only. Upon applying 32 N/ array comparison force, the MNs exhibited excellent insertion capability in Parafilm M[®] and excised neonatal porcine skin (approximately 90% and 80% of the pyramidal tips inserted, respectively) and the amount of drug delivered was around 12%. Consistent results were also reported upon using this MNs design to deliver other hydrophobic drugs [105,114]. To increase the amount of delivered drug further, Tekko *et al.* developed another new MN design with 256 (16x16) full pyramidal MNs (800 x300 x 100 μ m) (LxWx IS) (Figure 4A) [15]. Using PVA/PVP as a polymeric matrix, bilayer MNs with high drug loading of methotrexate nanocrystals (around 2.5 mg/ array) were fabricated. These MNs exhibited sufficient mechanical strength and good insertion capability (>70% of the height of the microneedles) and around 600 μ g of the loaded drug (25% of the dose) was delivered in vivo. Again, similar results were also reported upon employing this MNs design to deliver another hydrophilic drug- crude micronized cabotegravir sodium [105]. These two MNs designs, in addition to the other three, have been evaluated to deliver a hydrophobic drug model- micronized cabotegravir sodium [105]. Within this study, the authors, created another pedestal MNs designs bigger interspacing (300 μ m) and a fewer number of needles per unit area (11x11 microneedles) (Figure 4C). Another three conical designs; full conical MN(Figure 4D), MNs with cylinder base and conical tips (Figure 4E), and MNs with longer full conical MNs (1300 μ m) and wider diameter (500 μ m) (Figure 4F), were created to identify the MN properties. In terms of the insertion capability, the authors concluded that the MNs with full conical MNs exhibited slightly better insertion capability than the full pyramidal MNs of the same geometry. Increasing the length and the width of the conical MNs led to efficient insertion yet requiring the application of higher forces. Adding a cuboidal or cylindrical base to the needles did not significantly change the insertion efficiency of those MNs. Increasing the interspacing in the pedestal MNs was slightly beneficial in enhancing the insertion efficiency of the MNs. This is most likely due to the smaller aspect ratio (≤ 2) of the tips in comparison with those MNs with full conical and full pyramidal microneedles (>2). In terms of drug loading the amount of delivered drug per unit area in an excised neonatal porcine skin, the

study revealed that the pedestal MNs (256 microneedles) has significantly higher drug loading (around 3 mg/array) than other MNs designs. However, the amount of delivered drug was around (400 $\mu\text{g}/\text{array}$). Again, the MNs with 256 full pyramidal MNs and 256 full conical MNs delivered significantly higher drug amounts (around 600 $\mu\text{g}/\text{array}$) in comparison with other MNs designs. Although pedestal MNs and the full conical and full pyramidal MNs of the same geometry showed comparable insertion capabilities, the pedestal MNs was less efficient in delivering the drug in the skin. This is probably due that the pedestal MNs are affected by the skin elastic behaviour, which promotes the expulsion of the needle tips and therefore lower amounts of the drug been delivered in the skin.

Figure 4. SEM images of six polymeric MN array designed by 3D printed MN templates (0.5 cm^2) with different geometries. A) Full pyramidal (850 μm) 16x16 projections. B) Cuboidal base (250 μm) and pyramidal tips (600 μm in height) 16x16 projections. C) Cuboidal base (250 μm) and pyramidal tips (600 μm) 11x11 projections. D) conical (850 μm) 16x16 projections. E) Cylindrical base (600 μm) and conical tip (350 μm) 14x14 projections. F) Full conical 5x5 projections [74].

Based on the above-mentioned studies, it can be concluded that the optimum MNs, which could be used to deliver long-acting formulations, should fulfil certain criteria including:

- (1) It should be layered, where the drug-loaded only in the MNs projections, specifically in the inserted part of the projections, and no drug in the baseplate, as this would further minimise drug wastage.

(2) It should be prepared from robust materials to impart sufficient mechanical properties for insertion.

(3) It should be prepared from biocompatible/biodegradable polymers with MW <60000 Da, which allows fast clearance from the body after deposition.

(4) It should be long enough for transdermal drug delivery applications without causing pain (<1 mm).

(5) It should be designed to be applied on a specific anatomical location within the body.

(6) It should have a certain degree of flexibility to overcome skin contours

(7) It should present low insertion forces and high break force.

(8) Importantly, the MNs geometry should overcome the skin deformation and allow maximum insertion capability and high drug loading per unit area.

MNs with long projections (800-1000 μm), appropriate projection length/width aspect ratio (between 2-3) and interspacing of around 100 μm could be one of the options, but focused attention to the mechanical properties should be paid, to avoid such issue, MNs with pedestal MNs with the same geometry could be used [104,105]. Another critical factor that should be considered in MN design is the application method. There are two main methods of application, manual and applicator based. The range of forces that both methods can apply should be taken into consideration.

Table 1: MN-based long-acting therapeutics in pre-clinical development

Drug/protein Molecules	Type of MN and materials	Duration of release	Application/ Indication	Ref
PLGA/PLA based separable MN				
Levonorgestrel	PLA arrays with bubble and PVA-sucrose backing layer	1 month (<i>In vitro and in vivo</i>)	Contraceptive	[45]
Levonorgestrel	PLGA arrays with effervescent layer and PVA-sucrose backing layer	1 month (<i>In vitro and in vivo</i>)	Contraceptive	[47]
Levonorgestrel	Trehalose based porous PLGA arrays with PVA-PVP MN as a backing layer	2 weeks (<i>In vitro and in vivo</i>)	Contraceptive	[115]

Etonogestrel	PLGA arrays with PVA-PVP as a backing layer	2 weeks (<i>In vitro and in vivo</i>)	Contraceptive	[116]
Vitamin D3	PLGA-PLGA nano/microparticles loaded PVA-PVP MN	sustained over 5 days (<i>In vitro</i>)	Micronutrient	[39]
Doxycycline	PLGA/PCL nanoparticles loaded dissolving PVA-PVP MN	48 hrs (<i>In vitro</i>)	Skin infection	[117]
Drug micro/nanocrystals loaded MN				
Rilpivirine	Pure drug nanoparticles loaded PVA dissolving MN	1 month (<i>In vivo</i>)	Anti-HIV	[118]
Curcumin	Pure drug nanoparticles loaded PVA-PVP dissolving MN	15 days (<i>In vitro</i>)	Natural compound with a wide range of application	[119]
Methotrexate	Pure drug nanoparticles loaded PVA-PVP dissolving MN	72 h (<i>In vivo</i>)	Treatment of psoriasis	[15]
Doxycycline, albendazole, and ivermectin	Pure drugs nanoparticles loaded PVA-PVP dissolving MN	24 h (<i>In vitro and in vivo</i>)	Antifilaria drugs	[5]
Cholecalciferol	Pure drug nanoparticles loaded PVA-PVP dissolving MN	24 h (<i>In vitro</i>)	Micronutrient	[40]
Polysaccharide/protein polymer based slow dissolving MN				
Levonorgestrel	Silk fibroin microparticles loaded or pure silk fibroin MNs	Up to 100 days (<i>In vitro</i>)	Contraceptive	[120]
OVA	Sodium Hyaluronate/Chitosan Composite arrays with PVA/PVP backing layer	4 weeks (<i>In vitro and in vivo</i>)	Model antigen for vaccine	[121]
OVA	Chitosan MNs with PVA/PVP supporting array patch	28 days (<i>In vitro and in vivo</i>)	Model antigen for vaccine	[122]
OVA	Chitosan MN tips with PVP coated PLA supporting arrays	up to 14 days (<i>In vitro</i>)	Model antigen for vaccine	[100]
BSA	Chitosan MN	up to 8 days (<i>In vitro</i>)	Model protein	[123]
Isothiocyanate-dextran	Crosslinked Hyaluronic Acid nanoparticles loaded MN	up to 8 days (<i>in vivo</i>)	Model compound	[124]
Insulin	Pure silk fibroin MN	Up to 60 h	Diabetes	[125]

		(<i>In vitro</i>)		
Lipidic nanocarriers loaded/coated dissolving MN				
Cisplatin	NaCMC dissolving MNs coated with lipidic drug nanoparticles	72 h (<i>In vitro</i>)	Anticancer	[126]
doxycycline, diethylcarbamazine and albendazole	Solid lipid nanoparticles loaded PVP MN	48 h (<i>In vitro and in vivo</i>)	Antifilaria drugs	[127]
Hydrogel forming MN with drug reservoir				
Atorvastatin calcium trihydrate	Liquid drug reservoir with poly (methylvinylether-co-maleic acid) crosslinked hydrogel MN	24 h (<i>In vitro</i>)	Cardiovascular diseases	[128]
metformin hydrochloride	Lyophilised drug reservoir with poly (methylvinylether-co-maleic acid) crosslinked hydrogel MN	24 h (<i>In vitro and in-vivo</i>)	Diabetes	[64]
Methotrexate	Patch drug reservoir with PVA/PVP-based hydrogel-forming MNs	24 h (<i>In vitro and in-vivo</i>)	Rheumatoid arthritis (RA)	[129]

585

586 4.2. MNs formulation approaches for long-acting drug delivery

587 Lee *et al.* for the first time proposed the potential use of dissolving MN for sustained delivery
588 from the backing layer of the MN. They achieved sustained delivery ranging over hours to days
589 by encapsulating a model dye within the MN backing, which served as a controlled release
590 reservoir. This reservoir delivered molecules by a combination of swelling the backing with
591 interstitial fluid extracted out from the skin by inserted MN and molecule diffusion into the
592 skin *via* channels formed by dissolved MN. Moreover, they demonstrated that modifying MN
593 patch matrix composition can modulate the release kinetics in human cadaver skin *ex-vivo*.
594 Thus, MNs offer the important potential to be able to change release kinetics based on patch
595 type and materials, to necessitate different release profiles in order to achieve the optimum
596 therapeutic effect for different drugs administered for various indications [35]. Hormones and
597 other biomolecules (peptides and proteins) often necessitate a sustained release for a prolonged
598 time, which is achievable using polymeric MN technology. PLA, PLGA or poly(caprolactone)
599 (PCL), are often used for sustained drug release because of their different intrinsic properties
600 [39,130]. Slow dissolving MNs are also produced using biodegradable naturally derived

biopolymers, including silk fibroin and chitosan, to form the MN matrix to achieve the sustained therapeutic effect [81,131]. After skin insertion, they slowly degrade/dissolve, then releasing their drug cargo that may be sustained for days to weeks depending on the molecular weight and method of fabrication [35,36]. Dissolving polymeric MN can maintain slower drug release over a prolonged period by loading the particulate (nano/microcrystals) form of drugs, thanks to intrinsic slow dissolving properties of the drug particles [119]. Some approaches where drug-loaded lipidic or polymeric nanocarriers encapsulated in dissolving polymeric MN for intradermal deposition for slower or targeted drug release [39]. MNs can take advantage of chemical or biological triggers, to efficiently turn drug dosing on and off, to manipulate the properties of the polymer network or encapsulated drugs to attain sustained release [132,133]. Some of the MN-based long-acting therapeutics by use of different slow dissolving polymers and nanoparticles that in pre-clinical development is described in Table 1. In the following section, we draw attention to different approaches to achieve controlled/sustained drug release from MNs, either by controlling the release kinetics or targeting specific sites.

4.2.1 Nano/microparticles combined with MN

4.2.1.1 Polymeric Nano/microparticles loaded MN

Polymer-based, controlled release particulate systems include, solid colloidal nanoparticles (10–500 nm) or microparticles ($\geq 1 \mu\text{m}$) and, typically comprise of bioactive materials that are internalized through dissolving, wrapping, or adsorption and adhesion on the surface of the particles [89]. Nano/microparticles offer several benefits over conventional drug delivery systems. They permit sustained drug release over a prolonged period of time while protecting encapsulated materials against chemical or proteolytic degradation [134,135]. A best possible approach to increase the permeability of such sustained-release particulate system through the skin/eye is through the combined use of MNs. The development of combinatorial approaches to maximise the advantages of both particulate and MN delivery systems has increased over the past two decades [11].

Vora *et al.* reported this combinatory approach of PLGA nano/microparticles loaded PVP MN with a model drug (Vitamin D₃, VD₃). The MN bilayer structure was prepared from PLGA nano/microparticles with PVP hydrogel by centrifugation technique, to concentrate the particles in MN arrays (Figure 4a) that allow to deliver most of the drug loaded particles intradermally (Figure 4b). This PLGA nano/microparticle was prepared by single emulsion solvent evaporation technique. The multisize particles displayed

triphasic release kinetics that included initial burst release of drug from nanoparticles and drug on the surfaces of particles as well as sustained-release (more than 7 days) dependent on microparticle polymer degradation (Figure 4e). SEM images (Figure 4d) showed nano/microparticles specifically concentrated in MN tips and compactly packed. There was a discrete line between PLGA particles mixed with PVP polymer and plain PVP polymeric matrix clearly visible. After MN insertion in full-thickness porcine skin *ex vivo*, as shown in optical coherence tomography images (Figure 4c), particles embedded MN exhibited a 4.9-fold higher intradermal drug deposition compared to a needle-free patch [39]. This novel bilayer MN concept with slow releasing drug particles or polymeric particles concentrated tips could assist to deliver the maximum load and avoid the wastage of drug formulation.

Figure 4. PLGA nano/microparticle-loaded bilayer MN arrays for sustained delivery. a) Schematic representation of MN fabrication. b) Schematic representation of MN skin application. c) An optical coherence tomography image of MN inserted in Parafilm M® and neonatal porcine skin. d) Scanning electron micrograph (SEM) of PLGA nano/microparticles-loaded bilayer MNs and e) *In-vitro* drug release from PLGA nano/microparticles. Reproduced with permission [39]. Copyright 2017, Elsevier.

4.2.1.2. Lipidic nanoparticle loaded MN

The studies of lipid-based drug nanocarriers started in the early 1960s with the development of the liposomes [136,137]. It was several decades later when alternative lipid nanocarriers like solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) were invented. All

these methods are designed to encapsulate the drug, protecting it from degradation, controlling its release in a rate and site-specific manner [138–141]. Therefore, their use in conjunction with other drug delivery systems such as MNs results in an attractive new target for scientific exploration, as reviewed in this section. The first liposome-based MN system was reported in 2008 [142]. In an *in vitro* study, the authors pre-treated porcine skin with solid MNs and the applied deformable docetaxel liposomes, showing that the innovative treatment was able to improve the in-skin delivery of the drug. Also using a combination of liposomes and MNs, Chen *et al.*, augmented the plasma exposure of the anti-arthritis drug triptolide in comparison to oral treatment in rats [143]. In this work, the authors were also able to demonstrate an improved efficacy *in vivo*, where both joint swelling and immune markers were significantly reduced. Lee *et al.* evaluated the potential for sustained release of NLCs loaded into MNs [144]. To this purpose, the NLCs loaded with the model drug Nile red was placed into dissolving MNs made of hyaluronic acid. After a 24 h release study performed in Franz cells using dermatomed minipig skin, a radial spread pattern was observed, with 70% of the drug located in the skin. Using a similar approach, aconitine NLCs were loaded into dissolving MNs and assayed in a rat model of arthritis, where a significantly improved efficacy and decreased drug toxicity were observed [145]. The MNs seemed to form an in-skin drug reservoir, suggesting that the novel experimental formulations have the potential to release the active sustainably [28]. An interesting proof-of-concept study reported by Permana *et al.* aimed to target the lymphatic system with the conjunction of SLNs and to dissolve MNs [146]. The lipid-based nanocarriers (size < 100 nm) loaded with the antifilarial drugs doxycycline and albendazole sulphoxide were vehiculised in MNs formed by PVP and PVA and tested *in vivo*. The biodistribution assays revealed that the drugs effectively accumulated in the lymph nodes in concentration levels 4- and 7-fold higher than those observed for the control groups treated with oral formulations.

4.2.1.3. Metallic nanoparticles loaded MN

Inorganic nanoparticles, such as iron oxide nanoparticles (IONPs), gold nanoparticles (AuNPs), silver nanoparticles (AgNPs), quantum dots, silica nanoparticles and, carbon nanotubes, have been extensively investigated for their potential role in drug delivery [147,148]. Some of the unique physical and biological properties of these particles may allow the development of multi-function particles that could be used for diagnostic and therapeutic (“theranostic”) purposes [85]. Magnetic IONPs have been used to deliver ARV drugs due to their unique features, namely, controllable size distribution, biocompatibility, ability to bind

drug and sustain drug release, magnetically-guided site-specific targeting, and the ability to convert magnetic fields to heat energy. Saiyed *et al.* made 3'-azido-3'-deoxythymidine-5'-triphosphate (AZTTP) bound onto magnetic Fe₃O₄ nanoparticles by ionic interaction; the magnetic nanoparticle-bound AZTTP maintained its biological activity in peripheral blood mononuclear cells, and active NRTIs were targeted to the brain by applying an external magnetic force [86]. Magnetic IONPs could also be used for magnetic resonance imaging (MRI) to monitor drug distribution to target tissues *in vivo*. Superparamagnetic iron oxide nanoparticles (SPIONs) have shown to have the ability to track HIV-specific cytotoxic T lymphocytes (CTLs) [88] and to increase the cytotoxic potential of HIV-specific CTLs and improve the killing of HIV-infected cells in latent HIV reservoirs by employing magnetic field hyperthermia effects [89].

4.2.1.4. Nano/micro drug crystals loaded MN

The manipulation of particle size has been primarily used in the pharmaceutical industry to increase the dissolution rate of poorly water-soluble drugs (solubility <0.1 mg/mL) [149]. Firstly, the production of drug microparticles (<10 µm) was achievable mainly using comminution techniques. However, the development of novel formulation methods permitted the formation of drug particles within the sub-micrometre range, namely nanocrystals (NCs) [150]. NCs are drug nanoparticles with crystalline features, with minimal carrier materials or excipients, therefore capable of attaining a drug loading of close to 100% [151]. The term nanosuspension refers to the NCs dispersed in a liquid, whereas solid and re-dispersible NCs-based powders can be obtained by solvent removal [152–155]. NCs can be produced by two main approaches, “bottom-up” techniques, which consists of controlled drug precipitation from a solution, or by “top-down” techniques, which include the application of external shear forces [156]. Distinctive features of NCs include increased saturation solubility, dissolution rate and mucoadhesion, which leads to greater absorption and efficacy [157–159]. These advantages, coupled with the availability of flexible and scalable manufacture techniques, make of NCs an attractive option for the formulation of poorly soluble drugs.

The intradermal administration of micronised drugs and NCs using MNs has been reported in several recent publications. For instance, Vora *et al.* prepared a nanosuspension of the hydrophobic vitamin cholecalciferol by sonoprecipitation technique. The resultant nanosized suspension (average diameter of 305 nm) was loaded into dissolving MNs, which presented a significantly higher (7 fold) drug permeation *ex-vivo*, across dermatomed (350 µm) neonatal

porcine skin over 24 h [10]. Similarly, intending to potentially treat a series of local and systemic diseases, Abdelghany *et al.* loaded curcumin containing NCs into dissolving MNs [160]. In this work, the authors reported enhanced permeation of curcumin from the novel MN systems in comparison to the control (topical application). In a different report, NCs loaded with the antifilarial drugs doxycycline and ricobendazole (mean diameters below 100 nm) were incorporated into dissolving MNs composed of PVP and PVA [161]. *In vitro* studies demonstrated that the experimental MN formulations delivered the actives more efficiently than the control formulation consisting of a needle-free patch. In further studies, the contraceptive drug etonogestrel was loaded into dissolving MNs with the intended purpose of prolonged-release [162]. Here, drug microparticles were blended with aqueous solutions of PVA to cast the MNs. The authors demonstrated the ability of the novel formulations to deliver the drug for one week. Additional studies conducted by McCrudden *et al.* loaded an industrially prepared nanosuspension of the antiretroviral drug rilpivirine into dissolving MNs to develop a sustained-release formulation with improved patient compliance [163]. After successful mechanical characterisation, MNs were tested *in vivo* in Sprague-Dawley rat models, where they exhibited similar, if not superior pharmacokinetic behaviour to an intramuscular injection of the same nanosuspension. Based on the promising results obtained, the same researchers used a similar approach to deliver rilpivirine to the vaginal tissue, for the first time [164]. *In vivo* studies demonstrated that the mean plasma concentrations of the drug at the endpoint of the study (day 56) were similar to that of the cohort treated with the intravaginal MNs (116.5 ng/mL) and IM injections (118.9 ng/mL).

Itraconazole NCs were prepared by media milling using Poloxamer 407 as a stabilizer were incorporated into dissolving MAPs fabricated with PVP/PVA. The novel formulations were successfully characterised in terms of mechanical and physicochemical properties and afterwards tested in an *in vitro* model of skin candidiasis. This experiment demonstrated a significantly increased efficacy against the fungus when compared to control cohorts [114]. The advantages and potential applications of dissolving MNs and nanocrystal technologies have been thoroughly discussed in a recent review article [165].

The sustained release of dutasteride from dissolving MNs was reported by Giffen *et al.* In this work, the authors used a nanosuspension obtained by media milling and a blend of CMC, trehalose and polysorbate 80 to microfabricate the MNs [166]. Following optimisation formulation, a pharmacokinetic trial was conducted in rats and minipigs using IV and intradermal injections as respective controls, where they proved that the MN-based formulation

significantly increased the plasma half-life of the drug. These results together, indicate that the combination of micronised or nanocrystalline drugs with MNs is an appealing strategy for the sustained release of drugs *via* intradermal delivery. Dissolving MNs, in particular, are relatively simple to manufacture and, they have a demonstrated ability to deliver long-acting nanosuspensions importantly, do so without the generation of sharp waste [167].

Recently, Tekko *et al.* combined NCs and MN technology to localise and sustain the release of methotrexate (MTX) as a potential treatment for psoriasis in order to minimise the systemic exposure in order to avoid the drug systemic toxicity and side effects. The authors prepared a poorly water-soluble MTX-NC from its freely water-soluble salt MTX Na by a bottom-up method, namely, acid-base neutralisation accompanied with sonification. The MTX-NCs (average diameter of 700 nm) were then incorporated into the projections of dissolving MNs with a drug loading of 2.48 mg/array. The MNs projections dissolved within 20 minutes post-MNs application and deposited their payload in the skin. An *in vivo* study in Sprague Dawley rats revealed that the MTX-NCs-loaded MNs were capable of depositing approximately 25 % of the loaded MTX NCs in the skin. This in turn then acted as a drug depot and released the drug in a sustained manner over 72 h while minimising MTX systemic exposure in comparison with orally administered MTX Na (control) [14].

4.2.2 Slow dissolving MN

Biodegradable polymeric MNs were developed as a method for achieving sustained transdermal drug release. These MNs have potential as a patient-friendly substitute for conventional sustained release methods [168]. Most commonly used biodegradable MNs are described below, whereby the release of incorporated drugs can be sustained for days to weeks [169].

4.2.2.1. PLGA/PLA MNs

Biodegradable polymers of the aliphatic polyester family are PLGA and PLA, which have already been frequently applied in tissue engineering and drug delivery. PLGA, a copolymer of PLA and PGA, is the best defined biodegradable polymer in the controlled release of encapsulated drug and, is widely used for sustained drug release at desirable doses by implantation without surgical methods [170,171]. Additionally, the overall physical properties of the polymer-drug matrix can be tuned by controlling the relevant factors such as the ratio of lactide to glycolide, drug concentration and, polymer molecular weight to achieve a desired

dosage and release period varying upon the drug type [170]. Furthermore, both polymers are well-defined in safety, with various drug products containing PLGA approved by regulatory authorities and available for use in clinical practice [172]. Thanks to high biocompatibility, strong mechanical properties, and safe profiles, PLGA and PLA have already been employed in MNS fabrication for long-acting sustained delivery. As PLGA and PLA have a high melting point, heating at a high temperature above 135°C under vacuum is generally required to fill the microcavities of the moulds [133,173]. This relatively high temperature makes the fabrication method unfavourable, especially for the delivery of heat-sensitive compounds. MNs fabricated by drug PLGA and/or PLA mixture, are mostly manufactured through the solution casting method. Some of the most commonly employed solvents utilised in this process include: dioxane, tetrahydrofuran [45], N-methyl pyrrolidone [115], dimethylformamide [174], ethyl acetate [48] and dimethyl carbonate [48], diglyme [47] and, DMSO [175]. At times, water may also be added to the process in order to adjust the rate of the evaporation [45]. These organic solvents were removed by evaporation or, at 75–100°C within a vacuum oven [45,115,175]. Another commonly employed method to prepare PLGA and/or PLA MN is spray deposition. Researchers employed a spray disposition process for sequentially spraying of PLGA and PVP solution into the mould. However, it must be taken into consideration, that the use of organic solvent and heating might affect the structure and bioactivity of biomacromolecules during formulation [48,176]. Typically, therapeutics loaded in the PLGA tips are mainly hydrophobic compounds, such as levonorgestrel [45], etonogestrel [116], and rhodamine [46], predominantly the solubility of the mixture of drug and polymer in organic solvents. However, despite this, the hydrophilic β 3-adrenoceptor agonist CL316,243, has been incorporated within the system [174]. Biomacromolecules such as ovalbumin, bovine serum albumin and horseradish peroxidase were also loaded into this type of MN system, and it was found the protein retained 80% of the bioactive despite the addition of organic solvents. Interestingly, one study demonstrated the successful loading of mesenchymal stem cells into PLGA MN shells. This system consisted of an array of MNs with an outer PLGA shell and an internal gelatin methacryloyl-mesenchymal stem cells mixture. This device exhibited enhanced wound healing by localizing mesenchymal stem cells delivery with a minimal dose of cells [177].

To shorten the patch application time and quicken the implantation of PLGA/PLA needle tips, various two-layers and three-layer MN designs were attempted. Most researchers have used the dissolving material as a baseplate to separate them from insoluble PLGA/PLA tips [45,116,174]. As the upper part of the water soluble tips dissolves in the skin fluid, the PLGA

tips were separated and implanted inside the skin. The freeze-drying method is also employed to make the baseplate backing layer more hygroscopic to fasten the dissolution time required for quicker PLGA/PLA tips separation [46]. Prausnitz *et al.* recently developed rapidly separating/biodegradable MN, made of PLA and PLGA for the continuous release of levonorgestrel (LVN). In this study, the authors attempted to develop an air entrapped bubble structure between each PLGA/PLA MN and the water-soluble patch backing while fabricating the patches (Figure 5ia). Bubble structures allowed the MN to penetrate the skin upon compression, and to be ‘snapped off’ within 5 seconds of application. The MNs then remained embedded beneath the skin surface. The drug release is then controlled by a combination of diffusion and matrix erosion. The authors demonstrated that rats tolerated the MN patch and that upon removal it left a little visible residue, and that it maintained plasma concentrations of the hormone above the human therapeutic level (200 pg/ml) for one month (Figure 5ib) [45]. Moreover, the authors evaluated effervescent formulations that sandwiched between the PLA MN tips and the patch for *in-situ* effervescent generation and faster separation during skin insertion (Figure 5iia) [47]. Upon application to the skin, the PLGA and PLA MN tips implanted in the viable tissue, depositing the therapeutic cargo, from which sustained drug release is achieved (Figure 5iib). The release time has been reported to vary from days to 3 months [45,46,178]. The release rate of the PLGA/PLA tips could be affected by the amount of drug loading [178], the polymer chain composition [174] and, the addition of porogens, for example, trehalose [115]. It was found that the 10% w/w drug (2-methoxyestradiol) loaded PLGA MN implants released the drug completely within 8 weeks, whereas the 25% w/w drug loaded MN tip implants maintained therapeutic levels (0.1–1 mM) for 90 days. As reported by Aung Than *et al.*, drug-release kinetics of PLGA-MNs loaded with Cy5 could be readily modulated by choosing or mixing different PLGA molecules with different structure, chain length, and copolymer ratio [174]. Moreover, the addition of trehalose as a porogen in PLGA MN tips could tailor the faster release of LVN [115].

The most challenging issue with this system is the limited dosage. As reported in the literature, the dose loaded in a 10×10 or 11×11 arrays/ 0.5 cm^2 , were not able to exceed 0.5 mg [45,116]. Because of this, only very potent drugs such as hormones are appropriate for delivery by this system [39]. Vaccine antigens were also loaded in the system. However, the use of organic solvent or heat might adversely affect the protein structure. Localised therapies are also recommended for this system for future study.

Figure 5. A rapidly separable PLGA/PLA MN patch for the prolonged delivery of the contraceptive LVN, i) a) Bubble ii) a) effervescent MN design with the skin application process for sustained release of a contraceptive hormone. ia) and iib) Concentration of LVN in the plasma of rats after administration of the LVN-loaded MN patches with respective MN designs, as a function of time. Reproduced with permission [45,47].

4.2.2.2 PCL MNs

As a biodegradable and non-toxic material, PCL has been extensively utilised in a wide range of drug-delivery devices, notably in the biomedical applications of controlled-release drug delivery systems, absorbable surgical sutures, and three-dimensional scaffolds [179]. Attempts have also been made to utilise PCL for MN fabrication owing to its good solubility, low melting point, and exceptional blend-compatibility [180]. Despite a relatively low water solubility, PCL exhibits favourable solubility in organic solvents. Therefore, the solution casting method has been widely used in PCL MN fabrication. Generally, PCL is mixed with the drug in chloroform, acetone, or dichloromethane and, methanol. After the solution is cast on the moulds, the organic solvents are evaporated. A separable baseplate is often added to the PCL tips to enable the complete implantation of PCL drug-loaded tips. PCL/PEG blends tips MN were fabricated using the solution casting method. One of the most attractive advantages of PCL is its low melting point. The relatively low melting point of PCL made it possible for hot embossing PCL and drugs into the MN. PCL MN containing 20% furosemide was fabricated by the hot embossing method, and the release of furosemide was demonstrated by diffusion [181]. The low melting point of inserted PCL MN tips also provides opportunities for triggered

release behaviour to manufacture the stimuli-responsive MN. The inserted PCL MN tips were able to transit the thermal phase from a solid to a liquid at a relatively lower temperature such as 50°C. The thermal phase transition of PCL increases the mobility of the polymer chain and triggers drug release from PCL [182]. An electron-heating sheet was used to provide the heat to change the phase of implanted PCL MN tips. The melting of PCL tips was able to thermally modulate the release of the encapsulated metformin [183]. Besides, the heat for melting PCL tips can also be provided by near-infrared light and photosensitive materials. Photosensitive materials such as lanthanum hexaboride [184,185], silica-coated lanthanum hexaboride [182,186], Prussian blue nanoparticles [187], and Cu₇S₄ nanoparticles [130] were entrapped inside the PCL tips with drugs such as metformin [187], lidocaine [185] or DOX hydrochloride [182,184,186]. With the activation of near-infrared light, photosensitive materials were able to produce enough heat to melt the PCL tips. Thus, the release behaviour of the drugs can be precisely controlled by switching on and off the near-infrared light. Furthermore, magnetic nanoparticles can produce heat under an alternating magnetic field due to hysteresis loss. Biodegradable Fe₃O₄/PCL MNs were developed for potential skin cancer treatment with transdermal controlled drug delivery and magnetic hyperthermia [188].

4.2.2.3. *Silk MNs*

Silk-based biomaterials have excellent mechanical properties, biocompatibility, biodegradability, and the capability to protect and maintain the activity of biomolecules [189,190]. The degradation rate of silk-based materials and the diffusion rate of the encapsulated molecules can be self-controlled by adjusting post-processing conditions through the silk protein secondary structure [191]. Another advantage is that biomolecules or labile drugs can be incorporated into the silk-based matrix as a result of the aqueous and mild processing conditions by nano and microfabrication [14]. It is also well proven that biomolecules can be stabilised in dry silk films for extended periods [192]. The attractive properties afforded by silk biomaterials make it an ideal choice for the MN-based sustained and long-acting delivery system [131].

896

897 Figure 6 i) A) Schematic illustrations of sustained transdermal delivery of antigen using MN delivery system,
 898 composed of embeddable chitosan MNs and PLA supporting array. B) Bright-field micrographs of ovalbumin-
 899 loaded (b–b2) chitosan–PLA MN arrays/ C) In vitro release profiles of OVA-loaded MN with or without
 900 trehalose, conducted using Franz diffusion cells. Reproduced with permission [81] Copyright 2017, Elsevier. ii)
 901 A) SEM micrographs showing the structure and size of LVN -loaded silk MNs. B) *In vitro* release profiles of
 902 LVN from silk MN patches in (a) 1 mL PBS, (b) 200 mL of 0.5% SDS solution. Reproduced with permission
 903 [120] Copyright © 2020 American Chemical Society.

904 Yin *et al.* developed a 2-ethoxyethanol (ECS) modified silk fibroin (SF) MN with swelling
 905 characteristic to generate 3-dimensional (3D) porous network for sustained transdermal
 906 delivery. This modified SF MN was strong enough to pierce excised porcine skin and transform
 907 into semi-solid hydrogels with 50–700 nm porous network inside and, thus, provide
 908 substantially better transdermal drug release [193]. The release rate from silk-based MNs can
 909 be further regulated by altering the crystallinity. Wang *et al.* used the nontoxic proline treated
 910 SF to obtain MNs with Silk I crystal structure (random coils to β -sheets). Within this study,
 911 different ratios of proline to change the crystallinity of fibroin were evaluated, in order to
 912 improve the needles' swelling capacity upon encounter with the intradermal interstitial fluid.
 913 The needles were strong enough to penetrate the SC and created the microchannels. *In vitro*,
 914 insulin release experiments showed that the release rate of proline/fibroin MNs increases based

on the degree of swelling. The cumulative insulin release rate reached up to 80% with more than 60 h long sustained release that indicates a relatively high drug utilisation rate. Thus, potentially offering an effective and painless potential treatment route for diabetic patients [125]. Tsioris *et al.* reported a fabrication method to produce silk biopolymer microstructures with the necessary high aspect ratios required to fabricate MNs. Room temperature and aqueous-based micromolding allowed for the bulk loading of these MN structures with temperature-sensitive drugs such as peptides and vaccines, antibiotics, or any temperature-labile drug molecules. Controlled release of a model drug is attained by modifying the postprocessing conditions of the MN structures, mainly by controlling the silk protein secondary structure [131]. Yavuz *et al.* recently fabricated silk protein MN for the long-acting transdermal delivery of contraceptive hormone, LVN. Within the study, a comprehensive approach with silk MN was taken to tailor the release from months to more than a year (Figure 6ii). The authors achieved different sustained release kinetics by different loadings of the drug, *via* pre-encapsulation in silk microparticles or, freely inside the silk MNs. Sustained drug release extended up to 100 days when the drug was loaded directly inside the silk MN. However, the release continued for more than a year when the drug was encapsulated inside silk microparticles prior to fabricating the MN. As depicted in Figure 5B(a), drug release was controlled by drug solubility and no significant differences were found between the drug-loaded microparticles in the MNs and the drug-loaded MNs in terms of release rates under non-sink conditions. However, under sink conditions, the effect of the microparticles on release rate was observed over the same period of time (Figure 6B(b)). They also employed different molecular weights of silk as well as different silk concentrations (7 and 10%) to provide more control over the release profile. The higher the concentration of silk resulted in a slower release rate of the drug from MNs (cumulative release of $25.3 \pm 1.5\%$ after 100 days). However, there was no significant effect on the release profile of the drug (cumulative release of 40% to 50% after 100 days) from MN by varying the molecular weights of silk. Not only that, they studied the effect of solubility enhancers such as β -cyclodextrin (5% w/v) and a surfactant (Tween-20, 1% v/v) with the silk-drug solution (before casting the MN) on the release profile of the drug. Both formulations managed similar release profiles with comparatively enhanced release rate (~60%) compared to the silk MN without these drug solubilisers. Interestingly, all different MN formulations released LVN level above the require daily contraceptive dose (30 $\mu\text{g/day}$) [120]. These results indicate that long-acting contraception MN array patches are feasible to fabricate.

949 Chitosan has been commonly used as a material in the manufacture of both nanocarriers or MN
950 for sustained release because of its well-known biocompatible and biodegradable properties
951 [123]. Another advantage of the inherent cationic charge of chitosan is that it promotes
952 interaction with negatively charged functional groups on proteins or other drug molecules
953 [194]. These unique characteristics, combined with its desirable film-forming properties, make
954 chitosan appropriate as MN material for sustained delivery, especially for biomolecules [195].
955 In addition to the sustained release, chitosan possesses immune-modulating /stimulating
956 activity, which can act as immunoadjuvant for vaccine/gene delivery [196]. Xie *et al.* reported
957 a model molecule, calcein and bovine serum albumin (BSA) coated with different concentration
958 of chitosan solution on solid MNs. The study evaluated the effect of varying matrix thickness,
959 drug content and the matrix concentration of chitosan film upon the effect of sustained release
960 [197]. Chen *et al.*, first introduced in 2012, a chitosan MN patch for efficient and sustained
961 transdermal delivery of the model protein BSA with enough mechanical strength for successful
962 skin insertion. Investigation of the *in vitro* drug release of BSA from chitosan MN
963 demonstrated sustained release of BSA for 8 days. This chitosan MN did not affect the
964 secondary structure of BSA, and when the Alexa Fluor 488-labeled BSA loaded MN was
965 applied to the rat skin *in vivo*, the fluorescence of Alexa 488-BSA was observed at a depth of
966 300 μm under confocal microscopy. These proof of concept results suggested that the
967 developed chitosan MN could be potentially useful for transdermal delivery of macromolecules
968 in a sustained manner [123]. In a further study conducted by Chen *et al.*, fully embeddable
969 chitosan composite MNs with PLA supporting as a complete and sustained release depot of
970 encapsulated antigen for intradermal vaccination was evaluated (Figure 6i). Chitosan MN
971 separated from the PLA supporting array during insertion to rat skin *in vivo*, from which
972 sustained the release of OVA for over 14 days without the need for a transdermal patch. The
973 MNs fully penetrated the skin at a depth of 600 μm to target the model OVA antigens to
974 antigen-presenting cells in the epidermis and dermis [81]. Additionally, Chen *et al.* further
975 examined the capacity of chitosan MN for low-dose immunisation with sustained manner. The
976 system was composed of model OVA antigen-loaded chitosan MNs and PVA/PVP supporting
977 array as an additional base to attain superior MNs insertion into the skin. The chitosan MNs
978 exhibited a sustained antigen release for up to 28 days. In rats, applied chitosan MNs with low-
979 dose antigen had persistently high antibody levels for 18 weeks, greater IM injection.
980 Furthermore, OVA encapsulated chitosan MNs had higher immunogenicity compared to OVA

added chitosan solution [122]. Marin *et al.* fabricated MNs from CMC and chitosan. The fabrication was achieved by a polyelectrolyte complex mechanism based on the electrostatic interaction between anionic CMC and cationic chitosan. They described that the MN exhibited decreased hydration, thus slowing the protein release from the polyelectrolyte chitosan coating [198].

4.2.3 Metal MNs

Metallic MNs, are typically fabricated with stainless steel, gold, silver, metallic glass [199] or based on bio-composites and, are commonly used as hollow, coated, or solid MNs [200]. Hollow metallic MNs are usually used to deliver liquid formulations while coated MN with a small amount of a substance such as vaccines, proteins and potent drugs [201–203]. Another popular approach is named as micro-needling for cosmetic purposes, including the percutaneous induction collagen therapy [204]. However, to achieve long-acting release it is necessary to associate the metal MNs with a structured nanotechnology formulation [34,45] or, novel approaches as a highlighted by conductive MNs [205], which represent some of the most promising innovative approaches described for metallic MN patches. In an attempt to develop a new drug delivery system for short or long term release, Guoet *et al.* developed a new conductive polymeric MN containing a working electrode system integrated to an inert metal layer and a drug-loaded conductive film layer, with the purpose to deliver the drug through electrical stimulation [205]. The poke and patch approach was applied by Nayak *et al.* resulting in the extended-release of lidocaine microparticles, formed by electrostatic interaction between a hydrogel and water/oil emulsion under covalent crosslinking by glutaraldehyde. Stainless steel MN arrays were used to deliver lidocaine microparticles, achieving 2-fold delivery improvement when compared to the application of the same formulation without preceding treatment of the skin using metal MNs [203]. Koutsonanos *et al.* developed metallic MN coated with subunit influenza vaccine solution by a dip-coated method. This MN system was capable of achieving improved immune responses, conferring improved long-term protection in an *in vivo* mouse model compared with IM injection. Therefore, this MN method of vaccine delivery may present as a potentially beneficial approach to increase vaccine coverage and protection. The long-acting effect was observed after 36 weeks post-immunisation, where the MNs group exhibited 100% survival against 40% of mortality in the IM group [206]. The coated method is a successful strategy to penetrate the skin and easily release the compound through the skin layers, due to the small amount of drug in the tips, favouring the compound diffusion [207].

4.2.4. Coated MN arrays

The use of coated MNs for long-acting formulations is challenging due to their reduced drug loading capacity. In these MN formulations, the active substance may only be contained in a thin layer, which covers the surface of the MNs. Therefore, the potential application of this approach is limited to the use of highly potent agents. Despite this, some methods have been explored to control the release rate of coated MNs using diverse polymeric coatings. For example, Xie *et al.* coated MNs prepared by micro-electro-mechanical systems (MEMS) with a nanometre chitosan film loaded with the model drugs calcein and BSA [197]. Permeation studies demonstrated that after insertion in rat skin, the hydrophilic polymer permitted to form a matrix system through which the drug was released in a controlled manner. A similar concept was explored by Marin *et al.*, where they obtained CMC MNs which were subsequently coated with a chitosan layer to form a polyelectrolyte complex between both polymers, thus leading to potential applications on sustained drug release [198]. In different work, lidocaine was blended with dextran and loaded onto solid MNs by dip-coating. *In vivo* trials in domestic swine demonstrated that the drug rapidly dissolved and achieved therapeutically relevant concentrations within one minute after the MN application. Moreover, the in-skin concentration of the drug was found to be above the therapeutic threshold for approximately 90 minutes [208].

4.2.5. Stimuli-responsive smart MN arrays

Over the last decade, stimuli-responsive polymeric biomaterials have emerged as effective systems for therapeutic delivery. The design of the material may be used to tailor smart polymers to release therapeutic payload when particular stimuli are present, which may be either internal or external stimuli triggering. As illustrated in Figure 7, internal stimuli-responsive smart biomaterials include those that respond to specific enzymes [209], mechanical force [210] or changes in microenvironment pH [211]; external stimuli can consist of electro or magnetic fields [212,213], light [214,215] and, ultrasound [216]; with some smart biomaterials responding to multiple stimuli. As a result, stimuli-responsiveness may potentially enhance the spatiotemporal specificity of therapeutic release, thus resulting in increased efficacy, while simultaneously reducing side effects and off-target toxicity.

In recent years, smart biomaterials have been increasingly employed in MN mediated delivery of therapeutics. Incorporation of stimuli-responsive units into MN can be exploited to trigger both on-demand and sustained drug release by either manipulation of external stimuli, or

exploitation of naturally present internal stimuli. Hardy *et al.* developed a hydrogel-based MN-mediated transdermal drug delivery system, made from 2-hydroxyethyl methacrylate (pHEMA) and ethylene glycol dimethacrylate (EGDMA) as well as light-responsive ibuprofen-loaded 3,5-dimethoxybenzene conjugates [217]. The MNs containing the conjugate and drug were inserted into the skin and irradiated with UV light, stimulating cleavage of the conjugate and releasing ibuprofen as the hydrogel of the MN array swelled. This system remained intact *in vitro* and delivered up to three doses of 50 mg ibuprofen, over a prolonged period (up to 160 h) upon application of an optical trigger [217]. The release could also be turned on and off by turning the light on and off, respectively. Consequently, this type of system offers great potential as a stimuli-responsive delivery platform, where “on-demand” drug delivery is required, with triggered sustained-release analgesia as an obvious example. Similarly, it should be possible to design responsive drug delivery systems to facilitate drug dosing-on-demand to accommodate patient regimens as well as the delivery of multiple dosages at one time. Some photo/radio-sensitive nanoparticle variants possess interesting radiation responsive properties that have been used to design light-responsive drug release from MN arrays. Chen *et al.* designed silica-coated LaB₆@SiO₂ nanostructures (serving as near-infrared radiation (NIR) absorbers), which were subsequently incorporated into biodegradable PCL MNs [182]. This nanoparticle variant mediated a light to heat transduction, thus acting as a local heat source when the array was irradiated with external NIR. Following irradiation, the increased temperature caused a phase transition of the MNs thereby increasing the mobility of the polymer chains, thus leading to the melting of the polycaprolactone to trigger the drug release from the MN array. Rhodamine 6G was incorporated into MNs as a model molecule to ascertain this remote trigger behaviour. Furthermore, this NIR-light activated device was evaluated *in vivo* in rat models for the controlled release of DOX [182]. Recently, Liu *et al.* further explored the use of NIR for on-demand delivery of metformin from dissolving composite MNs coated with lauric acid, containing bismuth (Bi) nanodots stabilised by PVP as the photothermal conversion agent [218]. Upon exposure to NIR irradiation, light-to-heat transduction caused the lauric acid coating to melt, with the subsequent dissolution of the polymer upon contact with the interstitial fluid. Thus, enabling the encapsulated metformin to release into the skin tissue, producing a remarkable sustained hypoglycaemic effect in diabetic rats *in vivo* [218]. In bio-responsive MN, bio-responsive polymers and micro- or nanoparticles, which are sensitive to physiological signal, are utilised for drug delivery in response to biological stimuli. Bioresponsive MNs have garnered research interest in recent years, particularly concerning glucose-responsive smart insulin delivery for the potential treatment of

diabetes [219]. Recently, in work conducted by the same Group, Yu *et al.* further investigated the potential of a removable coin-sized ($\sim 5\text{cm}^2$) MN patch that released encapsulated insulin cargo in response to hyperglycaemic hypoxia *in situ* [132]. MNs were loaded with insulin and a non-degradable glucose-responsive polymeric matrix, achieved *via in situ* photopolymerisation. Under hyperglycaemic conditions, phenylboronic acid units within the polymeric matrix reversibly formed glucose-boronate complexes that, owing to their increased negative charge, induced swelling of the polymeric matrix and thus weakened the electrostatic interactions between the negatively charged insulin and polymers, encouraging the rapid release of insulin. Evaluation of the MN in insulin-deficient minipigs ($>25\text{ kg}$) resulted in extended blood glucose regulation beyond 20 h from a single MN patch ($\sim 5\text{cm}^2$). Consequently, MN-mediated responsive drug delivery systems have the potential to combine the essential features of sustained drug delivery in clinical applications.

Figure 7. Different type of stimuli-responsive MN

4.2.6 Hollow MNs

Hollow MNs are micron dimension needles quite similar to a hypodermic needle in shape. Hollow MNs materialised to inject liquids and suspensions for drug infusion into the skin or ocular tissue through the MN bore. Hollow MN has numerous advantages, for instance, they can be used to inject a wide variety of fluids or particulate system into the skin/ ocular site and offer the highest precision in dose delivery compared to other MN types. Additionally, hollow MN allows screening the different liquid formulations by simple steps while this is not the case with other types of MN [220]. This type of MN can be used to deliver particulate formulations like nanoparticles or microparticles for controlled/sustained delivery. Hollow MN has been

developed and investigated for the delivery of varieties of nanoparticles and microparticles by researchers. For the first time, Wang *et al.* investigated the microinjection of polymeric microparticles (2.5 and 2.8 μm) using hollow glass MNs. This work assessed the ability to use hollow MN to inject particulate system intradermally. The particles were effectively implanted intradermally into the skin of hairless rats (*ex vivo* and *in vivo*) by tailoring the optimal injection pressure [221]. Also, Häfeli *et al.* designed a silicon hollow MNs device (8 MNs) that attached with flexible PDMS reservoir to inject different aqueous suspension containing fluorescent polystyrene microspheres (0.7 μm in diameter) and blue polystyrene microspheres (0.93 μm in diameter) into the skin up to a depth of 70 μm [222]. In another study by Du *et al.*, four types of nanocarriers including PLGA, mesoporous silica, liposomes and, gelatin nanoparticles were used to encapsulate OVA and its adjuvant and, subsequently, administered intradermally by hollow MNs to assess the different physiochemical properties of these nanoparticles [223]. These reported studies prove that it is possible to deposit the nano/micro-particulate system for further sustained release application for varieties of chronic disease conditions.

Apart from the intradermal application, ocular drug delivery *via* hollow MN is also a promising research area, especially, when administering drugs to the back of the eye for sustained therapeutic effect. Hollow MN arrays in combination with micro/nano-carriers can play an interesting role in this niche area for long-acting sustained delivery. Jiang *et al.* successfully demonstrated the ability of hollow glass MN to infuse nano- and micro-particles into the sclera in a minimally invasive manner. Nile red encapsulated PLA nanoparticles were easily injected using an insertion-retraction method. However, latex fluorescent microparticles only injected in presence of hyaluronidase and collagenase spreading enzymes to disrupt scleral structure [224]. A similar hollow MN design was employed by Patel *et al.* to inject various different sizes of nanoparticles (20 nm to 1000 nm) to the interior suprachoroidal space of the whole eye (rabbit, pig and human) *ex vivo* with a different needle length and infusion pressure. Results demonstrated that particle size, MN length and, pressure played an important role in effective delivery into the suprachoroidal space [225]. Kim *et al.* demonstrated the use of hollow MNs to administer a core of high-density perfluorodecalin ($\leq 35 \mu\text{m}$ in diameter) particles surrounded and stabilised by fluorescein-tagged, polystyrene nanoparticles (PED) to the back of the eye of rabbits *in vivo*. After infusion of this high-density PED emulsion into the suprachoroidal space of the eye, at least 50% of the injected nanoparticles were detected to be near the macula or ciliary body depending on the eye cornea orientation (gravity-mediated targeting) after injection and 5 days later [226].

5. Application of MNs for long-acting drug delivery

The therapy of diseases such as diabetes, cancers, cardiovascular, neurological, chronic infectious and, musculoskeletal system diseases, can be significantly improved by long-acting formulations. Long-acting implantable formulations are also useful for contraception and vaccination. Long-acting MN formulations can be developed for controlled drug delivery to improve therapeutic effects, decrease dosing frequency, and prevent potential toxicity. Many therapeutic agents, such as biomacromolecules (e.g. peptides, proteins and gene therapeutics), drugs with low bioavailability and, drugs for local delivery are ideal candidates for creating long-acting micro-implants. However, not all drug molecules are suitable for these long-acting micro-implants. The ideal formulation should deliver the drug to the site of action at the correct therapeutic concentration for as long as the treatment is desired. The therapeutic and the physicochemical properties of a drug determine the preferred route of administration (oral or parenteral) as well as the most appropriate delivery systems [227]. This section briefly outlines examples of disease or drugs classifications that are suitable for long-acting sustained delivery by MN delivery.

Figure 8. Schematic representation of combination approaches with MN and Nano/microformulations for long-acting drug delivery for potential disease conditions

5.1 Proteins and Peptides

Protein/peptide therapeutics has been utilised in the treatment of many diseases in recent years and have received major research interest as a result. The main route of drug delivery for peptide and protein therapeutics is either through subcutaneous, IM or IV injections [228]. However, these routes suffer from proteolytic degradation and antibody neutralisation, which in turn, leads to short plasma half-lives. Therefore, this results in the necessity of repeated injection of the drug, albeit this comes at the cost of patient adherence which influences their quality of life [229]. To overcome the above limitations, long-acting injections or implants have been developed for such products. Examples include *in situ* injectable depot-forming systems, microspheres, biodegradable and non-biodegradable polymeric implants, implantable pump systems and, drug-eluting stents [230]. Minimally invasive peptide and protein delivery technologies are promising for product differentiation and value creation for industry, particularly for their potential in vaccination. In the last two decades, MN patches have made substantial medical advances in systemic delivery of potent biotherapeutics and vaccines [231]. MN delivery of genetic materials, in particular plasmid DNA (pDNA), to the skin represents a potential new method for the clinical management of genetic skin diseases and cutaneous cancers, and intradermal genetic immunisation [169].

5.1.1 Vaccine

Most vaccines are administered by the subcutaneous or IM routes using a needle and syringe; and to a lesser extent the ID route, which is used for the administration of *Bacillus Calmette-Guérin* (BCG) and rabies vaccines. However, there has been renewed interest in ID vaccine delivery in the last few decades. This is owing to the fact that the ID space of human skin, unlike that of the muscle and subcutaneous space, is a rich network of potent antigen-presenting cells (APCs) including Langerhans Cells (LCs) and many sub-sets of dermal dendritic cells (dDCs) with roles in both innate and adaptive immunity. Thus, suggesting that the delivery of vaccines to ID layers should be more effective with smaller amounts of vaccine antigen [232]. Another tactic to increase the immune response from vaccination is extending the antigen presentation, particularly for the inactivate/subunit vaccines that usually require one or more booster administration separated by months or years to induce lasting immunity. Long-acting extended delivery of an inactivated antigen could present the specific antigen over a prolonged period, thereby generating a stronger immune response. A new add-on for long-acting extended delivery of vaccine can improve immune responses by ID route as described previously [233]. ID injections are typically performed using the Mantoux technique, first described in 1910 by French physician Charles Mantoux for ID injection of tuberculin as a diagnostic tool for

screening of tuberculosis, in which a small 28 gauge needle is injected at a close angle (10–15°) compared to that subcutaneous (45°) injections [234]. As such, administration of ID injections is more complicated than conventional injection techniques, and, therefore, it requires special training to target the skin reliably [235]. Further issues arise with the potential of ID injection, which has been associated with adverse events such as pain, inflammatory changes and the development of abscesses. Considering all the issues with other routes and ID administration of vaccine by syringe, the concept of MNs has emerged as a technological solution for ID administration of vaccines [6,236,237]. Recently, MN technology has applied for safe and effective ID delivery of vaccines through the painless mechanical disruption of the SC [238]. MNs are arrays mechanically pierce the skin barrier, SC, to deposit vaccines in the ID space. MNs have been demonstrated as a potential strategy for pain-free vaccine administration in early clinical trials [239–241] These MN patches are a currently evolving technology to control vaccine kinetics.

As highlighted in Figure 8, the potential for successful MN assisted intradermal delivery of vaccine/immunotherapeutics formulation when combined with various nanotechnology approaches has been illustrated. Joyce *et al.* demonstrated for the first time the effect of different extended-delivery profiles on immune response using three different licensed vaccines (inactivated polio vaccine, tetanus toxoid and influenza) administered to the ID space of skin. The authors investigated the extended-delivery profiles by administering multiple fractional vaccine doses by ID injection or MN patch. Within the study, single doses of extended-delivery vaccination were compared to two-dose bolus vaccination. It was demonstrated that extended-delivery for one month further improves immune response compared to delivery over shorter times. Interestingly, the extended-delivery achieved through repeated MN patch vaccination further increases the immune response compared to injections [233]. This detailed proof of concept study investigating different vaccines approaches opened further research avenues to optimise the MN formulation, to achieve the long-acting extended profile after single MN application. Extended delivery of vaccines to the skin not only improves immune response but also has the potential for dose sparing and reducing the number of vaccine doses required to reach protective immune levels.

Boopathy *et al.* engineered implantable solid pyramidal MN arrays with silk fibroin protein tips with encapsulated a stabilised HIV envelope trimer immunogen and adjuvant, supported on a dissolving polymer base for sustained-release therapy. *In vitro* release studies demonstrated that the structural integrity of the antigen was preserved and, a molecular weight-

dependent release over time from the MNs. Briefly, upon MN application to the skin, within 5 minutes the vaccine-loaded silk tips were implanted in the deep ID space, from where they released vaccine over a period of time controlled by the crystallinity of the silk matrix. *In vivo* studies in mice, demonstrated that the subunit vaccine was released over 2 weeks in the skin, correlating with increased germinal center (GC) B cell responses, an approximate 1,300-fold increase in serum IgG titers and a 16-fold increase in bone marrow (BM) plasma cells compared with bolus immunisation. This early study proved that implantable MNs have the practical potential to substantially enhance humoral immunity to subunit vaccines [242].

Du *et al.* investigated hollow MN-assisted intradermal delivery of nanoparticulate vaccines to modulate the immune response of protein antigen. Within this study, four different types of nanoparticles were investigated for their effect upon kinetic release parameters, namely; PLGA nanoparticles liposomes, mesoporous silica nanoparticles (MSNs) and gelatin nanoparticles (GNPs). These different nanoparticles also used for comparison between those a model antigen ovalbumin (OVA) with and without an adjuvant poly(I:C). Hollow-MN applicator was also designed to inject nanoparticle suspensions precisely into murine skin at a depth of about 120 μm . Interestingly, OVA/poly(I:C)-loaded nanoparticles and OVA/poly(I:C) solution elicited equally robust total IgG and IgG1 immune responses. However, the co-encapsulation of OVA and poly(I:C) in nanoparticles significantly augmented the IgG2a response compared to OVA/poly(I:C) solution [223]. PLGA nanoparticles and liposomes induced stronger IgG2a responses than MSNs and GNPs, that proves the direct effect of small particle size and sustained release effect from the PLGA nanoparticles and liposomes for the antigen and adjuvant. In the case of cellular responses, the highest CD8⁺ and CD4⁺ T cell responses were induced by liposomes. This study demonstrated that the applicator controlled hollow MN delivery may be a potential choice for effective intradermal injection of long-acting nanoparticle vaccines for extended humoral and cellular immune responses [223].

With particular and recent significance, coronaviruses create a grave threat to global health as shown by Middle East Respiratory Syndrome (MERS), Severe Acute Respiratory Syndrome (SARS) and, COVID-19. SARS Coronavirus (SARS-CoV), MERS Coronavirus (MERS-CoV), and the novel coronavirus, now officially named SARS-CoV-2, are the contributing sources of the SARS, MERS, and COVID-19 disease outbreaks, respectively. The global pandemic caused by COVID-19 has emerged as a severe public health issue crippling world health care systems [243,244]. Reliable vaccines that swiftly induce potent and long-lasting virus-specific immune responses against these coronaviruses urgently required. Kim *et al.*

presented the rapid design and translational development of dissolving MN-based SARS-CoV-2 recombinant vaccines. Initially, they tested the pre-clinical immunogenicity of MERS-CoV vaccines in mice. The vaccine was delivered SC by traditional injection, or intracutaneously by dissolving MN to evaluate the virus-specific IgG antibodies in the mice serum. Specifically, dissolving MN delivered MERS-S1 subunit vaccines elicited strong and long-lasting antigen-specific antibody responses. Based on this, the authors also tested SARS-CoV-2 subunit vaccine loaded dissolving MN in mice. Most importantly, these MN delivered SARS-CoV-2 S1 subunit vaccines elicited potent antigen-specific antibody responses that were evident starting 2 weeks after immunisation. They anticipate clinical development of these dissolving MN delivered recombinant protein subunit vaccines against SARS, MERS and other emerging infectious diseases [245]. This MN-assisted vaccination could significantly speed up roll-out and distribution of such vaccines without stringent storage condition as well as lowering the burden on the global healthcare system.

Recently, the MN-mediated delivery of virus-like particles (VLP) vaccines has gathered greater interest. VLP is self-assembly particles (30–90 nm) self-possessed of viral structural proteins without a nucleic acid genome or a lipid envelope. Initial studies performed by Kang's group were conducted using coated MN patches with viral particles. For the first time, the ID route was utilised to immunise mice and effectively demonstrated that the size of antigens encapsulated in MN patches had no detrimental impact on the immune efficacy in mice. Moreover, the MN patch induced a significant long-term protective immune response with a high level of IgG in serum and IgA in lung alveolar fluid [246]. In further studies, MNs were utilised to deliver the commercially approved VLP vaccine Gardasil (composed of the L1 VLP capsid of human papillomavirus), for prophylaxis of human cervical cancer. To assess vaccine immunogenicity, Corbett *et al.* applied a novel dry vaccine coated densely packed MN patch (Nanopatch™) to C57BL/6 mice and compared the response to the intramuscular injection control. The dose delivered by the MN array was sufficient to produce a maximal virus neutralising serum antibody at day 28 post-vaccination and, a sustained response for 16 weeks, with higher titres than intramuscular injection delivery at equivalent doses of VLP vaccine [247].

Figure 9. Intradermal prolonged delivery strategies of various vaccines/immunotherapeutic formulation by MN

5.2.2 Gene/immunotherapy

Gene/immunotherapy offers great promise to treat a variety of skin (e.g., skin cancers, abnormal scar, psoriasis, hyperpigmentation wounds and infections) and systemic (cancer) diseases [248,249]. Genetic materials can be administered into skin after being coated on the surface of the solid MNs, embedded within the degradable/porous polymeric MNs, injected through hollow MNs, or smeared onto MN-treated skin with the aid of electroporation [250].

Qu *et al.* recently developed a gelatin methacryloyl (GelMA) MN for local and sustained delivery of pDNA to meet the increasing need for ID gene therapy. Within the study, Poly(β -amino ester) (PBAE) nanoparticles were utilised to achieve the intracellular delivery and high local transfection efficiency of plasmid DNA encoding EGFP (pEGFP) as the model cargo both *in vitro* and *in vivo*. After being embedded in the GelMA MNs, sustained release of DNA-encapsulated PBAE nanoparticles was attained and the release kinetics was found to be controlled by altering the degree of crosslinking of the GelMA hydrogel matrix. Here, The GelMA MN matrix provided protection for the encapsulated PBAE/DNA nanoparticles and, photocrosslinkability of GelMA was found to control both mechanical strength and DNA

release profiles. The authors used a C57Bl/6 mouse model to validate the *in vivo* gene delivery efficacy of the MN/PBAE/DNA. Mice skin harvested after 3 days of MN application to confirm the pEGFP delivery and local inflammatory reactions. MN/PBAE/ DNA enabled the transfection rate of cells in the region of interest was found to be ~31% without causing significant skin damage. These preclinical findings opened up the new avenue for localised targeted and sustained delivery of genetic materials through ID route [251].

The purpose of cancer immunotherapy is to generate a systemic immune response by the host to eradicate the tumour cells [252]. Considering the immunological advantages of skin and pain-free delivery of MN, the delivery of cancer immunotherapy has been explored utilising the application of MN patches, containing DNA/RNA, immunostimulatory adjuvants and/or antigens, as anticancer therapeutic approaches [253]. Recently, Zaric *et al.* reported the potential of dissolving PMVE/MA MN arrays laden with nanoencapsulated model antigen (OVA) to increase vaccine immunogenicity by targeting the antigen specifically to contiguous DC networks within the viable dermis. Following *in situ* uptake, skin-resident DCs, delivered antigen-encapsulated PLGA nanocarriers to cutaneous lymph nodes where they subsequently induced substantial expansion of antigen-specific T cells. Moreover, *in vivo* studies in mice models demonstrated that antigen-encapsulated nanoparticle vaccination *via* MN generated robust antigen-specific cellular immune responses, due to sustained antigen delivery from the vaccination site as a result of nanoencapsulation facilitating antigen retention in the skin layers. Furthermore, this immune system activation, mediated by the slow OVA nanoparticles release from the MN, prevented the development of ovalbumin expressing B16 melanoma tumours for 13 days. This approach was found to provide complete protection *in vivo* against both the development of antigen-expressing B16 melanoma tumours and a murine model of para-influenza, concluded the stimulation of antigen-specific cytotoxic CD8⁺ T cells that stemmed inefficient clearance of tumours and virus, respectively. Furthermore, nanoencapsulation provided enhanced antigen stability in MNs. Therefore, the use of biodegradable polymeric nanocarriers for selective targeting of antigen to skin DC subsets through dissolvable MNs presents a promising technology for improved immunotherapy efficacy, adherence, and coverage [254]. Wang *et al.* reported another approach to overcome these challenges by nanoparticle-embedded MN system that contains a dissolvable HA matrix and mesoporous silica-coated upconversion nanoparticles (UCNPs@mSiO₂). The mesoporous silica (mSiO₂) shell acts as the reservoir to protect siRNA and cellular uptake enhancer of siRNA. At the same time, the upconversion nanoparticle (UCNP) core allows the live tracking of MN skin

penetration and NP diffusion through upconversion luminescence or optical coherence tomography imaging. This proof of concept multifunctional MN system was used to deliver molecular beacons (MBs) and siRNA targeting transforming growth factor-beta type I receptor (TGF- β RI) that may be potentially used for abnormal scar treatment [255].

Qu *et al.* recently developed a gelatin methacryloyl (GelMA) MN for local and sustained delivery of pDNA to meet the increasing need for ID gene therapy. Within the study, Poly(β -amino ester) (PBAE) nanoparticles were utilised to achieve the intracellular delivery and high local transfection efficiency of plasmid DNA encoding EGFP (pEGFP) as the model cargo both *in vitro* and *in vivo*. After being embedded in the GelMA MNs, sustained release of DNA-encapsulated PBAE nanoparticles was attained and the release kinetics was found to be controlled by altering the degree of crosslinking of the GelMA hydrogel matrix. Here, The GelMA MN matrix protected the encapsulated PBAE/DNA nanoparticles and, photocrosslinkability of GelMA was found to control both mechanical strength and DNA release profiles. The authors used a C57Bl/6 mouse model to validate the *in vivo* gene delivery efficacy of the MN/PBAE/DNA. Mice skin harvested after 3 days of MN application to confirm the pEGFP delivery and local inflammatory reactions. MN/PBAE/ DNA enabled transfection rate of cells in the region of interest was found to be ~31% without causing significant skin damage. These preclinical findings opened up the new avenue for localised targeted and sustained delivery of genetic materials through ID route [256]. Duong et al. recently developed DMN-based polypeptide cocktails for the triggered delivery of ovalbumin-expressing plasmid OVA (pOVA) and immunostimulant, polyinosinic:polycytidylic acid (poly(I:C)) to augment cancer immunotherapy. To enhance the cellular uptake to APCs, pOVA and poly(I:C) were loaded into a nanosized polyplex prepared using cationic amphiphilic conjugates. MNs were fabricated from biocompatible and hydrophilic copolymers consisting of a cationic polypeptide and Poly (ethylene glycol) (PEG) as a matrix. The cationic nanopolyplex effectually transfected pOVA and poly(I:C) into the intracellular compartments of macrophages and dendritic cells. MN assisted cutaneous administration of cocktails in mouse model elicits a stronger antigen-specific antibody response than subcutaneous administration of the MN-free nanopolyplex. The MN cocktail therapy synergistically augmented the therapeutic effect in B16/OVA melanoma tumours [257]. In final remarks, the further development of MNs for gene therapy depends not only on the improved fabrication of MNs but also on the combined use of different delivery techniques (adjuvants, nanocarriers, targeting moieties) to achieve optimal and long lasting therapeutic outcomes [256].

5.3 Lymphatic targeting

The dermis is very well perfused with dense vascular and lymph capillary networks. In addition, the local capillaries and lymph vessels have less barrier properties to absorption owing to their reduced wall thickness and endothelial barrier. Lymphatic vessels situated in the dermis absorb and transfer interstitial fluid and administered macromolecules from the skin to bloodstream. Of particular importance, the lymphatic system with secondary lymphoid organs, mainly lymph nodes (LNs) play an essential role in absorbing several antigens and modulating the immune response *via* prophylactic or therapeutic interventions [258]. Skin-associated lymphoid tissue plays a vital role in vaccine delivery, due to the richness of immune cells, including dermal dendritic cells, T cells and macrophages. Therefore, the intradermal route of vaccination, particularly in use with MN, has shown prolonged and enhanced immune response compared with the IM route, allowing for an effective reduction in dose with long term protection [259].

Niu *et al.* reported a study where model antigens encapsulated PLGA nanoparticles (less than 100 nm) administered *via* hollow MN array for intradermal delivery in rats. Compared to IV and subcutaneous routes, intradermal delivery of polymeric nanoparticles *via* a hollow MN array resulted in an early burst transit through the draining lymph nodes and a relatively limited overall systemic exposure. Moreover, antigen-loaded NPs delivered by hollow MN array elicited a significantly higher IgG2a antibody response and a higher number of interferon (IFN)- γ secreting lymphocytes in comparison to antigen-loaded NPs given by IM injection and soluble antigen delivered through hollow MN array. Antigen-loaded NPs provoked better antibody response than the soluble antigen formulation because of the sustained release of the antigen at tissue level (depot in skin/draining lymph nodes), at the cellular level (nanoparticles internalised by dDCs), and at organelle level (nanoparticles in endolysosome/cytosol). This unique lymphatic targeted and sustained release property of antigen-loaded nanoparticles formulation *via* MNs might also apply to biologic drug development [260]. Previous studies using fluorescence-labelled dextran and liposomes verified the optimal size and charge characteristics for lymphatic delivery [261,262]. Nanoparticle uptake from the lymph bed capillaries, nanoparticles with sizes amongst 10 nm and 100 nm are held up by the lymphatic capillaries. In comparison, particles of less than 10 nm are absorbed by blood capillaries, and particles of larger than 100 nm are retained at the administration site [263]. Targeted delivery of therapeutics can be achieved utilising carriers with a specified affinity to the target tissue or directly administering the target organ [264].

As there is a vast abundance of lymphatic vessels situated in the intradermal space of the skin, it is the easiest way to access the potential of lymphatic targeting for a potential cure and prophylactic of infectious diseases. Several recent studies have explored minimally invasive MN devices as novel trans- and intra-dermal drug for lymphatic targeting. These studies have successfully shown increases in drug concentration in the lymph nodes compared to other injectable routes. Harvey *et al.*, for the first time demonstrated by the NIR imaging studies with Indocyanine Green (ICG) dye, that lymphatic absorption may be a significant contributor of uptake for biomolecules from MN delivery [4]. Additionally, our Research Group has previously developed MN formulations in combination with a long-acting rilpivirine nanosuspension to be delivered intradermally, with detectable levels of rilpivirine in the lymph nodes of treated rats, justifying the continued exploration of this approach [118]. Further to this, we recently explored the incorporation of novel lipid nanoparticles of multiple antifilaria drugs (doxycycline, diethylcarbamazine and albendazole) into dissolving MN for intradermal delivery, to evaluate the effect upon lymphatic uptake. The SLNs were prepared from Geleol[®] as a lipid matrix and Tween 80 as a stabiliser and with sizes of <100 nm. *In vitro* drug release was shown to be sustained for a longer duration over 48 h from SLNs, compared to pure drugs counterparts. The SLNs were mixed into a polymeric hydrogel, which was then cast to form SLNs-loaded MNs and, all three separate MN systems exhibited sufficient mechanical and insertion properties. Dermatokinetic studies further demonstrated that greater than 40% of the drug was deposited in the dermis of excised neonatal porcine skin for up to 24 h post-MN application, indicating the high possibility of the SLNs to be taken by the lymphatic system with longer duration of a slower release. In *in vivo* studies in rats, a 4 to 7-fold higher lymphatic concentration of each of the three drugs was achieved following intradermal delivery compared to oral administration. This way, targeting the drug to the lymph nodes, where filarial nematodes reside in diseased patients, prominent to an effective therapy for lymphatic filariasis [5].

5.3. HIV infection

Poor adherence to oral antiretroviral therapy (ART) remains an important challenge in the treatment of HIV. Moffatt *et al.* recently investigated the opinions from various end-users surrounding the translation of MN technology to general clinical practice, with a particular focus on the delivery of antiretroviral therapy. The consensus surrounding MN technology from patients with HIV involved in qualitative focus groups was found to be positive, with patients highlighting benefits pertinent to HIV, including discreet self-application and potential

sustained release thus avoiding daily pill taking and the associated side effects. Patient concerns focused on the need for varied MN dosing schedules and a reluctance to change from established antiretroviral therapy, which should act as a motivator for those involved in MN development to ensure a patient-centred MN product is delivered. The findings of this preliminary study provided an initial indication of MN acceptability and support of their clinical translation particularly for use within HIV, and, thus, highlighted that there was a compelling need for an alternative minimally invasive, long-acting method of antiretroviral delivery, to that of current oral antiretroviral therapy [265]. This work has been pioneered by Donnelly and co-workers. For example, McCrudden *et al.* developed dissolving polymeric MNs for the sustained delivery of rilpivirine to improve the adherence to prevention and treatment regimens of HIV. The MNs were fabricated using PVA polymer and an industrially manufactured long-acting rilpivirine nanosuspension that is currently under clinical development as an injectable, attached to a pre-formed bioadhesive baseplate. By conducting *in vivo* investigation in rats, the authors showed the comparable distribution of rilpivirine in plasma (extended for 56 days), localized tissue, and lymphoid tissue between nanosuspension delivery *via* MNs and intramuscular injections. Based on the results obtained from this study, a cautious extrapolation estimated that a patch size of approximately 28 cm² could be sufficient to maintain therapeutic plasma levels of rilpivirine in humans over 7 days [164]. Subsequent work from the same Research Group has led to the development of similar MNs for the intravaginal delivery of rilpivirine in a discrete manner. Encouragingly, positive results comparable to intramuscular injection were once again achieved [164].

In a further study from the same Research Group, Tekko *et al.* produced novel bilayer dissolving polymeric MNs with high drug loading (60%) for the sustained delivery of another ART drug- cabotegravir to improve the adherence to prevention regimen of HIV [266]. The MNs were fabricated using a blend of PVA/PVP and an industrially manufactured long-acting cabotegravir nanosuspension, attached to a drug-free PVA/PVP-based baseplate. The fabricated MNs sufficiently strong to pierce Parafilm M[®] and excised porcine skin models and could withstand up to 32 N of applied compression force. The *in vivo* study demonstrated that MNs was able to deliver a substantial amount of the loaded dose and maintained drug plasma concentrations above the human therapeutic concentrations for PrEP (Pre-exposure prophylaxis) of 4x P-IC90 (664 ng/ml) over 28 days study period. These studies again suggest that MNs could be also used to deliver the ART drugs in a sustained manner over prolonged,

thus demonstrating early promise for the future of MN-mediated delivery in the treatment of HIV, and supports the further investigation of this platform in delivering other ART candidates.

5.4 Contraceptives

To address the unmet worldwide need for family planning, scientists have been compelled to re-explore several approaches of modern contraception, focusing on those that are long-acting, reversible and, provide flexibility to a woman [267]. Most of the water-insoluble progestin based contraceptive progestogens are generally formulated as an oral pill, injection, implant, or vaginal ring. The last 3 dosage forms can deliver progesterones in a sustained release manner. However, either injecting/implanting or surgical removal of implants will raise the burden of treatment to a patient. As such, there is a strong need for long-acting contraceptives that can increase contraceptive accessibility and convenience for women. MNs are considered minimally invasive devices that can disrupt the SC barrier to achieve transdermal drug delivery and improve drug efficiency. There has been good progress toward the long-acting MN research for contraceptive agents in the last five years [268].

Prausnitz *et al.* published work with effervescence-triggered rapidly separating LVN loaded PLGA MNs. MN tips separated from the patch baseplate and implanted within the skin due to effervescence that was triggered upon contact with skin's interstitial fluid, as demonstrated in both rats and human participants. In rats, MN-mediated delivery of LVN exhibited sustained release above the relative human therapeutic plasma levels required to maintain adequate contraceptive control for more than 1 month. A placebo MN patch was well-accepted in human participants without any skin or allergic reactions. Women of reproductive age in three world regions expressed interest in and, a preference for long-acting contraception by MN patch compared to other available options. These findings signify that an effervescent MN patch could enable greater access to long-acting contraception [47]. He *et al.* reported novel long-acting MNs that can be inserted into the skin *in situ* by a relatively simple and rapid application. MNs were fabricated to entrap etonogestrel in the PLGA tips by a controllable casting-mould technique, thus avoiding any adverse effect of high temperature on the drug stability. A new formulation method of MNs has used the N-methyl pyrrolidinone as a solvent for the needle tip matrix. Following solidification at 70°C for 4 h, the needle tips were adequately strong to puncture the skin. Etonogestrel was shown to crystallise in needle tips uniformly, visualised by a polarising microscope. The ID insertion ratio of the MNs was influenced by the parameters of needle spacing and needle shape. Following optimisation of MN formulations, the drug

loading capacity was $153.0 \pm 13.5 \mu\text{g}$, and the drug utilisation rate was up to $92.6 \pm 8.1\%$. In rats, the pharmacokinetic study of the implantable MNs showed that the plasma etonogestrel level could be detectable until 336 h and, the AUC_{0/48h} only accounted for 37.8% of AUC_{0/∞} [116]. In additional studies conducted by He *et al.* investigating the potential sustained release of etonogestrel, dissolving MNs were fabricated with etonogestrel microcrystal particles incorporated for intradermal delivery over 1 week. *In vitro* release analysis showed that etonogestrel in the microparticles form could produce a greater sustained drug release in comparison to the nanocrystal form. Hydroxypropyl methylcellulose and PVA were employed to fabricate the fast-dissolving needle tips with 50% drug and drug-free flexible back layer, respectively. The tips of dissolving MNs dissolved in rat skin within 1 h with etonogestrel deposition efficiency of $63.8 \pm 2.0\%$. In rats, MN treatment demonstrated that the plasma levels of etonogestrel were a dose-dependent profile and were much steadier than ID injection. There was no statistical variation between the bioavailability of etonogestrel treated with dissolving MNs or ID injections [269]. Therefore, the MNs with contraceptive drugs (drug crystals, encapsulated in biodegradable particles, or PLGA/PLA tips) provided a perspective with self-administration features for intradermal sustained delivery [45,47,116,269]. This MN assisted long-acting delivery could offer practical choices for women to have access to safeguard against unwanted pregnancy.

5.5 Neurological disorders

5.5.1 Anti-psychotics

Treatment of schizophrenia and other psychosis, such as bipolar disorder, is currently supported by a range of antipsychotic drugs, however, their beneficial effect is often limited by the common experience of side effects, resulting in poor adherence to therapy [270]. There is a compelling need for a minimally invasive method of sustained administration of antipsychotics. However, to meet this need further advances in delivery technologies and assessment of these in clinical studies are required in order to broaden the clinical use of antipsychotic formulations for the transdermal treatment of psychiatric illness. In particular, employment of different strategies, such as the use of nanoparticles/vesicles in combination with permeation enhancers or MNs with or without iontophoresis may improve the absorption of antipsychotic drugs through the skin [271]. Moreover, several clinical trials regarding MNs have been conducted, or are ongoing, including studies with drugs acting on the central nervous system (zolmitriptan) [272]. For this reason, looking forward, MNs could represent an

attractive and valid option to improve antipsychotic drug delivery through the skin. In support of this proposition, dissolving MNs have previously demonstrated the ability to achieved high dose delivery of a hydrophobic nanosuspension in rat models, affording a sustained release of drug from over time the application site [118]. Thus, based on this study, it could be viewed that the dissolving MN intradermally deposits the drug nanosuspension, thus creating a micro-depot of the drug in the viable skin layers, from which allows prolonged drug administration. Subsequently, the incorporation of an antipsychotic drug nanosuspension, such as that of aripiprazole or paliperidone, within a dissolving MN system may be a possibility. Thus, upon application to the skin, the antipsychotic drug may slowly release over long periods of time to maintain constant plasma levels, thus affording a minimally invasive alternative method of sustained delivery. This novel dual-concept approach may, in theory, potentially circumvent limitations associated with parenteral and transdermal delivery at the same time. Notwithstanding, MN-mediated delivery of an antipsychotic agent, namely olanzapine, has been recently demonstrated *ex vivo* [65]. Kearney et al. demonstrated the enhanced release of olanzapine co-solvent loaded reservoirs in combination with hydrogel-forming MNs in a Franz-diffusion setup, with approximately 50% of olanzapine delivered at the 24 h from each of the two reservoir designs evaluated, thus indicative of a sustained release. This work provided early crucial proof-of-concept evidence that MNs are potentially a feasible alternative method to facilitate the delivery of hydrophobic antipsychotic agents. With further evaluation in future clinical studies, this delivery concept could potentially benefit not only therapeutic outcomes but also positively impact patient acceptance of therapy.

5.5.2 Alzheimer's disease

Alzheimer's disease (AD) is a progressive neurodegenerative disorder of the central nervous system (CNS), in which there is a continual deterioration of intellectual and social functions, memory loss, personality changes and an inability for self-care [273]. Despite the broad appeal of skin route of administration, rivastigmine (Exelon[®]) is the first and only transdermal patch marketed to treat all stages of AD [274]. While commercially successful, it still requires daily administration, and subsequent efforts to increase application time have been investigated, including completion of Phase I trials for the once-weekly donepezil transdermal patch [275], with further studies, are ongoing, such as of the recent advancements made in the 6-day dosage form of transdermal rivastigmine [276]. Sadeghi *et al.* in this study incorporated rivastigmine within chitosan microparticles further integrated into the patch acrylic adhesive, which provided sustained release of the active drug over almost 1 week *in vitro*. Subsequently,

demonstrating a promising feasible replacement from a 1-day dose to 6-day dose application of rivastigmine. While an opportunity presents in this instance to further enhance permeation and sustained release of nanoparticles by the exploitation of MN-mediated delivery, the field of AD research is not dissimilar to that of other neurological agents, such as antipsychotic drugs (Section 5.5.1), in that this platform has remained relatively unexplored in the potential delivery of agents for the treatment of AD. While MN-mediated delivery for the specific treatment of AD has yet to be explored in combination with nanomedicine technology, two independent studies have successfully demonstrated delivery of the anticholinesterase inhibitor, donepezil, with different MN-assisted approaches. Donepezil has a very long half-life and therefore lends itself readily as a candidate to achieve a sustained therapeutic release [277]. Inspired by the success of rivastigmine patches, Kearney *et al.* employed hydrogel-forming arrays with PMVE/MA reservoirs loaded with donepezil. Evaluation of the performance *in vivo* in rat models demonstrated successful delivery by this platform, producing detectable plasma levels of 51.8 ± 17.6 ng/mL at 24 h [278]. In a different MN-based approach, Kim *et al.* encapsulated a high donepezil hydrochloride content (up to 78% w/w) exclusively within dissolving MN tips without any severe loss of mechanical strength, and 95% of tip content was delivered through the porcine skin in 5 min. Besides, evaluation of pharmacokinetics in rats was cited as satisfactory as evidenced by the $C_{\max}$ exhibited by MN delivery 4 times higher than that of oral administration at 9 ng/ml. Furthermore, a total of 69% of donepezil entrapped within the MN tip was detected in plasma at 24 h [279]. While the results of both of these studies are cautiously indicative of a sustained release, sampling did not extend beyond 24 h to confirm this assumption in either instance, however, successful delivery of a potential treatment of AD was demonstrated in both cases. The benefit of transdermal products in the treatment of AD is well documented, and these studies offer substantial promise in support of future MN mediated delivery for AD therapy. However, further work is now required in evaluating this platform in conjunction with nanocarriers to fully realise this potential of the dual-delivery approach to attain a minimally invasive sustained-release alternative to currently available oral or transdermal therapy.

5.6 Diabetics/Insulin

Insulin is a compound delivered *via* subcutaneous injection to treat type 1 diabetes [283], in those patients who are specifically unable to produce enough insulin [284,285]. Not only is this route of delivery considered by many to be uncomfortable and painful, but it is associated with an increased risk of infection, and importantly, it generates a high amount of sharps waste

[283]. One of the most promising types of MN-assisted insulin delivery systems is the glucose-responsive MNs [286], explored in section 4.2.5 “*Stimuli-responsive smart MN arrays*” [286]. In a similar approach, Chen *et al.* [287] developed smart MN based on SF combined with semi-interpenetrating network hydrogel for glucose-responsive insulin delivery, aiming to replace the subcutaneous self-injection. The authors achieved sustained and stimuli-responsive insulin delivery, controlling the polymer degradation into the skin. The glucose-responsive system consisted of a two-layer patch, fabricated with silk fibroin combined and phenylboronic acid/acrylamide hydrogel (needles layer) and the base layer prepared only with silk fibroin. A porous system developed by submitting the MN to the lyophilisation improved the stimuli-responsive system, able to release insulin only when the hyperglycaemia was detected, based on the reaction of glucose binding reversibly and boronate ester in aqueous solution, releasing the insulin when the glucose is detected. The SF was found to act as a suitable polymeric matrix former capable of delivering sufficient amounts of insulin ID. Wang *et al.* [288] also describe MNs insulin-loaded formed with SF as a polymeric matrix. This material is biocompatible, non-toxic, degradable, and presented slow insulin release, indicating the MNs release was maintained up to 60 h, providing the future promise of the platform for the application of long-acting insulin delivery.

As an alternative to subcutaneous insulin delivery, dissolving composite MNs containing insulin-loaded CaCO_3 microparticles were developed by Liu *et al.* [289]. These CaCO_3 MPs containing insulin were obtained by precipitation in the presence of PVP. The new MNs (5, 10 and 20 UI kg^{-1}) was tested *in vivo* using diabetic rats and compared with subcutaneous administration (5 UI kg^{-1}). Therapeutic plasma insulin levels were found to be maintained for a longer period when insulin was delivered *via* MNs compared with SC injection of a similar dose [289]. This may be explained by the increased time required for the systemic absorption of insulin delivered *via* MNs. The extension of release rate achieved with MNs insulin microparticles loaded was beneficial to reduce the risk of hypoglycaemia [290].

Prolonged-release kinetics utilising MNs for insulin delivery has also been demonstrated by Kim *et al.*, in which they developed powder-carrying insulin MNs. The method of delivery was based upon creation of micro-cavities from MNs application to the skin and, subsequently, introducing the active agent on powder form on it using a micropillar implantation system to compact the insulin homogenously into the microcavities. The *in vivo* studies were able to prove the concept of the new MNs, achieving a prolonged release when compared with subcutaneous injection. This new approach can be applied to a range of powder drugs, with

further beneficial impacts upon minimising the side effects and improving the long-term stability, one of the issues related to insulin [291]. Seong *et al.* achieved the prolonged release of insulin using double-layer MNs with swellable tips composed by polystyrene-block-poly(acrylic acid) with a bullet-shaped array, and non-swellable polystyrene baseplate. To load the insulin only in the tips, a mild drop/dry procedure was applied, achieving 60% of release after 12 h *in vitro* studies. The *in vivo* studies showed a prolonged release from the swellable MNs resulting in a gradual decrease in blood glucose levels, explained due to the ability of the MN to uptake the body fluid in the tips, allowing the insulin release *via* diffusion [292]. Chen *et al.* developed a basal-bolus insulin regimen integrated MN patch for intraday postprandial glucose control, with multiple kinetics, which provided physiologic insulin coverage when tested in diabetic rats. The new technology comprised an array containing the short, intermediate and long-acting insulin, separated by three separated projections 9x9 for each type of insulin, totalling a patch with 81 MN projections. To develop the integrated MN patch, formulations containing the three different insulin were prepared separately. To deliver the immediate insulin, a hydrogel with gelatin was prepared. For intermediate insulin, a cross-linked blend of hydrogel composed with gelatin and genipin was developed. Moreover, to deliver the long-acting insulin, a cross-linked hydrogel containing hyaluronic acid and 1,4-butanediol diglycidyl ether was used. The *in vivo* studies showed sustained insulin delivery, because of the simultaneous bolus provided under the MN application, reducing the excessive intraday glucose fluctuations that occur in response to meal ingestion, showing the potential of this new MN system as a substitute for multiple daily injections [293].

5.7 Cancer

Traditional cancer treatment includes surgery, chemotherapy and, radiation. However, the effects of these procedures may damage not only the tumour tissue but also normal bodily tissues [294]. The exploitation of the properties of nanocarriers may overcome such limitations of conventional treatment, resulting from their nanometre scale, high surface-to-volume ratios, favourable drug release profiles, and targeting modifications, which may, in turn, may permit drug release in a sustained manner and subsequently increased drug localisation and cellular uptake within the target tumour tissue [295]. Current nanocarrier based drug delivery systems for cancer treatment, which are already under research and development, include liposomes, polymeric micelles, dendrimers, nanospheres, nanocapsules, and nanotubes [294]. Nanoformulations that have already been marketed include Doxil[®] (liposomal doxorubicin) [296], Abraxane[®] (nanoparticle-based albumin-bound paclitaxel) [297] and Onivyde[®]

(liposomal irinotecan) [298]. However, their administration, by necessity, is typically invasive (IV infusion) and may lack direct access to more restricted tumour sites, thus resulting in poor accumulation or non-localised distribution. Consequently, a minimally invasive cancer treatment associated with MN mediated delivery always provides broad appeal as a result of ease of use, non-invasive controllable administration and predominantly a potential synergistic effect [55]. Besides, some anti-cancer MN nanocarrier based systems have already demonstrated particular promise for the treatment of certain cancers. Cisplatin, a first-line chemotherapeutic agent, has recently been formulated within tumour-targeting pH-responsive lipid nanoparticles by Lan *et al.*, with the resultant nanoparticles embedded in dissolving MNs as a means to mediate the safe and efficient transdermal delivery for cancer therapy. Nanoparticles exhibited a high drug loading weight (80%), and substantially increased the solubility of cisplatin and enhanced its antitumour efficiency. Additionally, a sustained release over a period of 72 h in dialysis membranes *in vitro* was demonstrated. In *in vivo* studies utilising a xenograft tumour BALB/c murine model, MN-mediated delivery arrays significantly increased cytotoxicity and apoptosis in cancer cells with an apoptotic index of 58.6%, resulting in significantly reduced tumour volume and weight. Moreover, it was established that serum platinum, pulmonary toxicity, hepatotoxicity, and nephrotoxicity were not detected *in vivo*, indicating that the technique was bio-safe [126]. recently delivered DOX encapsulated PLGA nanoparticles to the oral cavity in the treatment of carcinoma. Stainless steel MNs arrays of 700 μm tip height were coated with nanoparticles of average diameters of 137 nm and applied to porcine cadaver buccal tissue. Evaluation of MN treated tissues utilising confocal microscopy proved that DOX could diffuse both vertically and laterally into the tissues and produced cellular cytotoxicity, with nanoparticles detected at a penetration depth greater than 1 mm from the point of insertion from the MN tip. In contrast, administration of DOX by hypodermic injection into porcine buccal tissue reinforced significant leakage of the injected volume (about 25 % of the injected 80 μl). Moreover, nanoparticles demonstrated a substantial prolonged release, as only 34% of the encapsulated DOX was released *in vitro* over a 24 h period. This sustained release of DOX, especially the release of the remaining 66 % of the drug still contained within the nanoparticles is a beneficial trait for chemotherapy because constant exposure of tumour cells to DOX is expected to result in high cytotoxicity [299]. MN-mediated delivery has also extended to some anti-inflammatory agents which possess residual adjunctive cytotoxic properties, such as that of ketoprofen, as demonstrated by Vučen *et al.* Ketoprofen nanocarriers composed of PLA were found to exhibit a sustained release over 7 days under the sink conditions using Slide-A-Lyzer dialysis cassettes. They demonstrated an

enhanced release over 24 h in porcine tissue following application to excised skin pre-treated with silicon MNs [300].

As well as chemotherapeutic agents, nanoformulated monoclonal antibodies have also been utilised for enhanced cancer immunotherapy by MN-mediated delivery. Self-degradable MN patches have been investigated by Wang *et al.* for the potential treatment of melanoma, through sustained delivery of anti-PD-1 (aPD1) in a physiologically controlled manner. MN-patches composed of biocompatible HA were incorporated with glucose oxidase (GOx) loaded pH-sensitive dextran nanoparticles encapsulated with aPD1. The generation of acidic environment promoted the self-dissociation of nanoparticles and subsequently resulted in the substantial release of aPD1. In this study, robust immune responses in a B16F10 mouse melanoma model were produced from a single aPD1-GOx MN patch application, in comparison to MN lacking a degradation trigger or that of intratumoral injection of free aPD1 at the same dose. Moreover, it was hypothesised that as a result of the superior immune response, that this administration strategy could be integrated with other immunomodulators (such as anti-CTLA-4) to achieve combination therapy for enhancing antitumour efficacy [301]. The studies described here demonstrate the sustained delivery of various therapeutic nanoformulation outlets involved in potential anti-cancer therapy, thus demonstrating early promise for the future of MN-mediated delivery in the treatment of cancer, and supports the further investigation of this non-invasive delivery platform.

5.8. Skin diseases

The skin is the principal protective barrier of the human body. However, it is susceptible to various diseases and disorders such as psoriasis, viral warts, cold sore, fungal infections, soft tissues infections and leishmaniasis. Most of these diseases are chronic, difficult to treat and, thus, seriously affecting peoples quality of life [302]. While in mild cases, topical therapy is mostly recommended, systemic therapy (oral or parenteral) for is widely indicated [303]. Unfortunately, topical therapy efficacy reduced by the skin barrier properties. For example, it has been demonstrated that when a topical cream spreads on the skin surface, only 10–20% or less of total drug loaded in cream could permeate through the skin [304]. Also, the efficacy of many of the systemic therapies is suboptimal this because drug administration through the gastrointestinal tract or circulatory system sometimes increases drug consumption or reduces local drug concentration per unit area of skin. This is mostly associated with numerous side effects and toxicity especially for drugs such as cyclosporin A (CyA), methotrexate (MTX)

and the antifungal agent- itraconazole [114,303,304]. Owing to limitations of the existing methods of treatment, researchers are committed to performing innovative investigations into drug delivery methods employing MNs technology alone or, in combination with long-acting formulation for efficient intradermal delivery of many of the therapeutic agents which are used to treat dermatological conditions [16,303–305].

Here, we focused on one of the most common skin diseases, namely, psoriasis as a case study, where we could track the up-to-date advancement made in using MNs-assisted delivery of long-acting formulations approach for localised and sustained intradermal drug delivery and minimising drug systemic exposure. Psoriasis is a chronic inflammatory autoimmune skin disease that affects 1-3% of the population [306,307]. Currently, several treatment strategies are in use, including photochemotherapy with UVA (PUVA), retinoids, CyA, MTX and recently biologics [308,309]. The latter three medications are widely used to treat the moderate to severe cases of psoriasis and, administered systemically (orally or parenterally), which are associated with many side effects and toxicity. Over the past two decades, many researchers have investigated utilising MNs alone or in combination with long-acting formulations for efficient intradermal delivery of the above-mentioned therapeutic agents to improve their efficacy and safety. Recently, Tekko *et al.* achieved the sustained release of MTX over 24 h with even reduced drug peak concentrations using a newly developed PVA/PVP-based hydrogel-forming MNs formulation [63]. The innovative approach achieved C_{max} (35.1 nM) of MTX in 24 h post-MNs application against C_{max} (57.4 nM) in 1 h for orally administered MTX aqueous solution in rats. Considering that some of the side effects (i.e. nausea and vomiting) of MTX are mostly related to its high spike blood concentrations [310], this novel MNs-mediated delivery approach has the potential to reduce/avoid these side effects, which are associated with systemic delivery. This drug delivery system was originally developed to deliver MTX to treat juvenile idiopathic arthritis; however, also it may be tailored to treat psoriasis, in which MTX is also licenced for use. In another study, Tekko *et al.* combined NCs and MNs technologies to localise and sustain release of MTX as a potential treatment for psoriasis to minimise the systemic exposure in order to avoid the drug systemic toxicity and side effects [15]. The authors prepared poorly water-soluble MTX-NCs from its freely water-soluble salt MTX Na by bottom-up method, namely acid-base neutralisation accompanied with sonification. The MTX NCs (average diameter of 700 nm) were then incorporated into the projections of dissolving MNs with a drug loading of 2.48 mg/array. The MNs projections dissolved within 20 minutes upon insertion and deposited around 25% of their drug payload in

the skin (*in vitro*). The *in vivo* study in Sprague Dawley rats revealed that the MTX NCs-loaded MNs were able to deposit approximately 25 % of the loaded MTX NCs in the skin, which acted, in turn, as a drug depot and released the drug in a sustained manner over 72 h while minimising MTX systemic exposure in comparison with orally administered MTX Na (the control). For example, after 24 h from MN application, approximately 312.70 µg/g of MTX was retained in the skin at the application site. This was 322-fold higher than the amount of MTX (0.942 µg/g) retained in the skin after oral administration of MTX Na. even after 72 h from MNs application, around 12.5% of the deposited drug was still retained at the application site. This approach was not only effective in delivering and retaining the drug for a prolonged period but also reduced the systemic exposure remarkably. Although the delivered MTX dose from MNs was approximately 25-fold higher than that delivered by oral administration, the maximal blood concentration of MTX was only 40% of that measured after oral administration of MTX Na.

Intradermal delivery of CyA using dissolving MNs has also been investigated. Benefiting from its intrinsic physicochemical properties, large molecular size (1 202.61 g/mol) and, hydrophobic properties (logP= 2.92), Jeong *et al.* developed hydroxypropyl cellulose-based dissolving MNs loaded with CyA for localised and sustained intradermal delivered [311]. The authors indicated that these dissolving MNs performed well *in vivo* using a rat model, with a longer period of therapeutic effect locally compared with oral administration. This was demonstrated by CyA administered with MNs maintained a CyA concentration in plasma above 5 ng/ml for 72 h compared to 24 h for CyA administered orally. The studies described here demonstrate that MNs-assisted delivery of long-acting formulations offers an outstanding opportunity to enhance therapeutic efficacy (by delivering the drug directly to the affected site and maintain the effective drug concentration for prolonged periods) and safety (by minimising their systemic exposure). Therefore, these studies support further investigation using this approach to deliver other therapeutic agents to not only treat psoriasis, but also other skin diseases and disorders.

6.8. Ocular diseases

MNs have been widely explored for the delivery of the drugs to an ocular tissue, to both the anterior and posterior segments of the eye [312]. The cornea and sclera are the outermost layers of the eye which provide the greatest challenge for drug diffusion to the inner ocular tissue, thus limiting the bioavailability of the drug [313]. As depicted in Figure 10, MNs find a

potential application in ocular delivery due to their ability to decrease the resistance to the diffusion physically. Hollow MNs, have received the greatest research interest in this aspect, as well as solid MNs, which have been extensively researched for periocular drug delivery. Other designs, including hydrogel-forming MN, as well as interlocking MNs, have also been studied for their ability to enhance ocular drug penetration [49].

Figure 10. A major route for MNs mediated intraocular long-acting drug delivery

Yiu *et al.* reported the assessment of suprachoroidal delivery of adeno associated viruses (AAV) using a transscleral Hollow MN system in non-human primates using, comprising of 30 gauge 700 μm MNs. The suprachoroidal delivery successfully leads to transient GFP expression on the retinal layer for up to 3 months following MN-mediated deliver in rhesus macaques. The study suggested that transscleral MN systems could be successfully used for efficient gene delivery for the management of retinal disorders [314].

MN could also be used for the preparation of intrascleral implants. Thakur *et al.* reported the preparation of intrascleral implant using poloxamer gel loaded with the fluorescein sodium dye as a model drug. This hydrogel was injected by using the hollow MN device, which was fabricated using the hypodermic needles of different lengths (400-600 μm). This study suggested optimum concentration of hydrogels could be used as an efficient tool for preparation of intrascleral implant that could balance the drug loading as well as the force of injection and sustained release [315]. Rowe-Rendleman *et al.* have used intrascleral injections of medroxyprogesterone, triamcinolone and, fluocinolone for studying the effect of steroid in the prevention of retinal neovascularisation caused by intraocular injection of lipid hydroperoxide

(LHP). The intrascleral injections were made with a micro-surgical approach by piercing the epi-sclera and depositing drug directly in the sclera for diffusion towards the choroid using 100 μ l syringe and 30 gauge needle as reported by Ueda *et al.* The study suggested that the intrascleral injections were well tolerated in the rabbit eye and did not lead to increase in the intraocular pressure, as well as did not posed any damage to retinal architecture [316].

One of the main challenges for drug delivery to the posterior segment is the limited delivery coverage leading to less profound therapeutic effect. Hwan Jung *et al.* reported the suprachoroidal delivery of collagenase enzyme along with latex microparticles leading to enhanced ocular coverage for distribution of microparticles. Hollow MN with a length of 750 μ m and 30 gauge was used for the for delivery of 50 μ l liquid containing microparticles and collagenase enzyme blend [317]. The same group also reported the delivery of hydrogel using a similar hollow MN system. HA-based (1%) microparticles along HA-based (4%) hydrogel loaded with model drug particles were delivered using hollow MNs. This strategy could be used for enhancing the sustained drug delivery effect of various transscleral and suprachoroidal drug delivery systems [318]. Jiang *et al.* also reported intrascleral drug delivery utilising hollow MNs for the delivery of various formulations. An in-house MN device containing a hollow MN and drug reservoir connected by a rubber tubing was developed. The hollow MN had an elliptical tip opening with a long axis of $\sim$ 100 μ m, and a short axis of $\sim$ 40 μ m and, the length of MN was controlled from the injector. Furthermore, this system was used for the infusion of sulforhodamine as well as dye loaded nanospheres and microparticles into the human cadaver sclera, in which MN of 700-1080 μ m length was used. The study suggested that a higher quantity of liquid could be infused in the medial sclera when compared to the anterior and posterior scleral tissue. Additionally, the study suggested that the use of such MN device could have a potential therapeutic application for delivery of small volumes of the drug as well as nanoparticles by the intrascleral route [224].

Kim *et al.* reported a miniaturised porous silica-based MN array backed on PVA membrane for management of ocular melanoma. The MNs were fabricated by standard photolithographic patterning of silica wafers followed by the formation of the backing membrane using PVA lamination. Finally, DOX was loaded on the surface of MN. The MN-assisted administration was found to sustain the release of DOX for up to 24 h by the application of chemical bonding. Maher *et al.* suggested the application of a self-adhesive and interlocking MN system for sustained ocular drug delivery. The MN consisting of dimensions of 1500 μ m length and 275 μ m base diameter, were fabricated using PVA. The interlocking mechanism leads to the

increase in the adhesion force, which can be further translated to sustained release of the drug. Although detailed *in vivo* studies are required for validation of interlocking MN systems, this could be used as a potential tool for sustained intrascleral drug delivery [319]. Saraswathy *et al.* reported the preparation of particle loaded hydrogel laden with HA-based MNs for enhanced ocular drug delivery. The moxifloxacin loaded HA MNs were prepared and sustained in the collagen-based cyrogel; the cyrogel was further crosslinked with 4-armed PEG succinate glutarate. The system was found to sustain the release of moxifloxacin for up to 7 days in *in-vitro* evaluation. Further *in-vivo* studies are required for the assessment of the biocompatibility of this system [320]. MN patches were used for delivery of amphotericin B (AmB) by Roy *et al.* The MN patches were prepared using a PVA and PVP mixture using PDMS template, and the dimension of MN was 552 μm length and with 1.64 μm diameter. Liposomal formulation of AmB was delivered following the application of solid MN to form pores on the corneal surface, which was found to lead to higher retention of AmB in the cornea. The MN-assisted drug delivery system also demonstrated complete clearance of the *Candida albicans* infection under 48 h in *in-vitro* conditions [321]. Thakur *et al.* have further reported a rapidly dissolving MN-based drug delivery system for the intrascleral drug delivery. The fluorescein sodium and FITC dextran (70 kDa and 150 kDa), were selected as model molecules. The MNs were prepared from drug-loaded PVP hydrogels and characterised for mechanical properties, and *ex-vivo* tissue penetration and distribution. It was observed that rapidly dissolving MN were able to dissolve up to 87% of the mass in less than three minutes. The biodistribution studies suggested that after MN dissolution, an intrascleral depot formed of FITC-dextran and, lead to an increase of more than 60% drug permeation across the scleral tissue. Thus, suggesting that it could be a potential intrascleral drug delivery system which could be used for minimally invasive drug delivery of macromolecules [312].

Than *et al.* studied the fabrication and evaluation of double-layered MN systems for transcorneal drug delivery. The MN patches were prepared using HA and methylated HA (MeHA), where the outer layers were made using the hydrophobic MeHA, and the inner core consisted of HA. The MN was able to sustain the drug release for up to 120 h. The drug delivery system was also found to be biocompatible, as it does not lead to any anatomical changes post insertion for up to 7 days as well as no change in corneal opacity. The MN system was used to deliver the DC101 by the intracorneal route. The *in-vitro* and *in-vivo* assay suggested that the MNs lead to a marginal reduction of up to 90% in the neo-angiogenesis when compared with topical and intraperitoneal application of the same antibody along with less macrophage

accumulation. The authors also achieved two weeks of sustained-release when HA-IgG conjugates are loaded in MeHA matrix. Based on this proof of concept, choosing or mixing with PLGA polymer matrix or conjugates, drug release kinetics from several days to months can be tailored [49]. Based on these sustained and long-acting MN approaches, various molecules can be readily delivered by localized, controlled manner that provides a pain-free, cost-effective and home-based solution for many ocular diseases.

6 Lab to market considerations and challenges

The primary purpose of the MN-mediated delivery systems is to reduce the burden of daily pills fatigue or painful injectable drug administration and, thus, improve patient compliance. Sustained-release drug delivery assisted by MNs can simplify drug administration, accomplish therapeutic concentrations for a specified period and, evade potential systemic toxicity. Although such an approach has many advantages in comparison with the conventional drug delivery systems, it has some limitations, which should be considered with a system designed to deliver such drugs. Firstly, unlike the conventional hypodermic needles, MNs are micron-sized devices and the amount of drug, which can be delivered, is limited. Secondly, to minimise drug wastage and increase delivery efficiency, bilayer MNs, where the drug is loaded in the MNs projections only, should be considered. Thirdly, to maximise drug loading per unit area in the MNs, poorly water-soluble forms or its nanocrystals should be the first option, whenever is possible, as using controlled release polymers such as PLGA and PLA, could reduce the amount of MNs drug loading capacity dramatically. Moreover, to avoid accumulation in the body, biocompatible/biodegradable polymers with molecular weight <60000 Da should be used to form the matrix of MN tips. Further special attention should be paid to MNs design and geometry which allow high drug loading, sufficient mechanical strength and, maximum insertion capability, such as, pedestal MNs or long MNs with intermediate interspacing (e.g. around 100 μm), but their length should not exceed 1 mm to avoid stimulating the nerve endings in the dermis.

Finally, the use of controlled-release technology is also reliant on the disease state or therapeutic effect desired. To transfer MNs products from the bench to the bedside, several challenges need to be addressed. Although the development of MNs assisted drug therapy has undergone substantial progress and contributed significantly toward trans-intradermal delivery, numerous limitations persist for large-scale manufacturing, regulatory requirements, and patient acceptance. As MN delivery systems are a comparatively new technology in the case

of commercialisation, their translational capability could be affected by complex formulation difficulties. Therefore, the creation of good manufacturing practice (GMP) facilities that set out and establish specific guidelines for MN formulation manufacture is necessary [322].

Table 2. Considerations for MN product development [322–324].

Pharmaceutical Considerations	Preclinical Considerations	Clinical evaluation for the commercialisation
Reduce complexity in the MN fabrication	Evaluation of MN application in an appropriate animal model	Evaluation of therapeutic efficacy, safety/toxicity (acute and chronic) in humans
Development of global regulatory or pharmacopeial standards for MN testing	Optimisation of MN insertion efficiency and application reproducibility in pre-clinical and clinical skin models /subjects	The requirement of clear regulatory guidelines
Optimisation of MN application with or without applicator device for reproducible insertion	An adequate understanding of <i>in-vivo</i> behaviour of MN insertion and drug deposition as well as drug absorption through the intradermal space	Understanding of the biological interaction of MN with the biological environment in the body of patients
Pharmaceutical stability (Chemical and Physical)	PK, metabolism, disease outcomes, and potential toxicities in small animals	Simplification of development pathways from invention to commercialisation to minimise time and expense
Whether a sterile or low bioburden device will be acceptable to regulators	confirmatory of drug PK and PD assessments in large animals include rhesus macaques and pig	Investigational new drug (IND) route
MN array patch design optimisation for the human use	Identifying the right animal model for the animal to human translation	
Scaled-up manufacture of MN patches with sizes suitable for delivery of high drug doses		

Successful MNs system development requires emphasis on reliable and repeatable application, a comprehensive survey of the user experience and, minute details of the MN fabrication process. MN enabled long-acting delivery, both hollow and solid MN, pose specific challenges. Answers to all these limits typically necessitate complex solution based on multidisciplinary approached with an in-depth insight of biology, skin physiology, physics, chemistry and bioengineering [325]. Mainly, converting nanomedicine facilitated long-acting MN formulations into the clinical product, there are further inherent challenges based on structural and chemical complexity of nanomedicines [324,326] Any added complexities to this MN technology would require demonstrating significantly superior benefits to the patients, due to the further costs and complexity in the manufacturing side. MN drug product-centric factors such as sterility of the MN, potential immunological effects, assurance of MN insertion,

deposition of MN material into the skin, the potential for MN re-use, uniformity of content and packaging, should be considered before technology transfer from lab to large scale product development for clinical studies [323]. Consideration to each one of these Research & Development criteria must be part of any systematic evaluation of GMP manufacturing to guarantee proper development and effective clinical translation. Multi-step MN production at GMP scale-up, continuous, and automatic on-line control with minimal human interference will be helpful to ensure product consistency, reproducibility and, quality. At present, most long-acting strategies are centred on improving the pharmaceutical and pharmacokinetic (PK) aspects in preclinical models. Small-animal models such as mice and rats provide screening of MN application, PK and, pharmacodynamics (PD) assessments. In these systems, improving product delivery, drug tissue targeting, metabolism, disease outcomes and, potential toxicities can be checked. Before proceeding to human clinical trials, evaluation in larger animal models, including rhesus macaques and pigs are now necessary for further in-depth and detailed confirmation of drug PK and PD assessments [327,328].

Those involved in charting the future evolution of MN assisted long-acting therapeutics should focus on not only the production efficacious delivery system but also patient-centred one. Although the development of nanotherapeutics has the potential to address the limitations of current MN long-acting technology. Largely, these hurdles can be categorised with respect to pharmaceuticals (formulation, scalability, usability), PK (absorption, disposition, safety) and pharmacodynamics (efficacy against disease), and clinical evaluation as described in Table 2. However, their actual utility in the clinic and acceptance by patients are being determined in broader populations. Various approved medical and cosmetic products using MNs that are sold around the world with low cost and good quality boost the confidence to explore the MN technology further. Based on industrial interest and possible benefits for patients with MN device technology, recently, the US FDA published draft guidance for quality control criteria for MN manufacture and acceptance for cosmetic applications [329], would be extended to therapeutics MN once some clinical trials progress with positive outcomes.

7 Conclusion/Future prospects

Recently, nanotechnology-based transdermal drug delivery platforms have gained renewed interest. Particularly, in combination with MN to ascertain a way to deliver a wide range of nanotherapeutics intradermally and, sustain the release of the therapeutic levels with only a single MN application. Urgently overcoming this unmet patient need is now necessary to

address both clinical needs and patient adherence. MN technology is in top first list amongst “10 Emerging Technologies 2020” by World Economic Forum [330]. Overall, MN technology is still in its early stage of clinical translation. First MN product with zolmitriptan (Qtrypta™) expected to be launch in the coming year that developed by Zosano Pharma Corp. This MN product NDA completed the first full review from US FDA and now the company have been trying to address the FDA concerns related to inconsistencies on drug plasma levels during the clinical trial from different manufacturing batches [331]. Therefore, simple, scalable, and user-friendly MN drug delivery platforms should be established. Once such a novel MN-assisted treatment is launched, it could be used to address the most challenging life-threatening and chronic diseases, such as HIV/AIDS, cancer, diabetes, cardiovascular disorders etc. At present, most long-acting MN strategies are focused on optimising PK and PD aspects in preclinical models [118,167,266]. Although MNs presents are able to deliver both hydrophobic and hydrophilic, small and large molecules for long-acting release, more studies need to be completed at the preclinical stage to support this. Additionally, efficient collaboration between academia, industry, non-profit organisations and, hospitals is now necessary to bridge the gap between the laboratory and meeting the patient. PATH, an international, non-profit, global health organization, has established the “Microarray Patches-Centre of Excellence” to bring all stakeholders under one umbrella to hasten MN manufacturing scale-up and, also the regulatory and clinical trial processes [332]. Finally, health and regulatory authorities should establish and enforce definitions (MN as a pharmaceutical product or device), quality test standards, guidelines and, regulatory issues for the MN drug delivery platform [325]. More investigational new drug (IND) type studies focusing on *in vivo* efficacy and long-term toxicity will need to be done. With more preclinical studies (and potential clinical studies) expected to take place over the next few years, we believe that more encouraging data will be reported to demonstrate the versatility of MN as a leading platform of choice for long-acting drug delivery. This could be the major step that is needed for the field to make the necessary progress towards fully realising the full potential of this novel drug delivery platform.

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