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Review

Microneedle arrays as transdermal and intradermal drug delivery systems: Materials science, manufacture and commercial development

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ABSTRACT

Transdermal drug delivery offers a number of advantages for the patient, due not only its non-invasive and convenient nature, but also factors such as avoidance of first pass metabolism and prevention of gastrointestinal degradation. It has been demonstrated that microneedle arrays can increase the number of compounds amenable to transdermal delivery by penetrating the skin's protective barrier, the *stratum corneum*, and creating a pathway for drug permeation to the dermal tissue below. Microneedles have been extensively investigated in recent decades for drug and vaccine delivery as well as minimally invasive patient monitoring/diagnosis. This review focuses on a range of critically important aspects of microneedle technology, namely their material composition, manufacturing techniques, methods of evaluation and commercial translation to the clinic for patient benefit and economic return. Microneedle research and development is finally now at the stage where commercialisation is a realistic possibility. However, progress is still required in the areas of scaled-up manufacture and regulatory approval.

Contents

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1. Introduction

For thousands of years, people have placed chemical agents on the skin surface, whether for healing, protective or cosmetic reasons. Historically, the skin had been viewed as an impermeable barrier to exogenous chemicals [1]. Topical drug therapy traditionally involved application of medicinal substances or drug-laden formulations to the skin only when its barrier had been compromised by disease or infection. In such cases, a direct route for drug absorption into viable skin was open. New possibilities for systemic delivery of medicines began to be realised once it was understood that intact skin is not a completely impermeable barrier. During the early 20th century, the increased skin permeability of more lipophilic agents was reported. By 1919, the barrier properties of the skin had been attributed specifically to the upper layers [2]. Subsequently, Scheuplein et al. investigated *in vitro* skin permeability to a wide range of substances [3]. They modelled skin as a three-layered laminate composed of the *stratum corneum*, epidermis and dermis, with drug permeation driven by Fickian diffusion. By separating the *stratum corneum* from the lower layers of the skin, this outermost 10–15 μm of tissue was determined to be the principal barrier to drug absorption. By the 1960s, systemic delivery of drugs applied topically to the intact *stratum corneum* began to attract attention.

The first transdermal system for systemic delivery—a three-day patch that delivers scopolamine to treat motion sickness—was approved for use in the United States in 1979 [4]. A decade later, nicotine patches became the first transdermal blockbuster, raising the profile of transdermal delivery in medicine and for the public in general. Advantages of this approach include:

- Prevention of gastrointestinal degradation and food-related inconsistency in absorption
- Avoidance of first pass hepatic metabolism
- Possibility for improved bioavailability
- Maintenance of relatively constant plasma concentrations for up to 7 days with the same patch
- Elimination of pain, discomfort, risk of infection and poor compliance associated with injections

Recently, the transdermal route has vied with oral treatment as the most successful innovative research area in drug delivery. Currently, 81 active clinical trials, studying applications ranging from vaccines to drug delivery to biofeedback loops, are ongoing. The worldwide transdermal patch market approaches $31.5 billion, yet is based on less than 20 drugs [5]. This rather limited number of transdermal drugs is attributed to the skin's excellent barrier function. Before being taken up by blood vessels in the upper papillary dermis, and prior to entering the systemic circulation, substances permeating the skin must cross the *stratum corneum* and the viable epidermis [6]. There are three possible pathways leading to the capillary network: through hair follicles with associated sebaceous glands, *via* sweat ducts, or across continuous *stratum corneum* between these appendages [7]. As the fractional appendageal area available for transport is only about 0.1%, this route usually contributes negligibly to steady state drug flux [7]. The intact *stratum corneum* thus provides the main barrier to exogenous substances, including drugs [6]. The corneocytes of hydrated keratin are analogous to "bricks", embedded in a "mortar" composed of highly organised, multiple lipid bilayers of ceramides, fatty acids, cholesterol and its esters [7]. These bilayers form regions of semicrystalline gel and liquid crystal domains. Most molecules penetrate through skin *via* this intercellular microroute. Facilitation of drug penetration through the *stratum corneum* may involve by-pass or reversible disruption of its elegant molecular architecture [7]. The ideal properties of a molecule penetrating intact *stratum corneum* well are [7]:

- Molecular mass less than 600 Da, when the diffusion coefficient in *SC* tends to be high
- Log *P* value between 1 and 3
- High, but balanced, *SC*/vehicle partition coefficient, such that the drug can diffuse out of the vehicle, partition into, and move across, the *SC* without becoming sequestered within it

- Low melting point, correlating with good solubility, as predicted by ideal solubility theory

Clearly, many drug substances do not do not satisfy these criteria. Those with Log *P* values below 1 are too hydrophilic to efficiently penetrate the *stratum corneum* by passive diffusion [7]. Those with Log *P* values greater than 3, which are the primary focus of the present application, are so hydrophobic that they become entrapped within the intercellular lipids of the *stratum corneum* and partition into the essentially aqueous environment of the viable epidermis below at such low rates that therapeutic plasma concentrations cannot be achieved [7]. Chemical penetration enhancers have no appreciable value in increasing transdermal flux of such compounds. Alternative enhancement strategies have, to date, focussed strongly on increasing transdermal delivery of hydrophilic drugs. This is understandable, given the ever-increasing number of biotechnology-derived protein and peptide molecules with therapeutic potential that have to be dosed parenterally due to gastrointestinal breakdown, poor absorption through biological membranes, rapid plasma clearance, peculiar dose-response curves and immunogenicity [7]. Accordingly, iontophoresis has been used to drive hydrophilic drugs through hair follicles and sweat glands, while electroporation, sonophoresis and suction, laser and thermal ablation create transient aqueous pores in the *stratum corneum* [7]. In practical and economic terms, however, these approaches suffer from considerable limitations.

2. Microneedles: characteristics, history and delivery strategies

2.1. Characteristics and history

Microneedle arrays (MN) are minimally invasive devices that by-pass the *SC* barrier, thus accessing the skin microcirculation and achieving systemic delivery by the transdermal route. MN (50–900 μm in height, up to 2000 MN cm^{-2}) [8] in various geometries and materials (silicon, metal, polymer) are produced using microfabrication techniques [7,9]. MN are applied to the skin surface and painlessly pierce the epidermis, creating microscopic aqueous pores through which drugs diffuse to the dermal microcirculation [7,10]. MN are long enough to penetrate to the dermis, but are short and narrow enough to avoid stimulation of dermal nerves or puncture of dermal blood vessels [7,10]. No reports on development of skin infection exist and we have shown that MN can be reproducibly inserted into skin by patients without additional applicator devices [11]. Solid MN puncture skin prior to application of a drug-loaded patch or are pre-coated with drug prior to insertion. Hollow bore microneedles allow diffusion or pressure-driven flow of drugs through a central lumen, while polymeric MN release their drug payload as they dissolve or biodegrade in the viable skin layers [12]. *In vivo* studies using MN have demonstrated delivery of oligonucleotides, hormones, reduction of blood glucose levels from insulin delivery, increase of skin transfection with DNA and elicitation of immune responses from delivery of DNA and protein antigens [10,12,13].

MN were first conceptualised in the 1970s [14], but it was not until the late 1990s when they became the subject of significant research due to advancements in microfabrication technology that enabled their manufacture [15]. In the first published paper on MN, Henry et al. described the use of silicon MN to facilitate the delivery of calcein (model drug) across excised human skin *in vitro*. Since then, these devices have been extensively investigated [7,10,12,16]. In 2004, it was suggested that MN arrays could be used to permit transdermal transport of macromolecules and possibly supramolecular complexes and microparticles [10]. Over the last decade, extensive research has been carried out on MN technology using a wide variety of materials and MN designs [7]. Moreover, a range of fabrication methods have been developed [9]. Five different types of MN designs are described in the literature [12], namely solid, coated, hollow, dissolvable and hydrogel-forming MNs.

2.2. Delivery strategies

Solid MNs are normally employed in the so-called 'poke with patch' approach [10]. Solid MN are applied to the skin and then removed, creating transient aqueous microchannels are created in the *stratum corneum*. Subsequently, a conventional drug formulation (transdermal patch, solution, cream or gel) is applied, creating an external drug reservoir (Fig. 1A). Permeation through these microchannels occurs *via* passive diffusion. The main limitation of this approach is the requirement for a two-step application process, which may lead to practicality issues for patients. The materials used to produce solid MN are typically silicon [17,18], metals and polymers [19].

Coated MNs are prepared by coating solid MN with a drug formulation prior to skin application. After insertion of coated MN arrays into the skin, the coated drug formulation will be dissolved and deposited in the skin (Fig. 1B). This delivery strategy is typically referred to as 'coat and poke' [10]. Coated MNs have been employed for the rapid cutaneous delivery of macromolecules, such as vaccines, proteins, peptides and DNA to the skin [12]. This type of MN allows a simple one-step application process, but its main limitation is the restricted amount of drug that can be coated onto the finite surface area of the MN structures. Accordingly, the use of coated MNs is restricted to potent molecules/drugs. Various techniques have been developed to efficiently coat the individual MN shafts in MN arrays [20,20–22].

The third type of MN are dissolving MNs. They are made by micro-moulding soluble matrices, generally a biocompatible polymer or sugar, including the active substance [7]. The skin insertion of the array is followed by dissolution of the MNs tips upon contact with skin interstitial fluid. The drug cargo is then released over time (Fig. 1C). The release kinetics of the drug depends upon the constituent polymers' dissolution rate. Therefore, controlled drug delivery is achievable by adjusting the polymeric composition of the MN array, or by modification of the MN fabrication process. Dissolving MNs present numerous advantages. The principal benefit is the low cost of polymeric materials and their relatively facile fabrication by micro-moulding processes at ambient temperatures, which should allow for straightforward industrial mass production. Various materials, including poly(vinyl alcohol) (PVA), poly(vinylpyrrolidone) (PVP), dextran, carboxymethyl cellulose (CMC), chondroitin sulfate and a sugars have all been used to produce this type of MN array [23]. Importantly, the use of water-soluble materials eliminates the potential risk of leaving biohazardous sharp waste in the skin. Moreover, safe MN disposal is facilitated, since the MN are, by definition, self-disabling [4,24]. On the other hand, the main limitation of this type of systems is the deposition of polymer in skin, possibly making these systems undesirable if they are likely to be used on an ongoing basis [25]. Biodegradable MN can also be included in this category. This type of MNs is produced using biodegradable polymers, including poly(lactic acid), chitosan, poly(glycolic acid), or poly(lactide-co-glycolide) (PLGA) to form the matrix. After insertion they degrade, rather than dissolve in the skin, releasing their cargo. Accordingly, release could possibly be sustained for months by choosing the appropriate polymer [26]. Since biodegradation typically produces small molecules by hydrolysis, polymer is not deposited in skin indefinitely. However, such MN typically require high temperatures during manufacture, which may damage biomolecular cargoes.

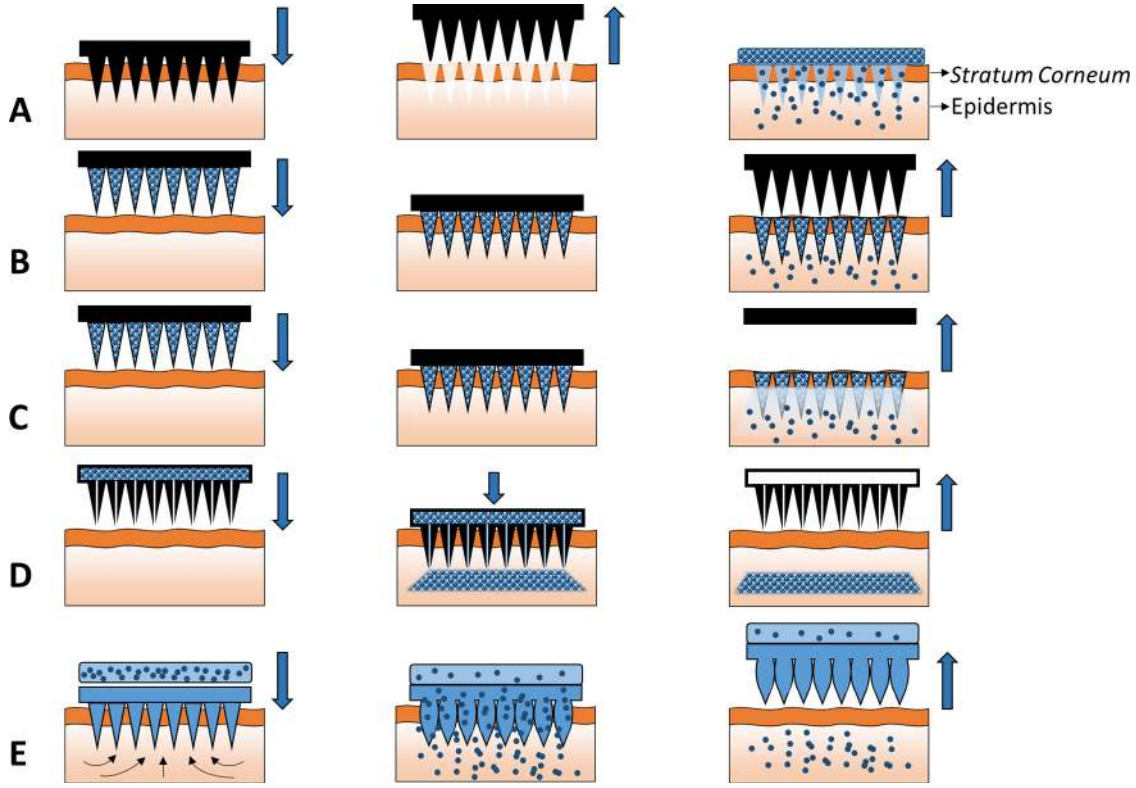

Fig. 1. A schematic representation of five different MN types used to facilitate drug delivery transdermally. (A) Solid MNs for increasing the permeability of a drug formulation by creating micro-holes across the skin. (B) Coated MNs for rapid dissolution of the coated drug into the skin. (C) Dissolvable MNs for rapid or controlled release of the drug incorporated within the microneedles. (D) Hollow MNs used to puncture the skin and enable release of a liquid drug following active infusion or diffusion of the formulation through the needle bores. (E) Hydrogel-forming MNs take up interstitial fluids from the tissue, inducing diffusion of the drug located in a patch through the swollen microprojections.

Hollow MNs allows the delivery of a particular medication into the skin *via* the injection of a fluid formulation through the inserted hollow needles (Fig. 1D). This type of MNs allows continuous delivery of molecules across the skin through the MN bore using different methods: diffusion or pressure- or electrically driven flow. Such systems are possibly capable of delivering larger amounts of drug substances in comparison to solid, coated and dissolving MNs [27]. Hollow MNs are made from a range of materials, including silicon and metal [27–29], glass [30], polymers [31] and ceramic [32]. The main limitations of hollow MNs are the potential for clogging of the needle openings with tissue during skin insertion [33] and the flow resistance, due to dense dermal tissue compressed around the MN tips during insertion [34]. The first limitation can possibly be overcome by using an alternative design to locate the bore-opening at the side of the MN tip [35]. Partial needle retraction following insertion may also enhance fluid infusion, due to relaxation of the compressed tissue around the tips [30]. However, use of liquid drug formulations will require a suitable, possibly complex, reservoir and liquid formulations are notoriously unstable, particularly at the elevated ambient temperatures found in the developing world.

A relatively new type of MN arrays are prepared from hydrogel-forming matrices. Such systems were first described recently by Donnelly et al. [8,36]. This novel strategy involves integrated systems consisting of crosslinked drug-free polymeric MN projecting from a solid baseplate to which a patch-type drug reservoir is attached. After application of the MN array to the skin, the inserted needle tips rapidly take up interstitial fluid from the tissue, thus inducing diffusion of the drug from the patch through the swollen microprojections (Fig. 1E). These systems are manufactured using aqueous blends of specific polymeric materials, namely poly(methyl vinyl ether-co-maleic acid) cross-linked by esterification using poly(ethyleneglycol) [8,25,37,38]. Garland et al. showed that drug delivery can be tailored by modulating the crosslink density of the hydrogel matrix [39]. Importantly, hydrogel-forming MNs are removed intact from skin, leaving no measurable polymer residue behind. However, they are sufficiently softened to preclude reinsertion, thus further reducing the risk of transmission of infection [40]. Other polymers that can be used to prepare hydrogel-forming MNs are chitosan, PLGA and poly(vinyl alcohol) [41,42]. With these alternative polymer systems, however, the drug is included inside the hydrogel-forming MN patch rather than in an external patch [42], thus limiting the quantity of drug that can be delivered.

3. Material types and biocompatibility

3.1. Material selection and properties

3.1.1. Silicon

Silicon was the material selected for the first MNs used for drug delivery because the technology needed to manufacture micron or submicron structures only became available with the advent of industrial high-precision microelectronics tools during the 1990s [7,9,15,33,43–45]. Silicon has proved very useful in manufacture of microstructures and microelectromechanical systems (MEMS) for a number of reasons [45]. Its main advantage is that there is much flexibility in the processes that can be used to shape it, meaning that microstructures in a variety of desirable shapes and sizes can be readily produced. Using monocrystalline or polycrystalline silicon allows tailoring of specific solutions to a broad range of requirements. Moreover, silicon offers many attractive physical

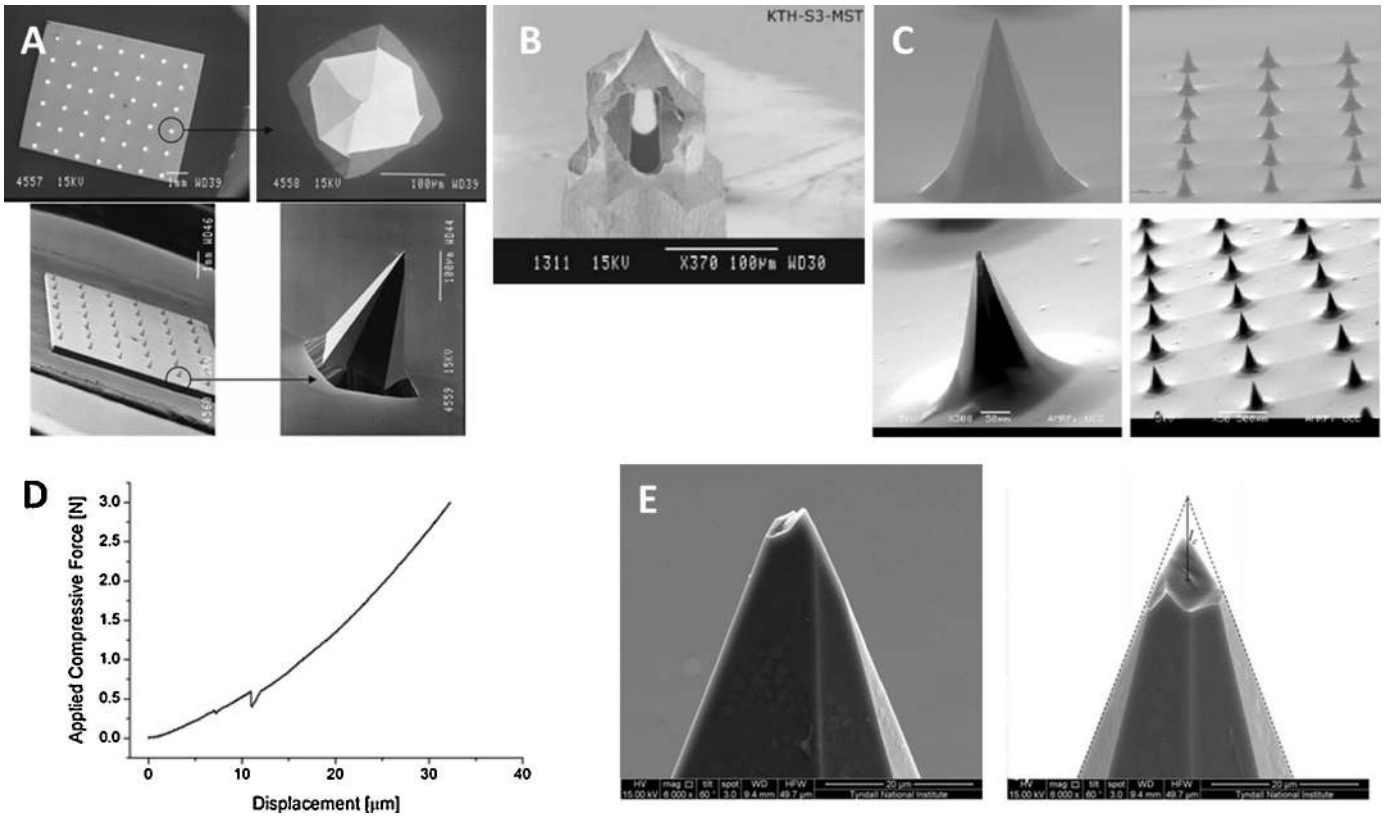

Fig. 2. SEM images of (A) solid, (B) hollow and (C) coated silicon MN arrays. (D) Force–displacement graph for silicon MN compression test showing discontinuities characteristic of structural failure. (E) SEM images of damage to needle after application of 0.3 N and 0.5 N compressive loads to the needle tip. lc is the distance from the needle apex to the intersection of the longitudinal axis of the MN and the broken plane. In this case, lc is 14 μm.
Source: Reproduced with permission of: [17,27,33,53,57].

properties, making it an attractive and versatile material. Finally, the manufacturing methods that exist for silicon substrates are precise and capable of batch production that reduce costs [43].

Silicon is an anisotropic crystalline material, the material properties of which depend upon orientation relative to the crystal lattice, yielding different elastic moduli, ranging from 50 to 180 GPa [46]. Therefore, in some cases, silicon can present an elastic modulus comparable to metals used for orthopaedic implants (see Section 3.1.2). Their considerable mechanical strength allows silicon MNs to successfully pierce the skin [15,47,48], facilitating transdermal drug delivery [17,33]. Silicon has been used extensively in manufacture of MNs with multiples shapes, heights and densities [49–51]. The three main types of MN that have been prepared using this material are: solid [15,17, 18,45,52], hollow [27,33] and coated MNs [53] (Fig. 2A–C). The main limitations of silicon are its high cost, elaborate fabrication, long fabrication times and complex multi-step processing [43,54]. Furthermore, there have been concerns with the biocompatibility of silicon [55,56]. Due to the brittle nature of the material [56], some silicon MN could become fractured in the skin [57] and this may raise health concerns. Biocompatibility is discussed further in Section 3.2. Numerous studies have been carried out to evaluate the reliability of silicon MNs in terms of their failure forces and investigation of the failure mechanism of the needle tips [47,48,57] (Fig. 2D and E).

3.1.2. Metals

Metals have been in medical use for decades. Classic examples are stainless steel (*e.g.* hypodermic needles) or titanium (*e.g.* implants and prostheses). Since conventional exploitation of these materials in production of medical devices, MN production should not present additional safety issues, thereby smoothing the way towards regulatory approval. The main metals used in productions of MNs are stainless-steel, titanium, palladium, palladium-cobalt alloys and nickel [58]. In addition to their good biocompatibility, such metals possess good mechanical properties. Young's moduli of the stainless steel used medical implants, SUS316L stainless steel, is around 180 GPa [59]. Young's moduli of Ti (pure titanium) and its alloys are generally smaller than those of stainless steels. For example, Ti and its alloy, Ti-6Al-4V ELI, are common materials in implants devices and have Young's moduli around 110 GPa [59]. A comparison of the mechanical properties of these types of metals can be found in Table 1. The elastic moduli of these are comparable to the highest possible for silicon (up to 180 GPa). Moreover, metals have higher fracture toughness and similar

Table 1
Strengths of materials used to make microneedles.

Material	Young's Modulus (GPa)	Ultimate tensile strength (MPa)
Silicon	110	7000
Glass	85	50
Nickel	214	586
Palladium	117	186
Platinum	147	117
Titanium	110	241
Stainless steel	200	1000
Ormocer®	17	30
PMMA	3	170
Maltose	31.1	–
SU-8	3	–

Source: Data obtained from: [166].

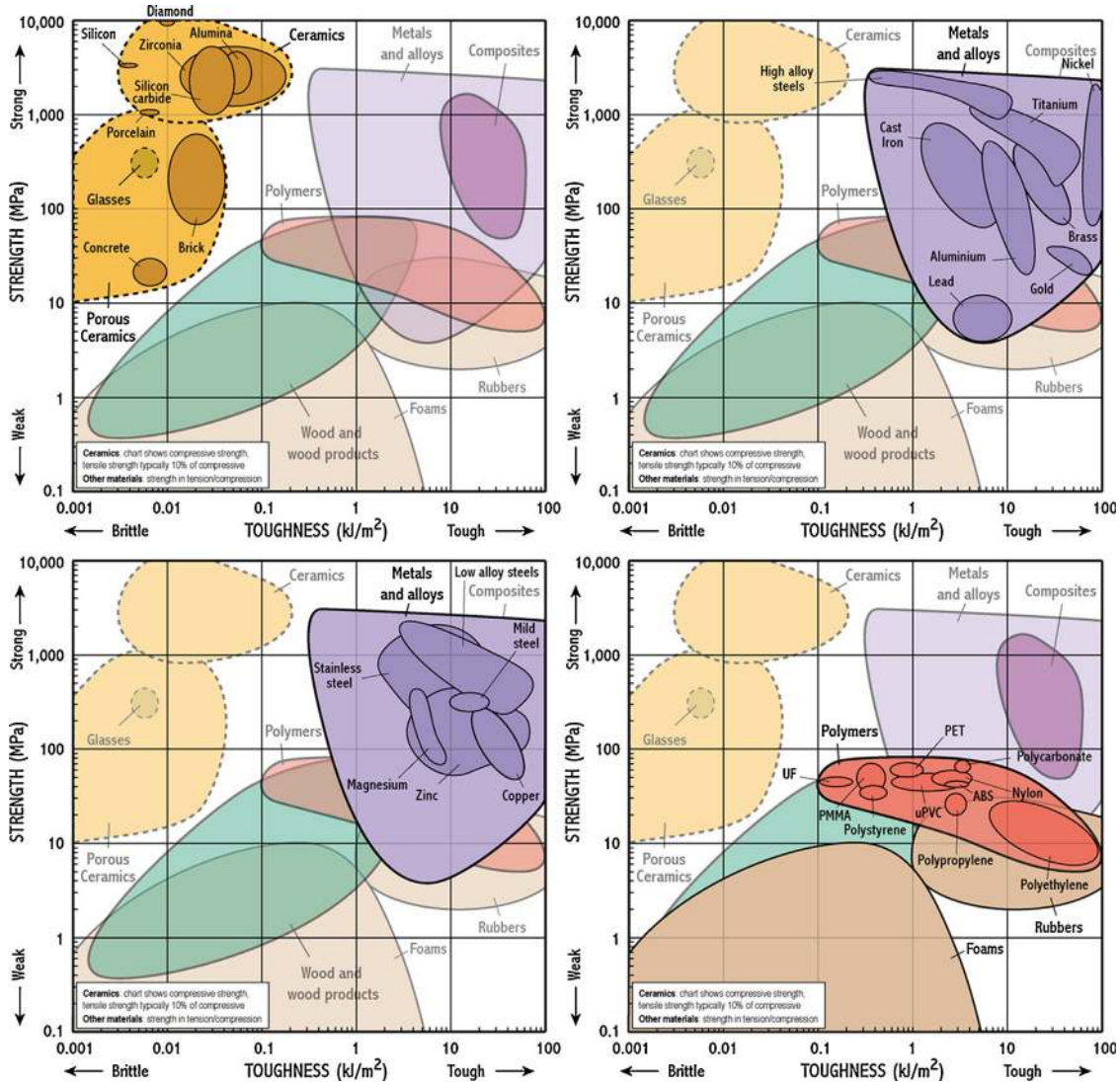

Fig. 3. Strength *versus* toughness graphs for different types of materials.
Source: Adapted from: www.materials.eng.cam.ac.uk/mpsite (Lovatt A.M., Shercliff H.R. and Withers P.J. (2000), "Material selection and processing"); data courtesy of Granta Design Ltd, Cambridge, UK.

values of yield strength (Fig. 3). Consequently, metals are possibly a more suitable material to silicon in MN manufacture.

Stainless steel was the first metal used in production of MN arrays. Most metal MNs have been obtained by manually pressing the tips of the smallest available stainless steel hypodermic needles (30/31 G) through a supporting material of defined thickness [7,60]. Less commonly, stainless steel MNs in a variety of designs have been produced using microfabrication technology [60–64,21] (Fig. 4A).

A good alternative to stainless steel is titanium. Despite possessing less robust mechanical properties to stainless-steel, this material is certainly strong enough for biomedical applications [59]. Titanium MNs have been produced, mainly as bio-sensors [65,66] and as transdermal delivery systems [67] (Figure 4B).

3.1.3. Ceramics

Another material that has been used to produce MNs is ceramic. Mainly they are produced using a micromolding technique by ceramic slurry cast into micromould [9] (see Section 4.1.2). Micromolding techniques offer the advantage of being able to develop device production as a low cost process, due to the potential for up-scaling the technology [9].

The main type of ceramic used to produce MN has been alumina (Al_2O_3) [68,69] (Fig. 4C). This material presents some advantages, principally chemical resistance. The Al_2O_3 molecule is one of the most stable oxides, because of the high energetic ionic and covalent bonds between Al and O atoms [70]. These strong bonds leave the ceramic unaffected by corrosion or adverse environmental conditions [70]. Under compression, alumina showed good resistance (Table 1), but under tensile stress it is shown to be brittle [70]. Table 1 shows lower strength resistance to tension than other materials, such as metals. Additionally, Bystrova et al. showed that Al_2O_3 MNs can be fractured during manual skin insertion [68]. Al_2O_3 MNs may be employed as a drug-coated solid MN array, following the 'coat and poke' (see Section 2.2) approach for release into the skin. However, due to the porosity of alumina, this type of ceramic MN, this type of MNs also holds a defined volume of active for controlled release through its open pore volume [69]. Other types of biocompatible ceramic used to prepare MNs are calcium sulfate dihydrate [Gypsum ($CaSO_4{\cdot}2H_2O$)] and calcium phosphate dihydrate [Brushite ($CaHPO_4{\cdot}2H_2O$)] [71] (Fig. 4D). These materials have been used before as drug delivering bone cements, so they present good mechanical and drug loading properties [72,73]. Additionally to pure ceramic MN arrays, during

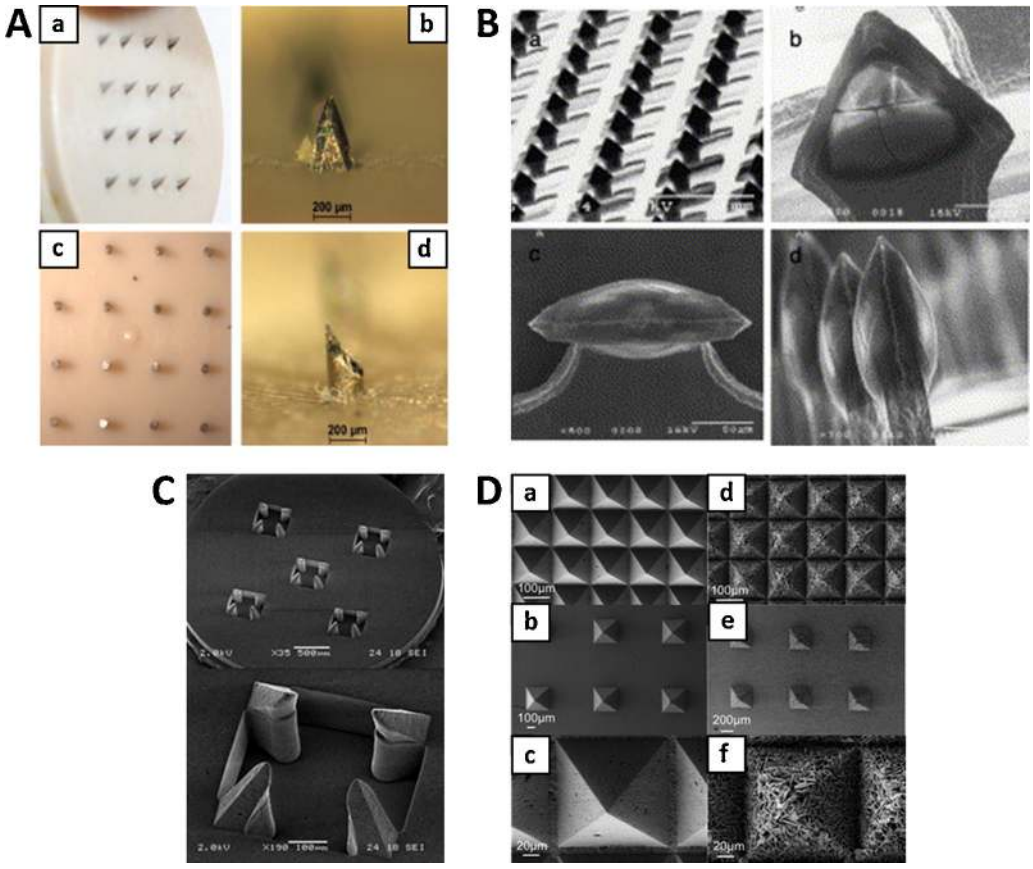

Fig. 4. (A) Different types of stainless-steel MNs: (a) hollow stainless-steel 4 × 4 MN array, (b) higher magnification of a single hollow stainless-steel needle, (c) solid stainless-steel 4 × 4 MN array and (d) a higher magnification of a single solid needle. (B) Scanning electron microscopy of titanium microneedle array coated with 80 μg of desmopressin per array. (a) Bar = 1 mm; (b–d) bar = 50 μm. Scanning electron micrographs of (C) alumina ceramic, (Da-Dc) gypsum and (Dd-Df) brushite.
Source: Reproduced with permission of: [63,67,69,71].

recent years, MN arrays have also been fabricated using an organic-ceramic hybrid material called Ormocer® [74,75]. This type of material contains organically modified silicon alkoxides and organic monomers forming a three-dimensional network [74]. An interesting characteristic of this type of materials is that its properties can be adjusted by varying the composition, the synthesis parameters tailoring the properties of the final material [76].

3.1.4. Silica glass

Silica glass is an alternative to the materials described above. Glass MNs can be quickly produced in various geometries for small-scale laboratory use. This material is physiologically inert, allows easy visualisation of fluid flow and, finally, can be fabricated with dimensions similar to those of microfabricated MNs [30,77,78] (Fig. 5A and B). Glass MN described in the literature are hollow in nature and have typically been used to bypass the *stratum corneum* and inject medicines [30,77]. Borosilicate glass presents lower values of elastic moduli (64 GPa) [79], making this material more elastic. However, silica glass is a brittle material and, as can be seen in Fig. 3, silicon and silica glass present similar fracture toughness. Fabrication of glass MNs is not time efficient, since it is typically performed by hand [80]. Glass MNs are still used today, but only for experimental purposes and are not viable for commercial use in drug delivery [30].

3.1.5. Carbohydrates

MN can be prepared easily by moulding hot melts/slurries of carbohydrate materials using silicon or metal MNs as master templates [81–85]. Drugs to be delivered are added to the mixture before casting the formulation into the moulds. Such MNs should dissolve upon skin insertion to release their drug payload [81]. Carbohydrates are good alternatives to the previously described materials, as they are cheap and, additionally, safe for the human health [85].

Maltose has been one of the most common sugars used to prepare MN arrays [82,83,86] (Fig. 5C–E). Other sugars, such as trehalose, sucrose, mannitol, xylitol and galactose have also been studied [81,87]. The mechanical properties of these MN arrays have not been extensively investigated. Galactose MN presented significant reductions in MN height when relatively small forces were applied to them against an aluminium block. Nevertheless, this was not observed during their relatively facile insertion into heat-stripped epidermis [81]. Consequently, this type of MNs seems to have enough strength to pierce the skin and deliver their cargos [82,86]. However this type of MN presents a range of disadvantages. Donnelly et al. reported inherent problems in the processing, storage, and use of MN arrays prepared from sugars [81]. The main drawback of this type of MN is that the need for thermal treatment during the manufacturing limits the number of compounds that can be loaded in the arrays. Additionally, during the release process, partially dissolved sugar sealed the MN-induced holes, thus limiting drug delivery. Finally, storage under conditions of temperature and relative humidity adversely affect this type of MN, as can be seen in Fig. 5E. Such difficulties are likely to preclude commercial development or clinical application of carbohydrate-based MN arrays. In addition to simple sugars, polysaccharides have also been used to prepare MN arrays. As they

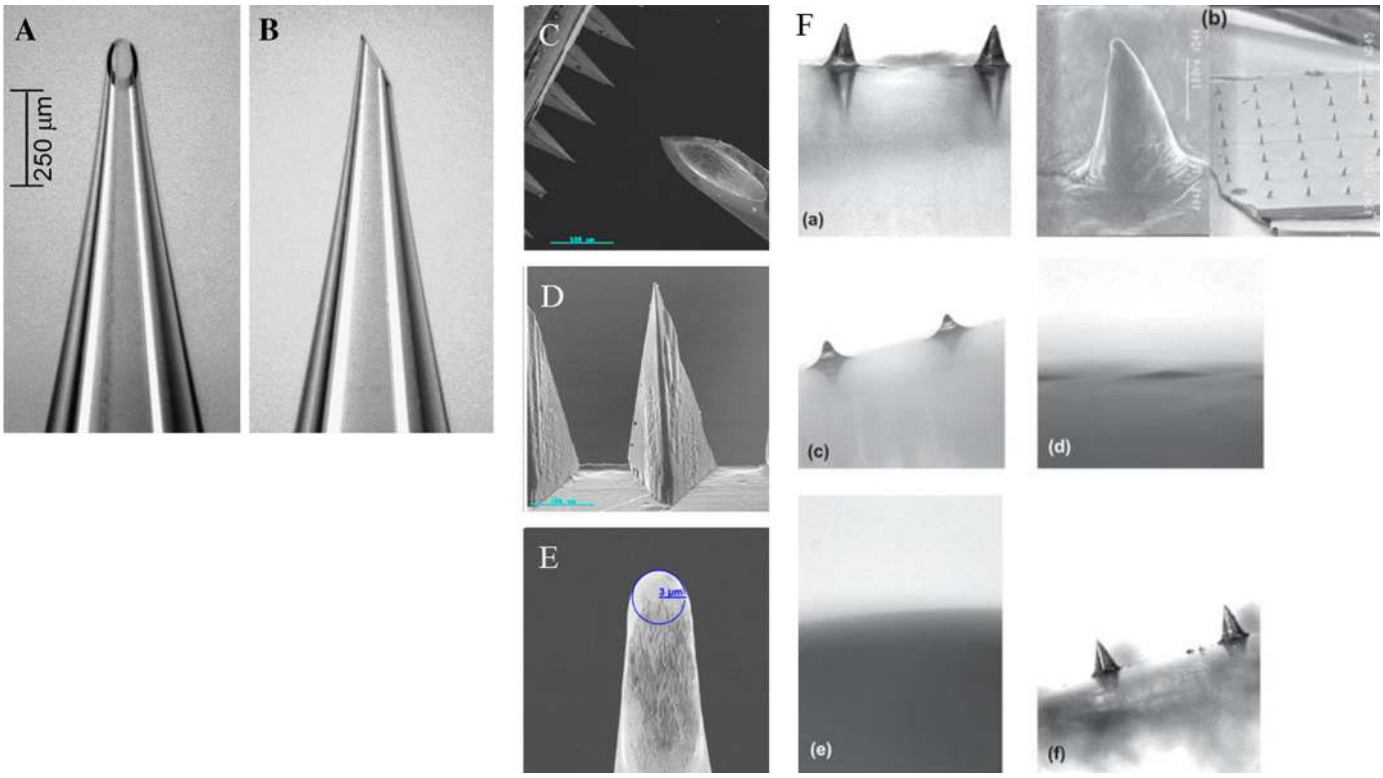

Fig. 5. (A) Front and (B) side views of a representative hollow, glass microneedle. (C) Scanning electron micrograph image of 500 μm long solid maltose microneedles shown opposite to a tip of a 26 G hypodermic needle, (D) in an individual array and (E) magnified view that show the radius of the tip. (F) Influence of the storage conditions for galactose MN arrays. (a) Light micrograph of galactose microneedles upon preparation. (b) Scanning electron micrographs of the same array. (c) Light micrograph of galactose microneedles after storage at a relative humidity of 43% for 1 h and (d) 6 h. (e) Light micrograph of galactose microneedles after storage at a relative humidity of 75% for 1 h. (f) Light micrograph of galactose microneedles after storage at a relative humidity of 0% for 3 weeks.
Source: Reproduced with permission of: [77,81,86].

are macromolecules, they will be included in the next section (see Section 3.1.6).

3.1.6. Polymers

Polymeric materials are promising alternatives to the previously described materials for MN production. Some polymers and polysaccharides are drawing increasing attentions because of their excellent biocompatibility, biodegradability, low toxicity, strength/toughness and low cost [7,26]. In general, polymers present lower strength than silicon, metals, ceramic and glass, but better toughness, than glass and ceramics (Fig. 3 and Table 1).

A wide variety of macromolecular materials have been efficiently used to fabricate MNs: poly(methyl methacrylate) (PMMA) [88–90], PLA [91], PGA [80], PLGA [92], poly(carbonate) [93], cyclic-olefin copolymer [31,94], poly(vinylpyrrolidone) (PVP) [95,96], poly(vinylalcohol) PVA [96], polystyrene (PS) [97], poly(methyl vinyl ether-co-maleic anhydride) [8,98], poly(methyl vinyl ether-co-maleic acid) [25], SU-8 photoresist [99] and polysaccharides [84,100–102]. Fig. 6 shows the structures of some of these polymers. Polymers are used mainly in production of dissolving/biodegradable and hydrogel-forming MN arrays. Nevertheless, to some extent, there are some studies using polymers for the production of solid [19,89], hollow [49,90] and coated MN arrays [103].

Dissolving/biodegradable polymeric MN arrays have been produced using different types of polymers [26]. The dissolution/biodegradation process (depending upon the composition of the MN material) releases the drug molecules from the matrix for local or systemic delivery (Fig. 7A) [7]. Macromolecular dissolving MN systems are prepared with polysaccharides (Fig. 7B). The main ones are carboxymethylcellulose, amylopectin, dextrin, hydroxypropyl cellulose, alginate and hyaluronic acid [26,102]. MN prepared using these macromolecules present sufficient mechanical properties to penetrate the skin and deliver their cargos (model molecules, lysozyme and insulin) [84,100,101]. In addition to polysaccharides, synthetic polymers have also been used to prepare dissolving MN arrays. The main ones are poly(methyl vinyl ether-co-maleic anhydride) (Gantrez AN-139®) [37,98,104,105], PVP and PVA [96]. MN prepared using Gantez AN-139® are strong enough to bypass the *stratum corneum*. Due to the plastic nature of the polymer, this type of MN s are able to resist compression forces up to 0.7 N/needle without breaking. Additionally, MN prepared using PVP and PVA possess sufficient strength to pierce the skin and deliver their cargos [96]. On the other hand, biodegradable MN arrays are produced mainly using PLA, chitosan, PGA or PLGA [26]. Biodegradable MN have been used to deliver different types of therapeutics, from small molecules to macromolecules or nanoparticles across the skin [26].

In addition to dissolving/biodegradable MN, the other main type of polymeric MN arrays are swelling or hydrogel-forming MN arrays [41]. This type of MN arrays provides drug release as a result of the polymer swelling when absorbing body fluid, leaving no measurable polymer residuals after removal from skin (Fig. 7C–E) [41]. The needle tips swell in skin to produce conduits. The drug can diffuse from a patch-type drug reservoir to the dermal microcirculation using these conduits, thus allowing prolonged transdermal drug administration [7]. The mechanical properties of these materials allows skin insertion and mechanical resistance to fracture when dry, making this type of hydrogel a good candidate for hydrogel-forming MNs manufacture [7]. The swelling of this type of device can be modified by adding $NaHCO_3$, a pore forming agent [106]. Besides polyanhydride type polymers, only mixtures

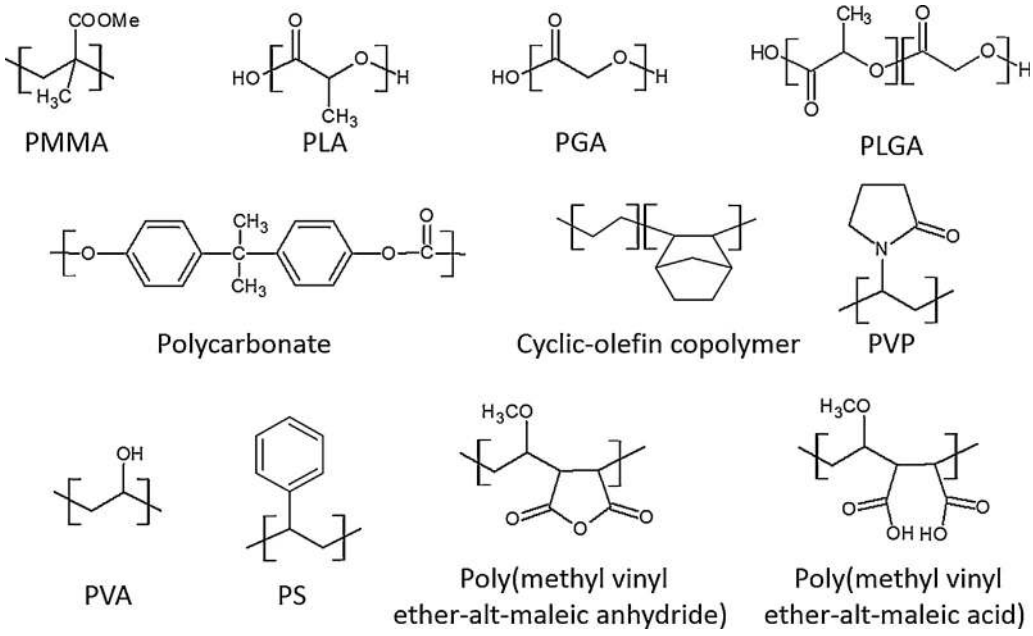

Fig. 6. Chemical structure of some of the polymers used to produce MN.

of polysaccharides (dextran, gelat) and PVA have been reported to be used successfully to produce hydrogel-forming MN arrays for drug delivery (Fig. 7B) [41,42,102]. Yang et al. reported the use of PVA, dextran and CMC to prepare hydrogel-forming MN arrays [42]. In this case, crosslinking was carried out by a freeze–thaw process. Using a similar process, Demir et al. prepared swelling MN arrays by crosslinking PVA and gelatin [102].

3.2. Biocompatibility and biodegradability of microneedle materials

As detailed above, MN arrays pierce the *stratum corneum* barrier, exposing the MN tips to the viable skin tissue. Therefore, it is mandatory that the materials selected for production of MN are biocompatible. The most important factor that differentiates a biocompatible material from any other type of material is its ability to exist in contact with tissues of the human body without causing an unacceptable degree of harm to such tissues. To the best of our knowledge, the skin biocompatibility of all MN materials has not been extensively evaluated.

3.2.1. Biocompatibility of silicon and silica glass

As described above, silicon and silica glass are brittle materials and a realistic concern about MN produced with such materials is the possibility of a breakage of the tips once they are inserted. Any broken pieces of such MNs will most likely be extravasated within four weeks, during the normal turnover of the epidermis [107]. Importantly, though there have been notable reports of silicon- and glass-related granulomas [108,109].

As silicon is one of the main MEMS materials, the biocompatibility of silicon has been examined during the last 20 years in a wide number of studies [110]. There are different studies assessing the biocompatibility of MEMS devices made of silicon for brain and subcutaneous implants [111–113]. Bayliss et al. showed that nanocrystalline silicon does "not exhibit significant cytotoxicity" [114]. On the contrary, other research works described some biocompatibility problems. Moreover, there are reports describing formation of granulomas [109] in subcutaneous tissue and an *in vitro* study demonstrated the formation of nodules on periodontal ligament fibroblasts when using a silicon-bearing bioglass. This phenomenon has been attributed to silicon release from the glass [115]. In conclusion, the biocompatibility of silicon is still uncertain. However, there is a silicon MN-based product already on the market, Microjet® (see Section 5.2.5) that received FDA clearance in 2010 [12]. This system consists of silicon MNs attached to a syringe and they are not inserted inside the skin for prolonged periods of time. As detailed above, silica glass can cause granulomas in the skin [108], but borosilicate glass apparently seems to be biocompatible for cortical implants [116]. Therefore, the use of silicon and silica glass for MN manufacture should be carefully studied, as there is currently not sufficient evidence of the biocompatibility of these materials.

3.2.2. Biocompatibility of ceramics

Ceramics have been used for repair and replacement parts of musculoskeletal systems during recent decades due to their good biocompatibility and high strength [117]. The precise type of ceramic used will, obviously, influence its biocompatibility. Alumina has been used for nearly 25 years in orthopaedic materials for bone and dental implants [117]. Therefore, its biocompatibility has been extensively studied [118–120]. Despite exhibiting good biocompatibility, some studies showed that alumina is not totally stable under physiological conditions and there is a risk of aluminium release from long-term bone implants [121]. However, this phenomena is not observed for short term implants [121], so MN arrays made of alumina should not present significant problems. Calcium-phosphate compounds used as bone substitute materials already have a firm place in clinical applications [122,123]. This type of ceramics is considered biocompatible, bioactive in the sense of osteoconduction and bioresorbable [124]. Additionally, calcium sulphates present similar characteristics [125]. Finally, a range of studies showed that Ormocer® presents good biocompatibility and is safe for use as a biomedical material [32,126,127]. Ovsianikov et al. demonstrated that this material does not adversely affect growth of human epidermal keratinocytes, a major cellular component of the skin [32].

3.2.3. Biocompatibility of metals

Metals used in production of MNs are normally biocompatible and have a dominant role to play in structural biomaterials used in

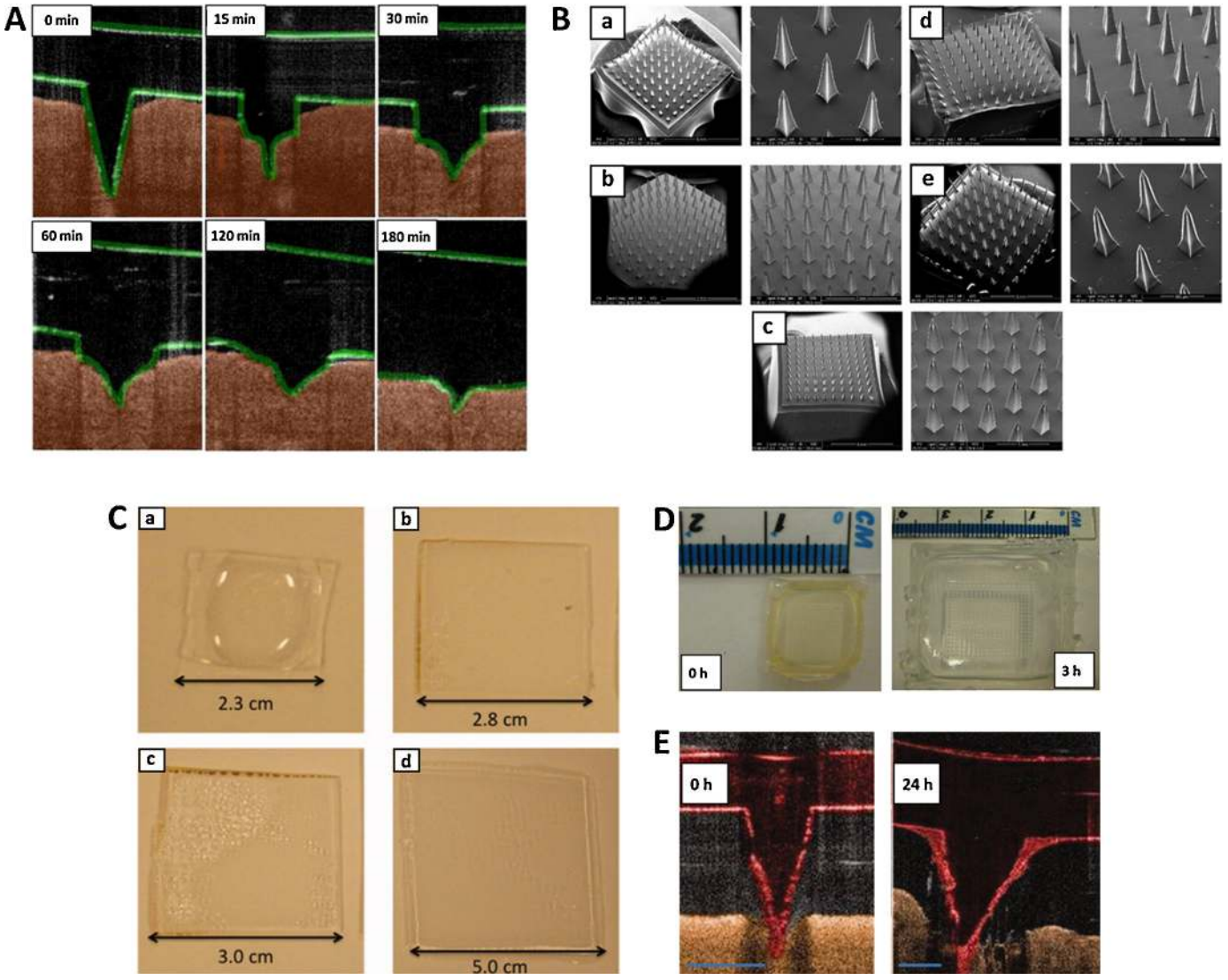

Fig. 7. (A) False colour optical coherence tomography images of the *in vitro* dissolution profile of Gantrez-AN 139® microneedles in porcine skin over a 3-h period. (B) SEM photographs of parts from 10 × 10 MN arrays made of: (a) alginat, (b–c) hydroxypropyl cellulose, (d) cross-linked PVA-gelatin, and (e) chitosan. (C) Digital photographs of equilibrium swollen hydrogels (with initial dimensions of 1 cm × 1 cm) prepared from aqueous blends of 15% w/w PMVE/MA and 7.5% w/w PEG 10,000 containing (a) 0%, (b) 1%, (c) 2%, and (d) 5% w/w of $NaHCO_3$ showing changes in respective dimensions. (D) Gantrez S-97® MN before and after swelling for 3 hs in PBS pH 7.4. (E) Representative optical coherence tomography images of hydrogel forming MN array before and after insertion into neonatal porcine skin for a period of 24 h. Scale bar represents a length of 300 μm.
Source: Reproduced with permission of: [25,102,105,106].

reconstructive surgery, especially orthopaedics, with more recent uses in non-osseous tissues, such as blood vessels [128].

Stainless steel is the generic name for a number of iron-based alloys that contain a high percentage (11–30 wt%) of chromium and varying amounts of nickel [128]. The properties and biocompatibility of stainless steel are affected by its composition [128]. The most common surgical stainless steel is 316L. In general, 316L stainless steel shows relatively good biocompatibility, but to a less satisfactory extent to other metals, such as titanium alloys, due to its greater corrosion rates [128]. However, the uses of MN do not typically require long application times, so corrosion is unlikely to present significant problems. The times of application of metal MN arrays will most likely be similar to those of stainless steel hypodermic needles that are considered biocompatible and, therefore, widely used [129].

Compared with stainless steel, titanium alloys have proven to be superior in terms of biocompatibility. This is mainly due to their excellent corrosion resistance [128]. Mutagenicity is not significant, as determined by *in vitro* mutation assays, indicating that titanium alloys are safe for humans and animals [128]. However, first generation titanium alloys have been reported to cause allergic reactions in the human body [130]. Second generation titanium alloys have been developed and investigated with great interest. They are considered to be relatively safe. So far, there is a lack of long-term clinical application data and follow-up reports on the biocompatibility of these titanium alloys [128]. Palladium and platinum present good biocompatibility [131,132]. However, they have been investigated to a much lesser extent in production of MNs. On the other hand, caution should be used when employing nickel as a material for fabricating MNs. Nickel is known to be carcinogenic and adverse allergic reactions have also been problematic in the past with nickel-containing biomaterials [133].

3.2.4. Biocompatibility and biodegradability of carbohydrates

Natural sugars are an important ingredient of many drug delivery systems, as they are safe for use in humans [85]. Maltose is

the main sugar used in the production of carbohydrate-based MN arrays. This sugar has been extensively used in different FDA-approved parenteral formulations [134]. Besides maltose, galactose is used in approved parenteral formulations [134]. Understandably, certain products containing maltose or galactose can produce interferences with blood glucose monitoring [134]. While maltose and galactose may be considered safe for MN production, this interference issue should be taken into account. Sucrose, mannitol, trehalose and xylitol can all be found in a diverse range of parenteral formulations as cryoprotectants, stabilisation agents or as parenteral nutrition products [135,136]. In addition to simple sugars, polysaccharides have been widely researched as biomaterials for a variety of biomedical applications, including in drug delivery and regenerative medicine [137]. Due to their chemical similarity with human extracellular matrix components, these polymers are recognised and accepted by the body [137]. If absorbed, polysaccharides can be eliminated by glomerular filtration in the kidney, provided they are below the glomerular threshold [138]. The molecular weight, as well as the size and the shape of the polymer has a major influence on its excretion and the rate of glomerular filtration. This phenomenon also apples to non-degradable synthetic polymers. Cellulose derivatives, such as carboxymethyl cellulose and hydroxypropyl cellulose are biocompatible and biodegradable [139,140] and have been employed in drug delivery formulations and as components of therapies for preventing post-surgical adhesions [141]. Chitosan has proven to be biocompatible and is biodegraded into non-toxic residues [142]. The polysaccharide chitosan is mainly degraded by lysozyme through the hydrolysis of the acetylated residues [138]. However, the rate of its degradation is strongly related to the molecular mass of the polymer and its deacetylation degree. Due to their biocompatibility, alginates have been extensively used for microencapsulation and for medical applications, such as tissue reparation, and in wound dressings [143]. Although alginates have been extensively investigated as biomaterials, one of their main disadvantages is their inability to undergo enzymatic degradation by mammals. There are, however, a number of ways to prepare alginate-based materials to increase their biodegradability [144]. Hyaluronic acid (HA) has been extensively studied in drug-delivery applications. A variety of commercially available preparations of HA derivatives and cross-linked HA materials have been developed for drug delivery [145]. This material can be considered biocompatible and biodegradable [145], since HA can be degraded within the body by free radicals found in the extracellular matrix, followed by endocytosis. Moreover, it can also undergo digestion by lysosomal enzymes to form mono- and di-saccharides, which can be further converted into ammonia, carbon dioxide and water *via* the Krebs cycle [144]. Starch-based polymers, such as amylopectin, are not an obvious choice for biomedical applications, as normally they are biocompatible but not easily biodegradable in human tissue [146]. The main reason for this is that the cellular energy storage polymer in humans is glycogen and not starch. Dextrins have not been extensively-used for biomedical applications. However, it has been proved that they are biocompatible and non-immunogenic, being degradable by α-amylases and undergoing renal clearance, thus avoiding tissue accumulation [147,148].

3.2.5. Biocompatibility and biodegradability of polymers

The majority of polymers used to produce MNs are biocompatible. In addition to their biocompatibility, some of the described polymers are biodegradable. This property makes them interesting materials to use for MN production, since they will degrade after piercing the skin [92].

PC and PMMA have been extensively used for medical purposes and are known to be biocompatible [149–151]. PMMA has been used for a wide variety of medical applications, such as in bone cements and intraocular lenses [150]. PC, on the other hand, has been used for medical apparatus, such as syringes, artery cannulas, as blood filter housings and for dental brackets [151]. In terms of stability, PMMA is not biodegradable [152], while PC can be biodegraded, releasing Bisphenol-A (BPA), the main raw material used in the production of the aromatic poly(carbonate) [153–155]. The release of BPA can be problematic, as this compound has been reported to induce hormone-related adverse effects [153].

PS polymers have been used for *in vitro* applications, including containers for a variety of liquids, cells and bacteria, and *in vivo* applications, such as in microspheres used as carriers of drugs and magnetic particles. Nevertheless, the biocompatibility of these materials is limited, so PS is typically not used where biocompatibility is a requirement [156,157]. Nevertheless Vesel et al. demonstrated that the treatment of PS with non-equilibrium oxygen plasma improve the biocompatibility of the material [156].

PVA and PVP are commonly used in biomedical applications [158,159]. PVA is used as a biomaterial, due to its biocompatible, nontoxic and non-carcinogenic nature, swelling properties and bioadhesive characteristics [158]. PVP is a hydrophilic, biocompatible polymer [159] and it is used in many biomedical applications, presenting an extremely low cytotoxicity, due to its water solubility [160]. PVP is biodegradable but to a lesser extent than PVA [160]. Additionally, PVP is a common excipient used in pharmaceutical formulations [135]. Another type of polymer with a wide variety of biomedical uses is SU-8. This epoxy-based polymer has been used in fabrication of microelectrodes for measuring electrical impedance in living tissues, monitoring neural spikes and for intraocular pressure sensing [161]. Nemani et al. studied the biocompatibility of this type of material *in vitro* and *in vivo*, demonstrating the suitability of SU-8 as an implant material, supporting cell viability [161].

Cyclic olefin copolymers are currently receiving much attention for their structural strength, optical clarity and biocompatibility [162]. Moreover, they are suitable materials for tissue engineering [163]. The biocompatibility of this type of material can be affected, *in vitro* at least, by modifying its surface [163].

PLA, PGA and PLGA are the most commonly used aliphatic polyesters in MN production. There is a wide variety of studies using such polymers in production of drug delivery systems, showing their good biocompatibility [164,165]. In addition to being biocompatible, the aliphatic polyesters are biodegradable (Fig. 8). This property makes them appealing materials to use for MNs, since they will degrade after piercing the skin [92]. The degradation rates of polyesters can be controlled, for example, by varying the ratio of PGA to PLA in the PLGA copolymer [164,166].

Poly(methyl vinyl ether-co-maleic anhydride) and its acid form (Gantrez® type co-polymers) have been extensively used as thickening and suspending agent for medicinal agents used in topical salves and ointments [167]. Additionally, it has been used for over 60 years as the bioadhesive in denture adhesives [167]. Due to its low toxicity it has been used in development of nanoparticle-based oral drug delivery systems [168–171]. The biocompatibility of these types of polymers has not been extensively studied. Nevertheless, some research works have demonstrated the *in vitro* biocompatibility of hydrogels prepared using Gantrez® co-polymers [172,173]. Additionally, McCrudden et al. performed *in vitro* biocompatibility and *in vivo* rat skin tolerance experiments using dissolving MN arrays, showing no adverse effects [104].

4. Microneedles design, manufacture and mechanical testing

4.1. Manufacturing technologies

In order to produce MN devices, investigators have used a plethora of manufacturing methods, including chemical isotropic

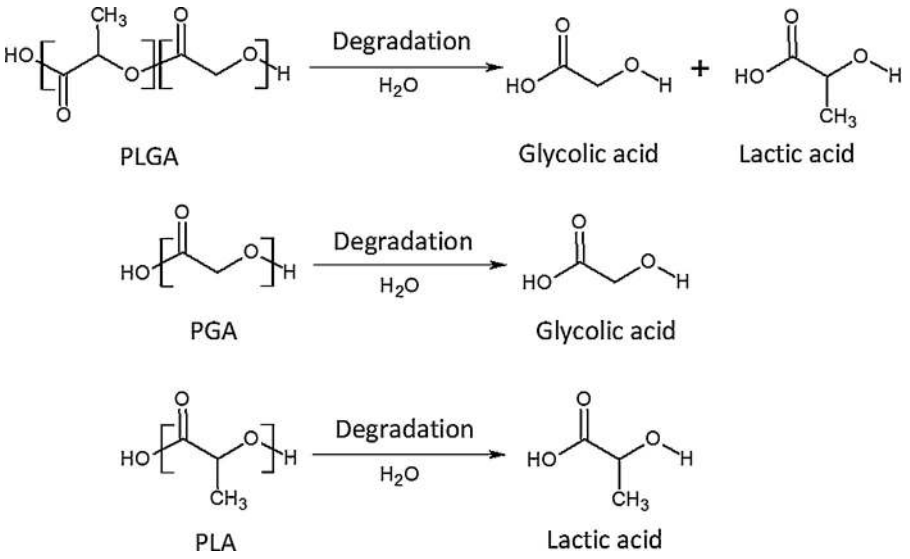

Fig. 8. Hydrolysis of PLGA, PGA and PLA.

etching, injection moulding, reactive ion-etching, surface/bulk micromachining, polysilicon micromolding, lithography-electroforming-replication, laser-drilling and two-photon polymerisation [7,9]. Furthermore, MNs have been produced in a wide range of designs [7,10,12]. The two basic designs are in-plane and out-of-plane MNs. Some designs combine in-plane and out-of-plane MN. For in-plane designs, the MNs are parallel to the fabrication surface, while for out-of-plane designs the MNs are perpendicular to the fabrication surface [174]. In the following sections, different methods used for MN fabrication are described.

4.1.1. Fabrication of silicon microneedles

Silicon MNs are produced mainly using MEMS technology. This type of technology utilises diverse tools and methodologies to create small 3D structures, with dimensions ranging from sub-centimetre to sub-micrometre. Originally, this technology was developed for production of integrated circuits. A MEMS process involves a series of sequential operations. The three basic techniques in MEMS technology are: application of a patterned mask on top of a film by photolithographic imaging; deposition of thin films of material on a substrate; and etching the films selectively to the mask [175,176].

4.1.1.1. Lithography and etching. The majority of processes in microelectronics and micromachining fabrication starts with lithography. This technique is used to transfer a master pattern onto the surface of a substrate (*e.g.* silicon wafer). For this purpose, this surface is previously coated with a photosensitive material by selective exposure to a radiation source (*e.g.* UV light). Indeed, the most widely used type of lithography is photolithography. In general, the subsequent steps are involved in the mask transfer onto the photosensitive-coated substrate (Fig. 9) [176]. The first

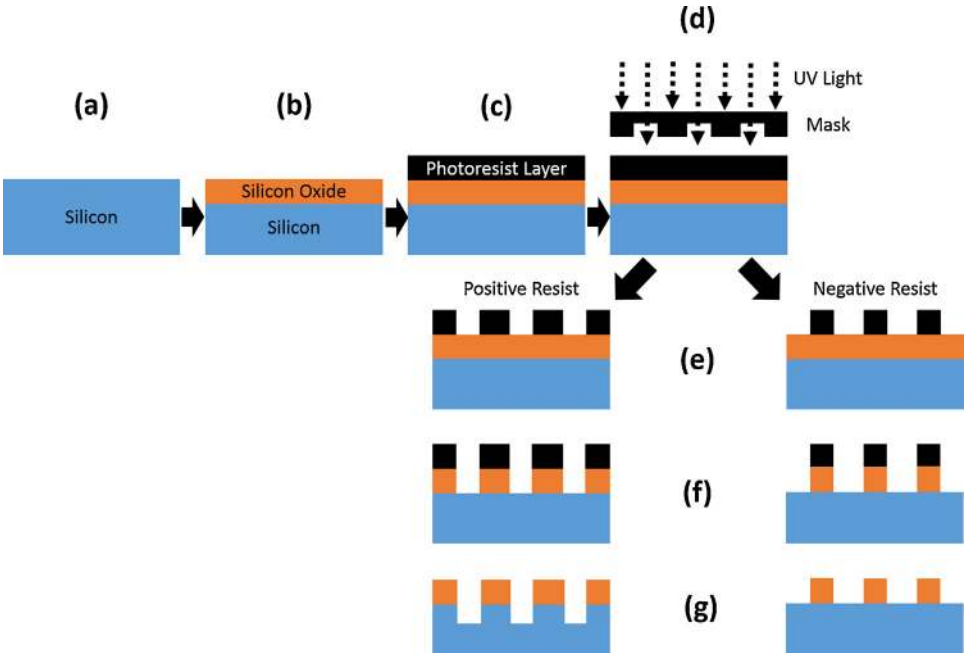

Fig. 9. Sequential processes in the transfer of a pattern to the substrate surface: (a) Si wafer; (b) Si wafer with oxide coating; (c) spin-coated photoresistive material; (d) mask guided UV light exposure on the photoresistive material; (e) development process to remove the soluble resist material; (f) etching of SiO_2 film; and (g) photoresist removal.

step, using a silicon wafer as a substrate, is to form a thin layer of oxide by heating between 900 and 1150 °C in the presence of steam or humidified oxygen. Subsequently, a thin layer of an organic polymer sensible to UV radiation, known as photosensitive/photoresist or resist material, is coated onto the oxide surface of the silicon wafer using a spin coating process (spun between 1500 and 8000 rpm) to yield a resist of defined thickness [176]. After the spin coating step, the solvent present in the resist layer is removed by heating between 75 and 100 °C for 10 min. In addition to solvent evaporation, this process promotes adherence of the resist layer to the wafer. Once the solvent is removed, the resist-coated wafers are exposed to illumination through a mask (normally UV radiation between 150 and 500 nm). This procedure allows an almost perfect transfer of the mask image onto the resist-coated wafers [175]. The radiation treatment prompts a chemical reaction in the exposed regions of the resist. After this reaction, the solubility of the exposed resist is altered. Thereafter, this region of the resist can be dissolved using a suitable solvent. This process can be either by wet (using solvents) or dry (using vapour phase or plasma) developments [177]. Then, an oxygen-plasma treatment, called de-scumming, is used to remove the unwanted resist left behind. Finally, and before moving to the next process, the wafers are post-baked with two purposes: to remove the residual developing solvents and to improve adhesion between resist and substrate interfaces.

Two main types of resists can be used: positive resists and negative resists. In the first one, the chemical bonds within the resists are weakened when exposed to UV light and, subsequently, the exposed resists become more soluble in the development solutions. In negative resists, these chemical bonds are strengthened when exposed to UV radiation. Normally, the mask used in photolithography is typically an optically flat glass or quartz plate, transparent to near UV, with a metal absorber layer. Additionally, there are other technological alternatives to create a desired pattern on a substrate, such as X-ray and charged-particle beam lithography [175,176].

Following lithography, it is necessary to etch the thin oxide layer previously deposited and/or the substrate itself (Fig. 9). In order to perform wet etching, the wafer is immersed in a liquid bath containing a chemical etchant to remove the desired material. The two main wet etching techniques are isotropic and anisotropic etching. The first type, attacks the material (*e.g.* oxide, nitride, aluminium, polysilicon, gold or silicon) at the same rate, and in all directions. They remove material horizontally under the etch mask. In contrast, anisotropic etchants attack the material (normally silicon wafer) at different rates, depending upon the directions. This process allows production of more controlled shapes. The crystal planes in silicon limit anisotropic wet etching. The etchants used for isotropic etching are normally hydrofluoric and nitric acids, in combination with either methanol or water [178]. The chemical agents used for anisotropic wet etching are potassium hydroxide (KOH) and tetramethyl ammonium hydroxide (TMAH) [179]. A critical aspect that must be considered is that the mask should not dissolve, or at least etch at a much slower rate than the material to be etched [175]. Conversely, dry etching is carried out at low pressure using inert or reactive gases. There are two main types of dry etching: reactive ion etching (RIE) and ion-beam milling (IBM). The first one is a chemic al process, while the latter involves physical treatment. In RIE, a plasma of reactive ions is created in a chamber and these ions are accelerated towards the material to be etched. In the case of IBM, inert ions are accelerated from a source to physically remove the material to be etched. When the entire substrate is showered with energetic ions, it is called showered-IBM (SIBM) and when the ions are focused to a spot of the material, it is called focused-IBM (FIBM) [175]. In order to create high aspect ratio (height-to-width ratio) structures, a deep RIE (DRIE) process can be used. This etching process is used in combination with chemical vapour deposition (film forming process) and it is called the BOSCH process.

4.1.1.2. Silicon MN produced using a lithography and/or etching process. As detailed above, Henry et al. were the first to publish on solid silicon MN fabrication by following a dry etch process (RIE with a chromium mask) [15]. Since then, numerous research groups have used both wet- and dry-etching processes in fabrication of a wide variety of solid silicon MNs. Wilke et al. fabricated solid silicon MNs using a wet-etch process. A standard P-type (doped with boron or gallium) silicon wafer was deposited with a 300 Å oxide layer and a 1000 Å layer of nitride. Subsequently, this double layer was etched using a plasma-etch to form 280 μm high MN arrays (with aspect ratio 3:2) [50]. The major disadvantage of this method of fabrication was the limitation of MN height and density.

Using the dry-etching technique, Paik et al. produced in-plane single-crystal-silicon MN arrays containing microchannels [180]. This array contained needles 2 mm in length, 100 μm wide, and of 100 μm thickness, strong enough to tolerate a 0.248 mN of out-of-plane bending moment and 6.28 N in-plane buckling load. These MN arrays, when integrated with a poly(dimethylsiloxane) (PDMS) microfluid chip, demonstrated efficient delivery of model solutes in both *in vitro* and *ex vivo* models. In a similar way, Roxhed et al. developed sharp hollow silicon MN tips with side-openings [27]. The needle tips were sealed with thin membranes to provide a closed-package system improving the shelf-life of the integrated MN device. To produce this device, MNs were etched on a 600 μm thick monocrystalline silicon wafers with a two-mask process employing an anisotropic DRIE etch through the BOSCH process and an isotropic plasma etch (Fig. 10A). The first process was the etching of the needle bore from the backside of the silicon wafer, followed by mixed isotropic and anisotropic etching from the front side, shaping the needle geometry. The side opening of the needles was produced in the intersection between the two-directional etchings. Two different MN designs were produced: a 310 μm long cross-shaped MN array and a 400 μm long circular-shaped MN array.

Electrochemical-etching was used by Rodriguez et al. to produce hollow silicon dioxide MN from an n-type silicon wafer [181]. This technique was used to fabricate MNs with different geometries and lengths (30–140 μm), with wall thickness from 70 to 110 nm and pore diameters between 2 and 5 μm. These needles were connected to a syringe to allow a delivery of the loaded dose with high precision. Another fully integrated microfluidic drug delivery device was recently demonstrated for treatment of hypertension [182]. The MNs in this device were produced using a DRIE etch technique, with a series of combined isotropic and anisotropic plasma etching steps, as described above.

Another example of a fully integrated silicon MN system was developed by Hafeli et al. [183]. An array of hollow out-of-plane silicon MNs was fabricated and then integrated with a reservoir made of PDMS. The silicon MNs were fabricated following a two-mask MEMS process. A 40 μm wide lumen was generated through a DRIE etch step through the back of the silicon substrate, thus obtaining arrays with 600 needles/cm^2 and shafts length of 200 μm. This array was attached to a PDMS reservoir with a volume capacity of 12.5 μl. The surface of the reservoir was treated with oxygen plasma and bonded with a silicon MN base. Subsequently, the drug suspension could be loaded into the PDMS reservoir by using an assembled MEMS syringe employing a 28 G hypodermic needle. This device is used by placing it on the skin and applying gentle finger pressure to the PDMS layer to deliver the content of the reservoir to the epidermal layer.

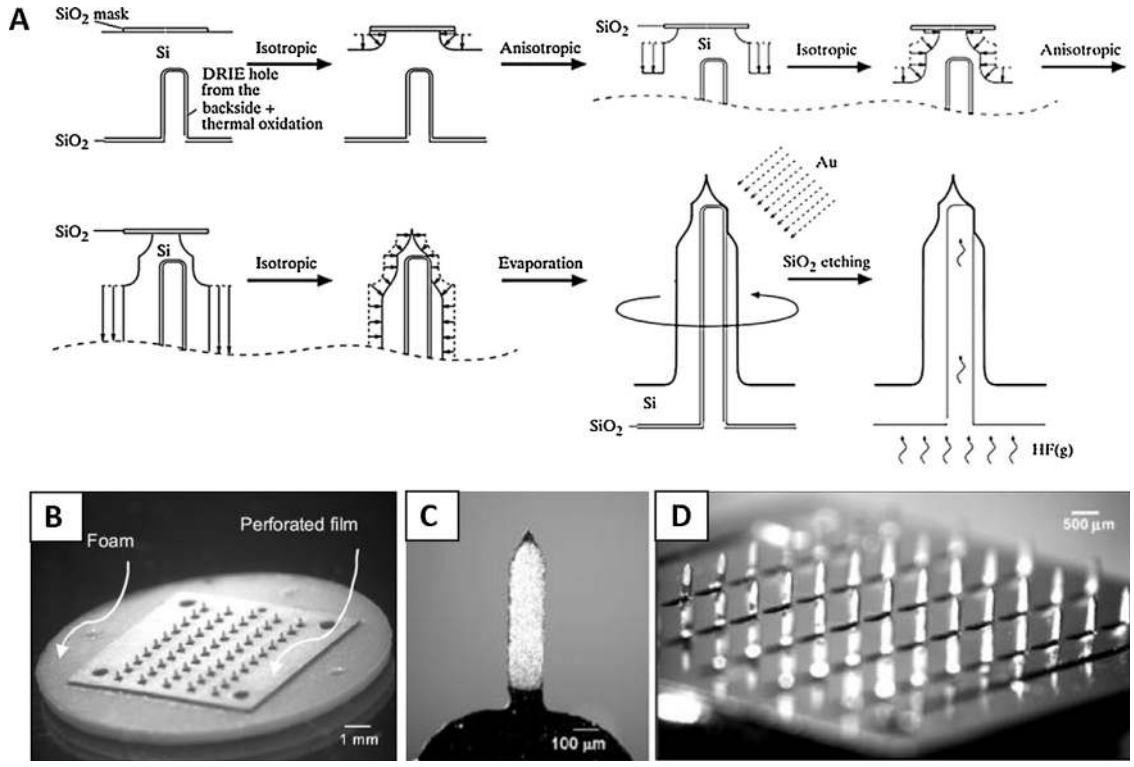

Fig. 10. (A) Process flow of the fabrication of circular side-opened hollow silicon microneedles. (B) 50 out-of-plane microneedle array. Vitamin B coated (C) single and (D) 50 stainless-steel microneedle out-of-plane array.
Source: Reproduced with permission of: [22,27].

In terms of processing, silicon is a particularly interesting material. It is a common microelectronics substrate, with extensive processing experience over more than 30 years. Nevertheless, as detailed above, these processes require long fabrication time, include complex multi-step processes are relatively expensive and require clean room processing [43,54].

4.1.2. Fabrication of metal and glass microneedles

In order to produce metal MN, a wide number of approaches have been proposed, such as electroplating (*e.g.* palladium), photochemical etching (*e.g.* titanium), and laser cutting (*e.g.* stainless steel). These techniques allow, in a similar way to silicon, routine fabrication of both solid and hollow MNs.

The simplest method to obtain metal MNs is by assembling conventional stainless-steel hypodermic needles or stainless-steel wires yielding hollow or solid MN respectively. Currently, the smallest used hypodermic needles are 30 G for conventional syringes (305 μm outer diameter) and 31 G for insulin delivery injectors (254 μm outer diameter) [184]. These hypodermic needles have been used to produce MNs by exposing defined lengths from a supporting material. This assembling process is normally carried out manually. For example, Verbaan et al. assembled commercially available 30 G hypodermic needles, forming 4 × 4 arrays, supported by a poly(etheretherketone) mould. These needles were adjusted manually to 300, 550, 700 and 900 μm heights (Figure 4A) [60,63]. Likewise, Dean et al. and Alarcon et al. used a 1 ml syringe barrel attached to a 1 mm long stainless-steel hollow MN for vaccine delivery in rat models *in vivo* [185,186]. The MN was prepared using a 34 G commercially available hypodermic needle. In these applications, the role of hollow MNs was similar in application to that of solid MNs. The drug was applied in the form of a patch/solution following MN treatment of skin. This type of design is limited because the only viable modifications are the needle height, width and density. An alternative to the use of hypodermic needles is the use of stainless steel wires. Verbaan et al. prepared 4 × 4 MN arrays by simply assembling stainless steel wires of 200 μm diameter and 300 μm height. In order to obtain sharp tips, the wires were cut tangentially (Fig. 4A) [60]. In addition, a simple design of solid metal MNs using acupuncture needles was proposed by Wu et al. [187]. In this design, the acupuncture needles were assembled on a silicon sheet, yielding 400 μm length MNs with a tip angle of 28°. Another approach using metal MNs was investigated by Badran et al. using a device called the Dermaroller® consisting of stainless steel MNs of different needle lengths (150, 500 and 1500 μm) protruding from a cylindrical assembly [188]. This device contains 24 circular arrays of 8 needles each (192 needles in total).

As an alternative to metal MNs, hollow glass MN can be produced [30,77]. This type of MNs can be prepared by pulling fire-polished glass pipettes using a micropipette puller. Finally, the resulting blunt-tip MN can be bevelled (at an angle of 35–38°) and cleaned using different solutions. Normally, these MNs are attached to syringes and used to administer infusions. The production of metal and glass MNs using a manual process is not time efficient and clearly not feasible for industrial purposes.

Unlike assembly of stainless steel wires, or needles, Martanto et al. used an alternative method. An infrared laser was used to fabricate arrays of MN shafts from 75 μm thick stainless steel sheets [189]. In this technique, computer software was used to draft the shape and orientation of the arrays. Subsequently, the needles were cut using the laser (1000 Hz at an energy density of 20 J cm^{-2}), taking 4 min per array. After this, each needle was manually bent at an angle of 90°, creating an out-of-plane MN array, followed by electropolishing using a mixture of glycerol, phosphoric acid and water (6:3:1). Examples of this type of MN arrays can be seen in Fig. 10B–D. This strategy is promising and presents more advantages than the manual assembly of needles because it allows MNs of different designs and dimensions to be

produced. Nevertheless, it requires specialised instruments for fabrication, which makes the process more expensive.

In addition to laser cutting and manual assembly, metal MNs can be produced using MEMS technology. Saluja et al. prepared titanium MNs following a photolithography/wet etching process (see section 4.1.2.) using different titanium foil thicknesses (75, 127 and 250 μm) [190]. It was observed that the 127 μm thick titanium foil produced MNs with a length of 454 μm and a width of 225 μm. Cormier and Dadonna [67] prepared titanium MN arrays (321 MNs/cm^2, area 2 cm^2, height 200 μm, base-width 170 μm and thickness 35 μm) by chemical etching, followed by bending the microprojections at an angle of 90°, as detailed above.

Contrasting the previously described methodologies, the commonly used MEMS are based on the inherently planar geometries of 2D substrates. Various techniques have been reported to create MNs with limited height, due to restrictions of substrate projection lithography [191]. In order to overcome this limitation, and to create relatively ultra-high-aspect-ratio (UHAR) metal MNs, an alternative method called 'drawing lithography' has been employed. This method involves formation of a thin layer of a thermosetting polymer (SU-8 2050) following by controlled drawing using pillars of defined patterns. This process allows creation of 3D solid long polymeric needles that can be used as a mould to finally create hollow metallic nickel MNs. The resulting hollow MNs have heights of 600, 1200 and 1600 μm.

4.1.3. Fabrication of ceramic microneedles

Ceramic MNs are produced mainly by micromolding techniques. These techniques will be explained in detail in the next section. A ceramic slurry is cast inside a mould followed by a sintering process [68,69]. This type of process allows device production at a low cost, as this technology can be easily scaled up. Bystrova and Luttge prepared MN arrays using this process, obtaining a wide variety of array geometries [68]. The first step of the process consists of casting an alumina slurry into a PDMS microneedle mould. Additionally, the multiple replication of the PDMS mould offers a low cost production mould which can be reused for ceramic micromolding. Such a micromolding process was used by Cai et al. to prepare calcium sulphate dihydrate and calcium phosphate dihydrate MN arrays [71]. Two types of needles, each with different dimensions, were designed (Fig. 4D): densely arranged pyramids 100 μm in height, 150 μm in base width and with 160 μm between tips, and sparsely-arranged pyramids 200 μm in height, 285 μm in base width and with 820 μm between tips. A similar micromolding process was followed by Gittard et al. to prepare 5 × 5 Ormocer® MN arrays using a two-photon polymerisation-micromolding technique [74]. In the first step, two-photon polymerisation was used to fabricate a master structure of the MN array on a silanized glass coverslip. The mould contains MNs with base diameter values of 200 μm, lengths of 500 and 500 μm MN centre-to-MN centre spacing. The second step consisted of replication of the MN array by means of a micromolding process using a silicone elastomer and curing agent. In the third step, Ormocer® was cast into the elastomer moulds using a vacuum process and the material was finally photo-polymerized within the mould to produce MNs.

4.1.4. Fabrication of carbohydrate and polymeric microneedles

Polymeric MNs have been produced using a wide variety of mould-based techniques. Some examples of these techniques are: casting [90,94], hot embossing [192], injection moulding [31], investment moulding [94], drawing lithography [193], laser micromachining [194] and X-ray [88] methods. Fig. 11 shows how some of these techniques work. Drawing lithography, on the other hand, is a unique additive process to fabricate MNs. This method produces 3D polymeric structures extended directly from

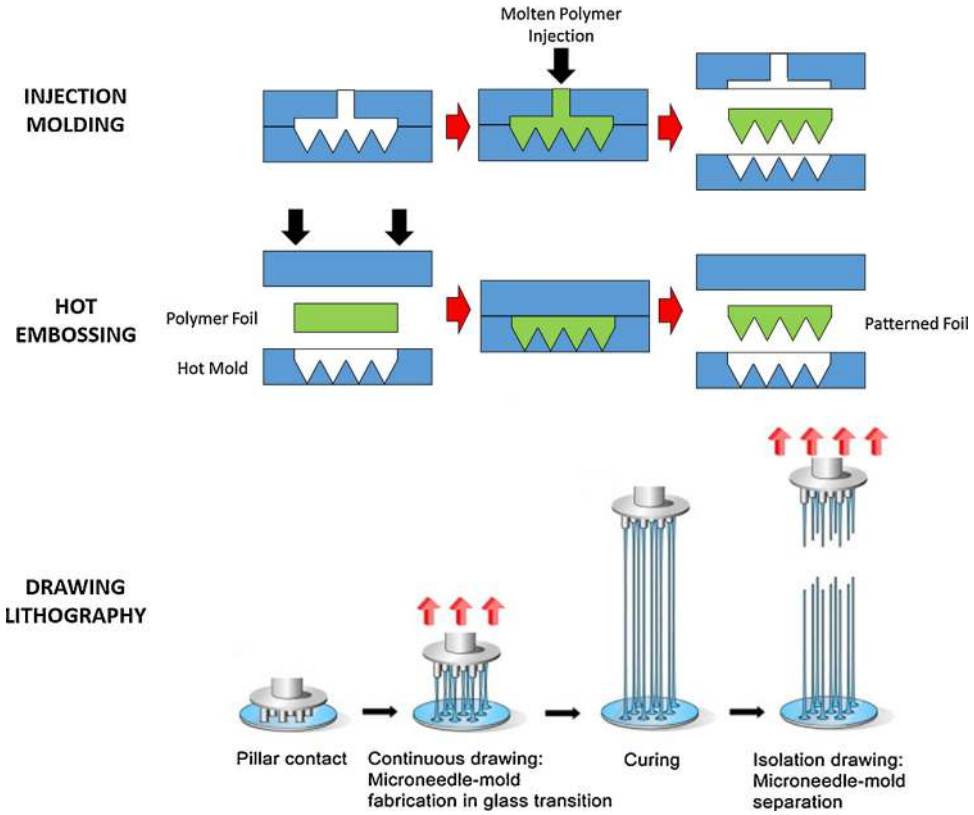

Fig. 11. Schemes of injection moulding, hot embossing and drawing lithography processes.
Source: Reproduced with permission of: [193].

2D viscous polymeric materials. The polymer is cooled down until it becomes a viscous glassy liquid. After contact of the drawing pillars on a coated glassy liquid surface by plate moving, 3D structures are selectively elongated from the 2D glassy liquid-plate as a result of the longitudinal upward movement of the drawing pillars. Then, the polymer is cured and finally isolated (Fig. 11).

During recent years, an extensive number of publications have addressed the fabrication of polymeric MNs. Generally this type of MNs, unlike silicon or metal, depends on the dissolution, degradation or swelling of MNs after skin insertion by contact with the interstitial fluid in order to deliver a drug or vaccine payload. The following sections will describe the techniques used in the fabrication of a wide variety of MNs.

4.1.4.1. Micromolding-based fabrication of polymeric and carbohydrate microneedles. Micromolding involves the replication of a master structure using moulds. To date, the majority of moulds for MN production are made from PDMS. The main reasons for the selection of this material are its flexibility and accurate reproducibility of master structures [7]. In order to prepare moulds, master structures can be produced using different materials (*e.g.* silicon or metal) and the used as positive master templates. A wide number of polymers have been micromolded to prepare MNs. However, carbohydrates were initially the main candidates considered as potential MN materials [85]. Carbohydrates change to yellow-brown coloured substances, known as caramels, when heated at temperatures around their melting points. This caramelization process involves the removal of water, followed by isomerisation and polymerisation steps, yielding a hard and brittle material. Miyano et al. reported, for the first time, the use of natural sugars in the fabrication of MN arrays [85]. In this work, powdered maltose was heated to 140 °C for 1 h and then powdered drug was added and mixed uniformly over one minute and, finally, stored in a dry environment at room temperature. In order to form the MN arrays, a small quantity of this maltose-candy containing drug was then placed onto a casting MN mould at 95 °C and cast into 500 μm high MN arrays. The resulting MN arrays contained ascorbate-2-glicoside, sodium salicylate and calcein. These sugar MNs should be stored at controlled humidity, as they dissolved within a few hours at a humidity of more than 50%. Nevertheless, they retained their shapes for at least three months at 40% humidity. Moreover, in another work, Miyano et al. [195] developed hydrolytic MNs using maltose and PEG (Mw = 600 Da), obtaining MNs with lengths ranging from 500 to 2000 μm. Another example of micromolded maltose MNs was reported by Kolli et al. [86]. In this study, the drug nicardipine hydrochloride was not incorporated into the MN array itself. Instead, it was included in a liquid reservoir patch that was applied to the skin on top of the inserted MN array. It was shown that this type of MN array enhanced the flux of nicardipine hydrochloride *in vitro* across full-thickness rat skin.

Diverse authors have studied the fabrication and use of MNs from carbohydrate materials using silicon or metal master templates [85,86,100,195,196]. However, Donnelly et al. reported a series of difficulties and limitations in the use of carbohydrate materials for MN preparation following a micromolding process [81]. As described previously, high processing temperatures are required to produce hot-melts, which are extremely viscous and resistant to flow. These high temperatures (160 °C for galactose) can cause loss of the loaded active pharmaceutical ingredient (API). Besides, the materials produced after cooling are extremely hygroscopic, causing problems with storage and handling. Therefore, the authors suggested that carbohydrate-based MN arrays are not the solution to the limitations associated with silicon and metal MNs.

Apart from sugars, diverse materials have been also used to prepare MNs using micromolding processes, with macromolecules the main alternatives. For example, Lee et al. fabricated pyramidal MN arrays made from carboxymethyl cellulose, amylopectin and bovine serum albumin (BSA) using casting techniques. These MNs were able to insert into cadaver skin and then dissolved within minutes [84]. Additionally, in the same research group, Chu et al. reported the use of a mixture of polymers: PVA and PVP, for the production of MN arrays following a micromolding process [96]. A 10×10 array with 300 μm × 300 μm × 600 μm (width, length and height) based on a pyramidal MN master template was produced using photolithography (see Section 4.1.2) and then used to create PDMS micromolds. Using these moulds, three different MN designs were produced, namely solid, bubble and pedestal MNs. Here, it was shown that incorporating a bubble into the base enhanced the drug encapsulation in the MN tip, whereas incorporating a pedestal at the base of the MN improved skin insertion. The main objective of this work was to load the drug only in the MNs tips and, therefore, increase MN loading, with minimal wastage. This approach is particularly interesting and effective for potent drugs. Additionally, the same research group used a combination of micromolding and photo-polymerisation techniques for production of PVP microneedles [95]. For this purpose, vinyl pyrrolidone (liquid monomer) and azobisisobutyronitile (free-radical initiator) were applied to PDMS moulds (arrays of 15×15, 750 μm height, and 250 μm base diameter). In order to induce the polymerisation reaction, the moulds were placed under a UV lamp for 30 min at ambient temperatures. The resulting PVP MNs were strong enough to pierce the skin and dissolved in less than one minute. Alternatively, methacrylic acid (MAA) has also been copolymerized, producing MNs of greater fracture force and slower dissolution rates when tested in porcine skin *in vitro*. In other studies, the same research group used three types of biodegradable polymers, PLA, PGA and PLGA, in combination with a micromolding process for the production of MNs with different geometries [24,92,197]. Three different types of master structures, namely bevelled-tip, chisel-tip and tapered-cone MNs were used to form PDMS micromolds [24]. The micromolds were then used to fabricate biocompatible and biodegradable polymeric MNs. All three MN designs presented strong enough needles to easily penetrate human cadaver epidermis, increasing calcein and BSA permeability by almost 2- or 3-orders of magnitude. Additionally, using similar micromolding techniques and the same biodegradable polymers, Park et al. produced sharply tapered MN arrays, with enhanced insertion properties [197]. The PDMS micromolds used in production of these MN arrays were demonstrated to be reusable for producing polymer MNs at least 100 times. This finding clearly illustrates the advantage of micromolding techniques and their potential cost-effectiveness when compared to expensive and complex MEMS processes.

Poly(carbonate) has been used quite often in MN production. Based on the micromolding method, Han et al. used a casting technique to design a prototype MN array [93]. This prototype was produced in a series of steps. During the first step, in-plane MNs were fabricated using inclined UV lithography and electroforming. Subsequently, these in-plane MNs were then aligned parallel to each other to form an out-of-plane MN array. Using these MN arrays, a negative mould of PDMS was fabricated. Finally, biocompatible poly(carbonate) out-of-plane MNs were produced using a hot-embossing machine. The same research group proposed MN with shaft tips shaped so as to allow a reduction of the skin insertion forces and an increase in fracture forces and drug loadings [192]. PLA was the selected polymer for the production of these arrays, obtaining MN with height, base width and thickness of 880 ± 20, 710 ± 15 and 145 ± 15 μm, respectively. Moulds containing PLA pellets were heated to 190 °C and exposed to a pressure of 20 kg/cm^2 for 10 min, followed by cooling to room temperature. Finally, the replica MN arrays were then demolded. In

this case, the authors coated the array with an API formulation. This process presents a significant advantage over sugar MNs: the APIs are not exposed to high temperatures.

Hollow polymeric MNs have been produced using micromolding techniques. For this purpose, an alternative technique called investment moulding (a combination of investment, casting and injection moulding) was reported [94]. Cyclic olefin copolymer was used to produce hollow in-plane MNs 280 μm in height, 130 μm in base width and of 35 μm bore diameter.

Using similar microinjection moulding techniques, Sammoura et al. fabricated in-plane, open-channel MN devices made of a cyclic olefin and ethylene co-polymer [31]. The main components of this design were the shank portion (4.7 mm), with an open-channel (cross-sectional area of 0.1 mm × 0.1 mm) and the base portion, consisting of a reservoir. This MN device was applied into beef liver, drawing approximately 0.04 μl of liquid immediately from tissues.

X-ray radiation can also be used in lithography. In fact, a technique known as deep X-ray lithography (DXRL), can create high-aspect ratio hollow microstructures with sharp tips [7]. Therefore, this technique can be used to create moulds. Using a DXRL process, Moon et al. prepared high-aspect ratio and sharp tip PMMA MN arrays [88]. These arrays presented needles ranging from 750 to 1000 μm in height, with base diameter from 270 to 400 μm and bore diameter between 70 and 100 μm. These PMMA MN arrays could be used to take blood samples from the skin on the back of the hand. However, this method did not present any replication technique for the MN arrays. The limitation in the replication technique was solved by Perennes et al. In this work, they manufactured sharp bevelled-tip PMMA hollow MNs by the DXRL process and employed a replication technique for PMMA hollow MN arrays using soft PVA polymeric micromolds [90]. Saw-toothed hollow PMMA MN arrays were produced after a two-step DXRL application on 2.7 mm thick PMMA sheets. Subsequently, they were electroplated with a metal layer to ensure the rigidity of the device. These MN were then used as master templates to produce the PVA moulds. These moulds were cast with liquid PMMA and left for 3–4 h at room temperature for polymerisation. The PVA mould was then dissolved by immersing the system in water at 40 °C for 2 h, leaving behind the PMMA MN arrays. PVA was selected as the raw material to produce the moulds because PDMS did not create efficient moulds, due to a lack of stiffness of the polymerised PDMS. A similar technique was used by Matteucci et al. to create reusable PVA masters for PMMA MN production [198].

The main ways to produce micromolds for MN production are lithography, etching or the replication of a master structure. However, there is another alternative for this purpose: laser-based micromolding techniques [98]. Lasers have been used for material processing extensively during the last 30 years. Due to its monochromatic and coherent nature, a laser beam can focus its entire power onto a spot of reduced diameter [175]. Certain lasers have enough power to selectively ablate the material by interacting with the chemical bonds within the material. They have been used for different purposes, such as heat treatment, welding, ablation, deposition, etching, lithography, photopolymerization, microelectroforming and focused-beam milling of plastics, glasses, ceramics and metals [176]. Therefore, this technology is ideal for microstructuring of medical devices. Donnelly et al. used, for the first time, laser processing to produce micromolds for MN array production [98]. By using a pulsed excimer laser beam controlled by a computer, a defined pattern can be ablated in silicone sheets. The novel laser-based fabrication method described can produce holes of the required dimensions (11 × 11 arrays with a base diameter of 300 μm, height of 600 μm and spacing of 300 μm) in around 5 minutes (Fig. 12). These moulds were reusable more than 50 times in micromolding of poly(methyl vinyl ether-co-maleic acid)-based soluble polymeric MNs. Therefore, this method is ideal for fast and cheap mould production. This method have been extensively used by the same research group for production of different types of MN arrays that are able to bypass the *stratum corneum* delivering a range of different APIs [8,25,37,40].

Micromolding techniques have also been applied to production of hydrogel-forming MN arrays. Donnelly et al. prepared such devices using poly (methyl vinyl ether-co-maleic acid) crosslinked by esterification by PEG (Fig. 12D) [8]. A formulation containing both polymers was cast into silicone moulds and then dried at ambient temperature. To allow the crosslinking reaction between the polymers, the moulds were placed inside a convection oven at 80 °C during 24 h. Recently, crosslinking times for this process have been reduced to around 45 min by using microwave radiation [199]. Additionally, different types of crosslinking processes could be performed, such as freeze–thaw. Demir et al. cast a formulation (gelatin and PVA) into moulds and the system were then frozen at −20 °C for 12 h, followed by thawing at 25 °C for 12 h [102].

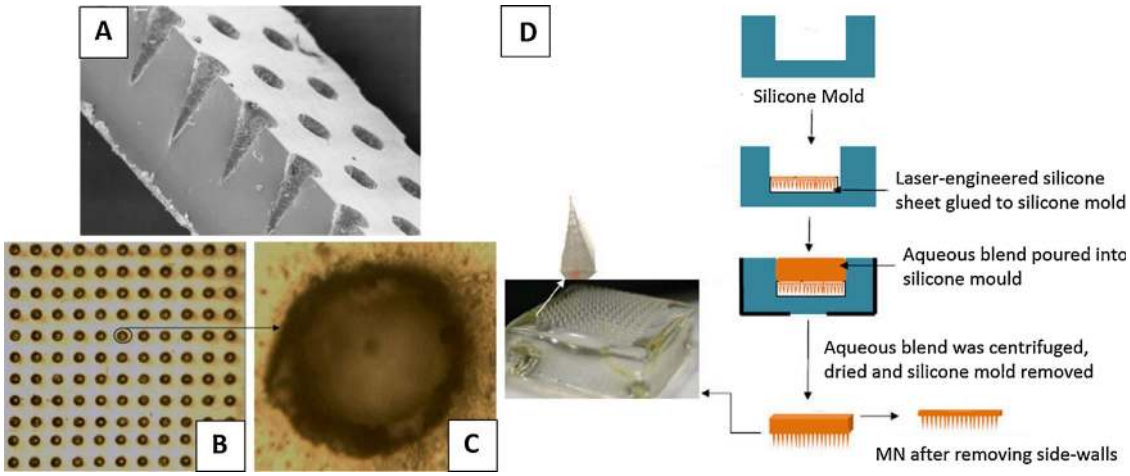

Fig. 12. (A) SEM of the cross-section of a silicone micromold after laser engineering, revealing cone-shaped holes. (B) Digital microscope image of laser-engineered silicone micromold (11 × 11) on 1.0 mm thick silicone sheet. (C) Digital microscope image of a single hole. (D) Diagrammatic representation of the steps involved in preparation of laser-engineered MN arrays.
Source: Reproduced with permission of: [98].

4.2. Microneedle design

The main purpose of MNs is to penetrate into the skin or any other biological tissue without breaking or bending. An optimal MN design should present a low insertion force and high fracture force. In order to achieve this feature, different factors can be optimised, such as material, fabrication method or design. 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 MN height, the skin could fold around the MNs, leading to partial or incomplete piercing of the *stratum corneum* [63]. Nevertheless, some MN arrays that showed good strength failed to enhance transdermal drug delivery as they were not able to properly pierce the skin. The main factors affecting fracture forces and skin insertion are: type of material, needle height, tip-radius, base diameter, needle geometry, needle thickness, and needle density [200]. Consequently, understanding the interaction between MNs and skin will allow design of optimal MN arrays for clinical applications.

Understanding the mechanical properties of the skin, especially that of the *stratum corneum* and viable epidermis is mandatory in development of MN technologies. Mechanical properties of skin were evaluated by Kendall et al. by penetrating intact *stratum corneum* and viable epidermis with micro- and nanoprojections [201]. The storage modulus, Young's modulus and the break strength of murine skin were investigated. It was pointed out that the values of these mechanical properties decreased with depth through the *stratum corneum.* Additionally, the authors showed that there are some factors (*i.e.* thickness variation at different body sites, and variation due to age, sex, race and body mass index) that need consideration in order to ensure consistent application of MN arrays. The age factor has been reinforced in recent work by Kelchen et al. [202].

A clinical study evaluated the efficiency of intradermal (ID) injection using 1.5 mm long 30 G MNs on 342 adult subjects. This study evaluated the efficiency of intradermal injection as a function of age, sex (205 women and 137 men), ethnic origin (101 Caucasian, 118 Asian, and 123 Black Africans), body mass index and skin thickness [203]. It was observed that, irrespective of the age, race, body mass index or gender, the 1.5 mm long MNs, when inserted perpendicularly, could be efficiently used for ID vaccine delivery. Moreover, it was demonstrated that skin thickness varied less between people of different body mass index, age, sex and race than it did between different body sites on people with similar demographic characteristics. Although these variations in skin thickness directly influence MN-based drug delivery, the other main factor to consider is MN geometry. Notably, however, this study was focused on the use of an injection system with needles longer than 1 mm. This is not the common scenario for the majority of MN devices, which are typically comprised of much shorter needles.

To the best of our knowledge, the first study to demonstrate the effect of MN geometry was reported by Davis et al. [200]. The skin insertion forces varied in a linear way with interfacial area of the needle tips. Additionally, the fracture force increased with MN wall thickness, wall angle and tip radius. Finally, the safety margins between fracture force and insertion force were high. In another study, Davidson et al. studied the most effective MN geometry for effective skin permeation [204]. The influence of a range of parameters, such as MN thickness, MN diameter, coating depth of drug on MN, penetration depth, spacing between MNs and array pattern were all evaluated. MN designs evaluated in this study can be seen in Fig. 13. The main parameters that significantly affect skin penetration were found to be: needle height and centre-to-centre spacing of MNs. The study concluded that wider, longer and more densely packed MNs lead to greater effective skin permeability. In addition to this work, different studies used theoretical models to evaluate numerous factors in MN performance. For example, a theoretical model was proposed to evaluate the influence of injection velocity, blood perfusion rate and tissue porosity on MN-based transdermal drug delivery [205]. Teo et al. showed that sharpness and type of material should be considered for the design of MNs [206]. Different MN geometries were studied and tested. Some examples are: straight-walled solid MNs, straight-walled hollow MNs, and solid MNs with sharp tips. The main finding was that, regardless of the sharpness of the tips, the insertion of needles by hand was not very consistent and presented some difficulties. In another study, the optimum MN length was proposed to be between 50 and 200 μm [207]. In addition, Stoeber and Liepmann pointed out that the length of the MN must be longer than 100 μm [208]. Opposite to these findings, Pastorin et al. reported that MN arrays with lengths less than 50 μm were sufficient for successful ID vaccine delivery [209]. Needle length is crucial for drug diffusion from the MN tips to the dermal microcirculation. Therefore, increase in MN length will reduce the length of the diffusion path, increasing uptake of the drug by the dermal microcirculation. Furthermore, needle length should not be too short to be ineffective or too long to contact the nerve endings in the deeper layers of dermis, causing pain. There are a wide variety of factors affecting MN insertion and geometric and design factors will be strongly affected by the type of material. However, all of these findings obviously cannot be extrapolated directly to individual types of MN arrays.

Two studies by Al-Qallaf et al. investigated the influences of a variety of variables related to MNs and their impact on drug transport through the skin using mathematical models [210,211]. Some factors considered in this study were: MN length, duration of application, size of the patch and application to different anatomical regions. The main objective of this work was to determine the influence of these factors on the blood drug concentration. The main findings of these studies points out that the blood concentration of the drug molecule can be increased by increasing the MN length or the patch surface area. Optimised designs for both solid and hollow MNs were proposed. In these designs, higher skin permeability was observed by decreasing the aspect ratio of needle height over needle radius and the number of MNs was increased. Notably, however, these findings were obtained using a theoretical model which would need to be experimentally validated. Overall, the optimum MN design should be long enough for transdermal drug delivery applications without causing pain; should present low insertion forces and high break force; its density should be tailored to deliver the desired amount of the active compound; it should be designed to be applied on a specific anatomical location within the body; it should have a certain degree of flexibility to overcome skin contours; it should avoid skin deformation to allow complete MN penetration. Another critical factor that should be taken into account 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.

4.3. Microneedle mechanical characterisation

A crucial step in the development of successful MN devices is mechanical characterisation. MNs normally experience a wide range of stresses, including those experienced during insertion or removal. Therefore, it is mandatory for such devices to possess inherent strength to avoid failures [47]. There are a wide number of failure modes that these stresses can cause, including MN bending, buckling and base-plate fracturing [52]. Therefore, mechanical characterisation should be performed in order to ascertain that the designed MN are safe prior to use. As there is no single test

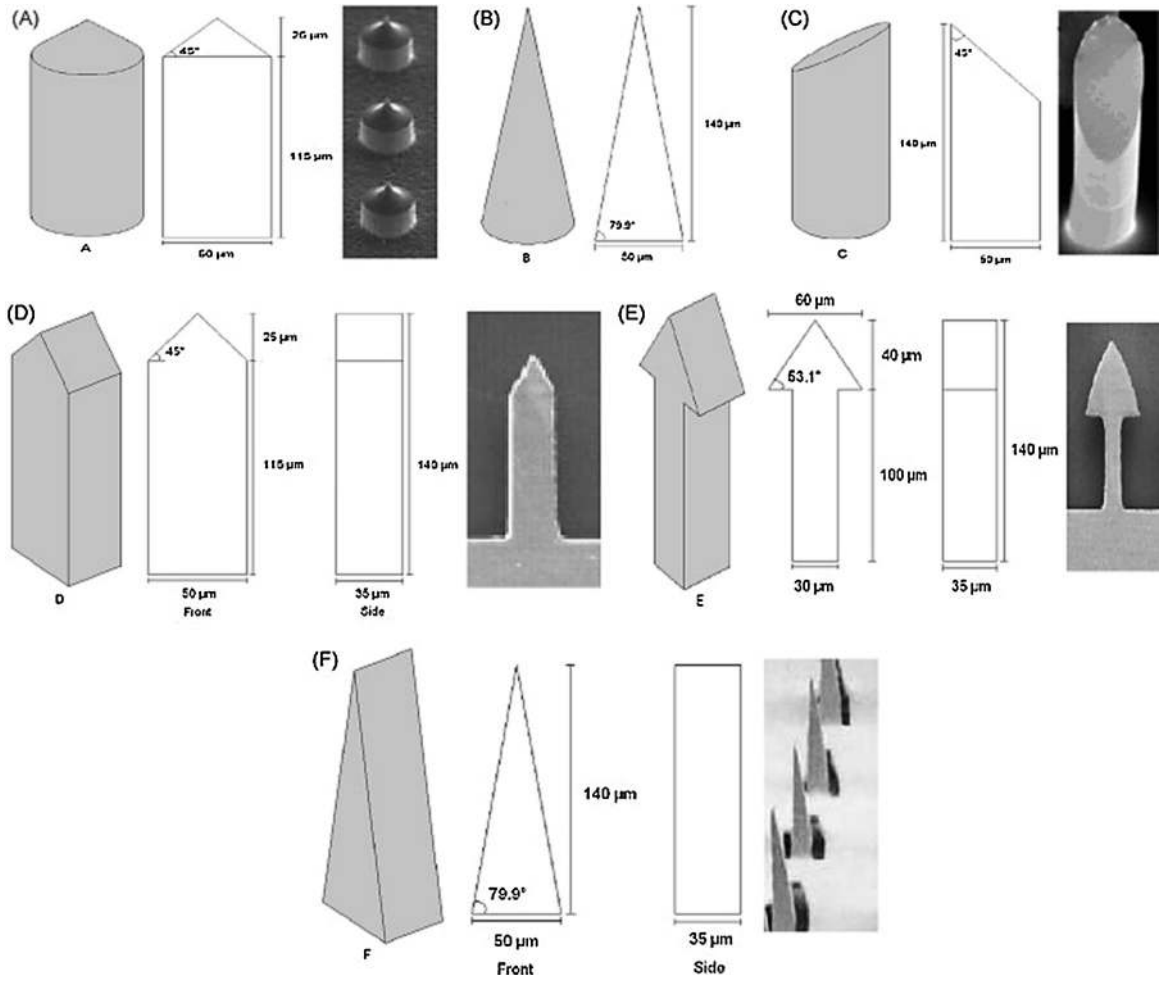

Fig. 13. Different MN designs.
Source: Reproduced with permission of: [204].

simulating MN insertion *in vivo*, characterisation consists of a range of tests. One of the first MN mechanical tests described was developed by Zahn et al. [52]. This test consisted of a single, hollow, silicon MN and a force gauge that gradually increased the vertical force being exerted at the MN tip (0–20 g range) until it fractures. Since then, numerous methods and tests have been developed for MN mechanical characterisation.

4.3.1. Axial force mechanical tests

Axial force mechanical tests involve applying a force to the MNs perpendicular to the base-plate [98]. This type of test normally needs the use of a mechanical test station, which records both displacement and force while the MNs are pushed against a hard surface at a defined rate [24,102] (Fig. 14A). When MN fracture occurs, a sudden decrease can be observed in the force-displacement curves generated. The maximum force exerted immediately before this drop is normally taken as the MN failure force [24]. Images of the MN arrays can be taken before and after fracture to provide insights into the mode of failure [212]. Some axial force tests reported in the literature should be viewed with caution, as they only use a single MN [52,213]. The failure force of a MN array obviously cannot always be assumed to correlate with that of a single MN. Additionally, it is important to clarify that this test does not accurately simulate the forces experienced by MNs during insertion into the skin. In this test, MNs are pressed against a hard surface, leading to a concentration of the forces at the MN tip. During insertion of MN into skin, the forces are distributed over a greater MN area, as the flexible skin wraps around the MN projections [212].

4.3.2. Transverse force and shear strength mechanical tests

Skin surface irregularities and its natural elasticity often lead to incomplete insertion of MN arrays, with transverse bending of the needles also frequently observed. Therefore, a transverse fracture force test is necessary to probe the behaviour of MNs during application [24,102]. Using a mechanical test station, a transverse force (applied normal to the MN *y*-axis) is applied at a defined point on the MN shaft until the MN fractures (Fig. 14). Again, a sudden drop in the force-displacement curves indicates MN failure [98,102]. When this test is applied to a row of MNs rather than to a single MN, the force should be divided by the number of MN in the row to calculate the transverse fracture force per individual MN [98]. The main limitation of this test as reported in the literature is the requirement to align the metal probe with a defined length manually [98]. This can lead to experimental inaccuracies, even if the test is performed with the aid of a microscope camera [102].

4.3.3. Base-plate strength and flexibility tests

The tests described above are focused on mechanical testing of the needles themselves, but assessment of the MN base-plate strength is also important. Clearly, fracture of the base-plate during patient application is not acceptable. Therefore, base-plates need to be flexible enough to conform to the topography of the skin without fracturing [98]. For this purpose, a three points bending

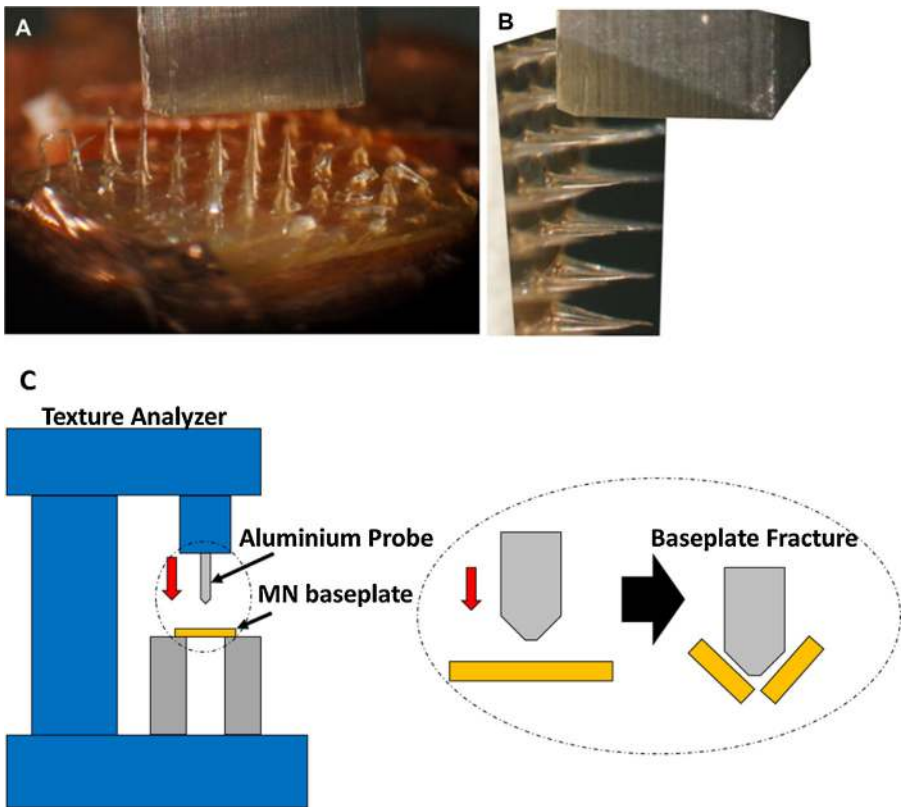

Fig. 14. (A) Digital photograph of MN pressed against the metal mill during axial fracture force. (B) MN shafts were transversely pressed against the metal mill for measurement of the transverse fracture force. (C) Illustration of the Texture Analyzer set-up for base-plate strength and flexibility test.
Source: Reproduced with permission of: [102].

test has been used [98]. Donnelly et al. used a Texture Analyzer to apply forces using a metal probe to base-plates placed between two aluminium blocks (Fig. 14C). A maximum peak observed in the force-distance curve represented the force required to break the base plate. Additionally, the baseplate bending upon fracture was calculated in order to evaluate the flexibility of the base-plate (Fig. 14C).

4.3.4. Importance of microneedle mechanical test results

The importance of the experimental testing of MN failure forces can only be properly assessed when compared with the equivalent insertion forces [24]. For this purpose, the parameter 'margin of safety', the ratio of MN fracture force: insertion force, is a valuable index [24,213,214]. Naturally, human or animal skin tissue is required to measure the insertion forces. In the majority of studies researchers evaluate the ability of the MNs to pierce the skin using techniques such as microscopy or histological analysis. However, these experiments do not give information about the insertion forces required to pierce the skin. However, there are some published studies where the MN insertion forces were measured. For example, Davis et al. measured the insertion forces of metal MN (radii varying between 30 and 80 μm) in human volunteers using a force-displacement measurement device [213]. The obtained insertion forces (0.08 and 3.04 N per needle) suggested that this process allows manual insertion. Using the same method, Khanna et al. measured the insertion forces of 4×4 hollow silicon MN arrays using cadaver skin [215]. In this case, the obtained insertion forces ranged between 4.75 and 0.1 N determined mainly by the tip sharpness. Loeters et al. described a method to measure insertion forces using a MN integrated with electrodes device [216]. This method was used to evaluate the skin insertion forces of a 9×9 MN array, showing that 2.6 N were required for this purpose. Using electrical impedance measurements in the skin, Roxhed *et al.* evaluated the insertion force of a novel design of ultrasharp MNs in human skin [217]. The results showed that the required forces were lower than 10 mN. Another critical parameter for MN design as pointed out above is the fracture force. For all types of MNs, the fracture forces should be significantly greater than the force required for insertion into the patient's skin. As described above, MN array designs can be optimised to increase their 'margin of safety' value [24,214].

In order to define the margin of safety, the nature of the insertion process should be studied. MN can be inserted manually or by using an applicator. A wide variety of applicators can be found in the literature [7]. When using an applicator, the insertion conditions are controlled. When using manual insertion, the process obviously presents more variability. Consequently, the range of forces that patients can apply by hand should be taken into consideration to define the margin of safety. In a recent study, Larrañeta et al. concluded that the range of manual forces applied by 20 volunteers, following instructions for MN insertion, ranged between 10 and 50 N, with an average value of 20 N.

MN mechanical characterisation tests are commonly used to ascertain the mechanical strength of MN arrays. However, due to the range of MN geometrical dimensions, the diversity of tests employed and the range of measuring equipment utilised, direct comparison between different MN designs cannot be made. To overcome this important limitation, MN technology would benefit from the development and consolidation of standardised mechanical tests.

4.4. Microneedle Insertion measurements

There are different techniques to demonstrate successful MN insertion. Normally, these require biological tissue and/or complex

techniques. The most used is the staining of MN-treated skin using coloured dyes, such as methylene blue or trypan blue [80,194,218]. These dyes stain only cells of the viable epidermis and not the *stratum corneum* identifying MN-created microchannels. Alternatives to the staining are transepidermal water loss [194,219] or electrical impedance measurements [213,217] after MN insertion. Both techniques can ascertain if the barrier function of the *stratum corneum* has been reduce. However, such techniques cannot provide quantitative information on MN insertion depth. In order to obtain insertion depth data, histological cryosectioning with adjunct staining [216,217], confocal microscopic imaging [212] and optical coherence tomography [105] can be used. The latter is the best option, as it does not require skin excision or mechanical manipulation of samples and allows measurements in real time [105]. However, all these techniques require the use of biological tissue. Recently, Larrañeta et al. proposed an artificial membrane as a skin model for MN insertion studies [38].

The relevance and interpretation of MN insertion studies is limited by a number of factors, such as the materials and geometry of MN arrays, use of varying experimental protocols and different skin models. These limitations could lead to divergence of results using different methods of analysing MN penetration, even though the MNs and skin samples used remain the same. Therefore, standardised MN test protocols should be developed.

5. Microneedles: applications and translation to clinic

5.1. Microneedle applications

5.1.1. Drug delivery

The wide variety of MN types described previously reflects the extensive nature of research into MN technology. During the last 20 years, a wide variety of studies have demonstrated the substantial drug delivery enhancing effects of MNs.

As detailed above, Henry et al. published the first research work using MN for transdermal drug delivery [15]. This work described the use of solid silicon MNs for the delivery of calcein (model drug) using the 'poke and patch' approach (see Section 2.2). Since then, this approach have been used extensively employed, using mainly silicon and metal MNs. McAllister et al. reported the use of metal MNs for the delivery of different molecules, including macromolecules, such as BSA and insulin [80]. Photosensitisers [5-aminolevulinic acid, 5-aminolevulinic acid methyl ester and meso-tetra(N-methyl-4-pyridyl)porphine tetratosylate] were also delivered after a pre-treatment with solid polymeric [220] and silicon MN arrays [17]. Additionally, Stahl et al. showed the transdermal permeation enhancement of non-steroidal anti-inflammatory drugs (diclofenac, ibuprofen, ketoprofen, paracetamol) using a pre-treatment with MN roller devices [221]. In some studies, this strategy was used in combination with other enhancement strategies, such as iontophoresis or sonophoresis, showing a synergistic effect in the enhancement of transdermal delivery of macromolecular compounds [187,222].

Coated MNs have been used in transdermal permeation enhancement of a wide variety of compounds, such as fluorescein sodium [223], salmon calcitonin [224], desmopressin [67] and parathyroid hormone [225]. In addition to drugs, coated MNs have been also used for DNA delivery. For this purpose, DNA solutions can be dip coated onto MNs [218]. This system may present an attractive and effective treatment option for genetic skin diseases and cutaneous cancers. Additionally, metal MNs have been coated with RNA, leading to *in vitro* and *in vivo* silencing of gene expression [226].

Dissolving MNs have been extensively used to enhance transdermal and ID delivery of numerous drugs and biopharmaceutical molecules. Small molecules delivered using dissolving MN include caffeine, lidocaine, metronidazole [227], ibuprofen sodium [104], sulforhodamine B [84] and 5-aminolevulinic acid [81]. Macromolecules delivered using dissolving MNs include insulin [228,229], low molecular weight heparin [230], ovalbumin [231,232], leuprolide acetate [233], erythropoietin [196] and human growth hormone [234].

To date, insulin has been the most extensively delivered molecule using hollow MNs [29,30,235]. However, hollow MNs have also been used for ID delivery of smaller molecules, such as sulforhodamine [77].

Hydrogel-forming MN arrays have been used to deliver clinically relevant doses of a low potency, high dose drug substance and also for rapid delivery of proteins [25]. Studies carried out with hydrogel-forming MN arrays the show their ability to enhance percutaneous delivery of a wide variety of molecules, such as small hydrophilic drugs (*i.e.* theophylline, caffeine, methylene blue and metronidazole) and high molecular weight compounds (*i.e.* insulin and BSA) [8].

5.1.2. Vaccine delivery

The viable skin is viewed as an ideal target for vaccine delivery. It contains a large network of immunologically active cells and antigen-presenting cells (APC) responsible for initiating adaptive immune responses (Fig. 15) [236,237]. Additionally, skin vaccination presents a dose-sparing effect when compared to conventional intramuscular (IM) vaccination. This effect is due to the higher numbers of APCs present in the skin, leading to induction of stronger immune response with lower antigen levels [238]. As a result, MN vaccination has become an area of intense activity in recent years.

The main type of MN used for vaccination purposes is dissolving MN. Cell-culture-derived influenza vaccine has been loaded and delivered using trehalose and carboxymethyl cellulose dissolving MNs with considerable effect [239]. MN vaccination against influenza was further exemplified by using diverse approaches, such as delivering trivalent influenza vaccine in combination with ovalbumin using carboxymethyl cellulose MNs [240] or N-vinylpyrrolidone MN containing inactivated influenza vaccine [241]. In addition to influenza vaccination, Zaric et al. combined ovalbumin (as model protein)-loaded PLGA nanoparticles in combination with dissolving poly(methylvinylether-co-maleic acid) MNs to target dendritic cells, inducing considerable Th1 CD4+ and potent cytotoxic CD8+ T cell responses [242]. Hyaluronic acid MNs (Microhyala®) were used by Matsuo et al. to investigate vaccine efficacy against tetanus, diphtheria, influenza and malaria, obtaining in all cases immunisation results comparable with the parenteral route [243]. Additionally, the same research group used the hyaluronic acid MN system to try to develop a vaccine for Alzheimer's disease, delivering an amyloid-beta 42-amino acid peptide as an antigenic stimulant [244]. Even though results did not show improved brain function in those patients treated, it demonstrated that efficient anti amyloid-beta immune responses can be attained after MN application.

Coated MN arrays have been used successfully for vaccination purposes. For this purpose, different types of solid MN arrays can be coated with antigen-containing formulations. Stainless steel MNs coated with a vaccine against the Edmonston-Zagreb virus was used to successfully raise neutralising antibody titres equivalent to subcutaneous injection [245]. Influenza vaccination was also explored using coated MNs. Kommareddy et al. developed an influenza antigen coated MN system that elicits comparable antibody titres to IM injection [246]. Additionally, Zhu et al. and Wang et al. developed influenza vaccine-coated MNs, showing good immunisation results, in some cases superior to IM delivery [247,248]. Following a different vaccination strategy, Vrdoljak et al. delivered live virus vaccines, achieving equivalent immune

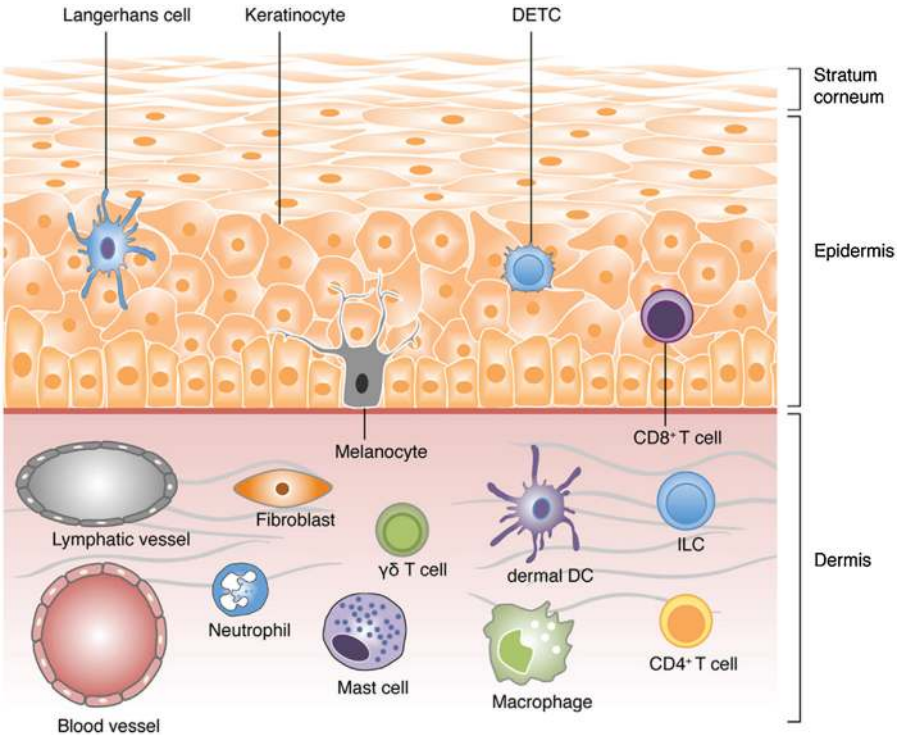

Fig. 15. A schematic view of the different cell types populating the skin. The stratum corneum is composed of dead keratinocytes and acts as a barrier. The epidermis is a dense and poorly vascularised region that comprises mainly of keratinocytes with few melanocytes. The major immune cells in this compartment include Langerhans cells (APCs), dendritic epidermal T-cells, and CD8 T-cells. The dermis is a highly vascularised region, rich in collagen, and elastin fibres, with low cell density. It comprises of fibroblasts, T-cells (CD4 αβ, and γδ), innate lymphoid cells (ILCs), dermal DCs (dDCs), macrophages, mast cells, and neutrophils (non-exhaustive list).
Source: Reproduced with permission of: [236].

responses to that of a conventional vaccine delivery approach (needle and syringe) using equivalent doses [249]. Vaccination with DNA and RNA in combination with MNs is beginning to show potential. DeMuth et al. developed a lipid nano-capsule loaded with DNA vaccine for a model of human immunodeficiency virus, achieving similar results to that of gene gun technology [250]. Additionally, Kim et al. in a more resent study, proposed an Alzheimer's disease vaccine be delivered using coated MN arrays [251]. The study showed that a robust humoral response, comparable with subcutaneous injection, could be achieved.

Finally, the last type of MN used for vaccine delivery is hollow MN. This type of vaccination is similar to conventional parenteral vaccine delivery, as one or more hollow needles are used to deliver the vaccine liquid formulation. In this case, the needles are between 1 and 1.5 mm in length and the injected volumes are less than 200 μl [252]. These systems have been designed to improve the Mantoux technique, consisting of insertion of a dermal needle into a patient's skin using a shallow angle. This technique is difficult to perform, leading to inconsistent responses [253]. Anthrax vaccination using rabbit models showed a dose reduction up to 50-fold when administered using hollow MN instead of IM injection [254]. Additionally, the rabbit survival profiles were equivalent to those injected using the IM route. Dose-sparing studies conducted with a rabies vaccine in healthy humans demonstrated that MN delivery showed an adequate immune efficacy in comparison with IM injection [255]. Another therapeutic approach was the inclusion of multiple vaccine-components combined into a single product. Morefield et al. successfully used hollow MNs to deliver a combination vaccine against anthrax, botulism, plague and staphylococcal toxic shock in rhesus macaque monkeys [256].

5.1.3. Patient monitoring and diagnosis

Blood extraction for patient monitoring or diagnostic purposes presents some drawbacks, including needle-phobia, need for trained healthcare staff and risk of infection associated with conventional needles, amongst others. MN have the potential to overcome these limitations. MN can be designed in a similar way to hypodermic needles, allowing interstitial fluid extraction from the skin [257]. Interstitial fluid represents a good medium for analyte monitoring, as the concentration of molecules in this fluid can often be correlated accurately with their blood concentrations. Therefore, MNs offer the possibility of a pain and blood free monitoring/diagnosis system (Fig. 16A).

The first MN extraction systems were based on capillary action of interstitial fluid. Novel strategies involve the use of more complex extraction mechanism, such as vacuum or osmotic pressure [257]. Glucose monitoring have been investigated using hollow glass MNs. The designed MN device was used to extract interstitial fluid from hairless rats and human volunteers using a vacuum over 2 and 10 min. Results from this study showed that the concentrations of glucose in interstitial fluid can be correlated with blood glucose [258]. An alternative to MN interstitial fluid extraction is the use of longer MN (upper-end of the micron scale) for capillary blood extraction (Fig. 16A). This method offers a less invasive procedure than conventional hypodermic needles. Hollow metallic MNs (1800 μm height, 60 μm inner diameter, 120 μm tip diameter and 150 bevel) were used by Li et al. for blood extraction. The authors claimed that this device allows blood extraction with low risk of pain inducement. This device was used to extract 20 μl of blood from mouse-tail vein [259]. A different approach for interstitial fluid extraction is the use of hydrogel-forming MN arrays. Once they are inserted, they swell, taking up skin interstitial fluid, depending on the degree of polymer cross-linking. Subsequently this swelled patch can be analysed in order

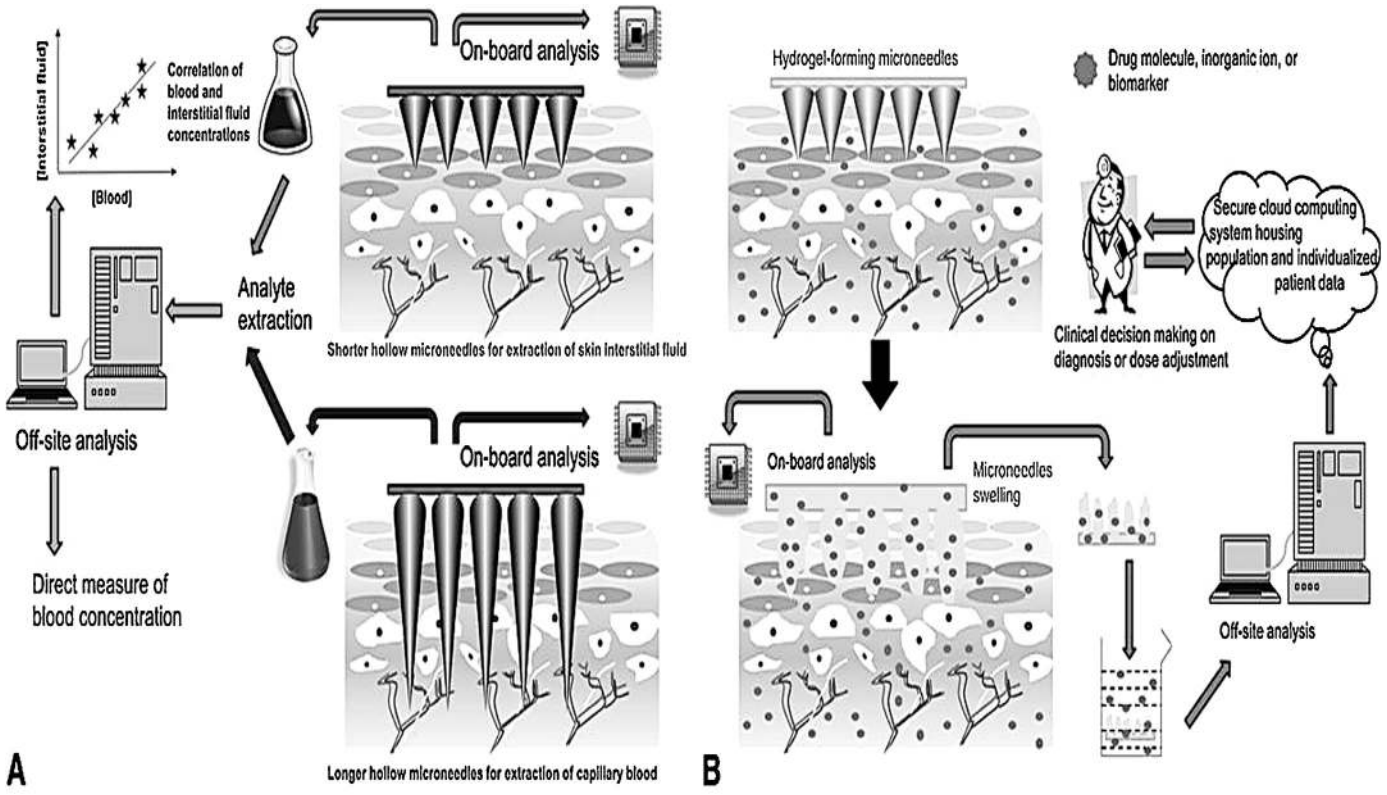

Fig. 16. Microneedle-based strategies for minimally invasive patient monitoring through blood or interstitial fluid extraction with on-board or off-site analysis. (A) Hollow MN for extraction of interstitial fluid or blood enabling indirect and direct quantification of plasma levels of analytes of diagnostic or therapeutic interest. (B) Hydrogel-forming MN for interstitial fluid capture and the possibility of "connected health" applications.
Source: Reproduced with permission of: [257].

to quantify different analytes and/or biomarkers (Fig. 16B) [257,260]. The development of integrated systems, which negate the need for off-site analysis after extraction of fluid and instead rely on the use of integrated sensors, has also been explored for many of the devices described previously (Fig. 16).

A different approach in the use of MN for monitoring purposes was proposed by O'Mahony et al. [261]. A MN system for electrocardiography signal optimisation was proposed. The *stratum corneum*, due to its high electrical impedance, causes significant interferences in the signal acquirement when using this technique. Therefore, with a view to optimisation, alternative approaches have been investigated, such as application of electrolytic gels or skin abrasion. The use of electrocardiography in combination with silicon MN arrays (25 needles with 300 μm in height) connected to two electrodes allowed the recording of images with comparable quality to conventional ones. This system has other potential applications, such as electroencephalography and electromyography.

5.1.4. Cosmetic applications

MN can be used for cosmetic applications, mainly for treatment of skin blemishes and the delivery of active cosmetic ingredients. Therefore, the cosmeceutical industry has shown great interest in MN technology [262,263]. Kim et al. developed a hyaluronic acid-based dissolving MN patch for the intradermal delivery of ascorbic acid and retinyl retinoate [264]. These MN systems were tested using human volunteers, displaying improved skin appearance in terms of roughness and wrinkle appearance. In order to obtain these results, MN were applied twice a day for 6 h at a time. Such conditions are, obviously, not ideal for cosmetic purposes. Recently, Kumar et al. showed an enhancement of local delivery of eflornithine (used to reduce facial hirsutism) *in vitro* and *in vivo* [265]. For this purpose, the skin was treated with MN followed by local administration of an eflornithine cream. Importantly, combination of MNs and commercially available topical products intended for application to intact skin should be treated with caution, since some adverse reactions, such as granulomas, have been reported by using non-sterile topical products [266].

MN technology can be used to treat different types of scars by dermabrasion [267–271]. MN dermabrasion consists of the use of MN to pierce the skin multiple times to induce collagen growth. Fabbrocini et al. treated patients with acne scars using a Dermaroller® treatment, reporting aesthetic improvement, with a reduction in scar severity in all subjects [269]. Patients were treated twice (eight weeks apart) with the needle roller (up to 2 mm in length), creating 250–300 microscopic holes/cm^2. To date, the skin recovery mechanism has not been fully elucidated, but it has been hypothesised that the treatment with MNs increases production of collagen and elastin. Nevertheless, this treatment is not optimal as, due to the needle length, it induced skin bleeding. Similar results were obtained by Majid using a dermarroller for treatment of atrophic facial scars [272]. Additionally, Park et al. used a disc MN therapy system for treatment of dermal scars (*striae distensae*), obtaining noticeable improvement in 7 out of 16 patients [268]. However, this treatment has the same limitations as those described previously for acne, namely skin bleeding and painful treatment.

In addition to the use of Dermaroller®, an alternative approach for the treatment of acne scars has been reported, the fractional radiofrequency MN system. This system was introduced recently as a new device for facial rejuvenation [273]. This system works by creating radiofrequency-induced thermal zones without epidermal

injury. After damage to the reticular dermis, dermal thickening occurs. This system was clinically evaluated by Chandrashekar et al. [273]. The results suggested that this novel technique is efficacious for the treatment of acne scars, as it reduced the severity of the scars in more than 80% of volunteers.

5.1.5. Other potential applications

There are increasing reports of alternative uses of MN beyond those previously described. Recently, Yang et al. reported the use of MN system improving skin graft adherence [274]. For this purpose, conical shaped MNs composed of a non-swelling core and a swellable tip were prepared using poly(styrene) and poly(acrylic acid) polymers. This medical device showed enhanced tissue adhesion strengths in comparison with the conventional staple system used for this purposes. This MN system can be inserted with minimal forces and allows the loading of therapeutic agents in the tips for a combined tissue adhesion and drug delivery strategy.

The use of MNs for alternative target areas other than the skin was studied by some authors. Thakur et al. [275] investigated the use of hollow MNs for the injection of thermoresponsive poloxamer formulations into rabbit sclera. The formulation contained fluorescein sodium as model compound. This MN-based system allows intra-scleral injection in a minimally invasive fashion when compared with traditional methods. The injected formulations forms a gel *in situ*, creating a drug reservoir that allows sustained release of loaded molecules. An interesting alternative for the use of MN arrays was proposed by Lee et al. [276]. They demonstrated the therapeutic performance of MN devices in treating neointimal hyperplasia that often occurs at anastomosis sites after grafting surgery. MN were inserted into vascular tissue layers in a rabbit balloon injury model. MN were coated with drugs, such as paclitaxel. These studies confirmed that the use of these MN arrays leads to a significant reduction of neointimal hyperplasia formation after both 2 and 4 weeks following balloon injury.

5.2. Translation to clinic

5.2.1. Microneedle safety

MN are not equivalent to conventional transdermal patches, as they are not applied to the skin surface. As explained previously, MN works by piercing the outermost protective skin layer. In some cases, MN penetrate into both the viable epidermis and dermis, normally sterile areas of the body [277]. Therefore, it is mandatory that MN products do not contain microbial loads that can cause skin or systemic infections. Additionally, the bioburden of these products should be controlled and minimised to avoid immune stimulation of the immune cell population present in the viable epidermis and dermis [278]. Microbial penetration through MN-created holes is minimal and significantly lower to that elicited using conventional hypodermic needle puncture [279]. Consequently, in normal circumstances, the use of MN should not cause infection. Wei et al. showed similar results *in vivo*, using MN laced with *Staphylococcus aureus* [18]. Certain materials used for MN production can show antimicrobial properties, further minimising infection risks [40]. In order to guarantee patient safety, a possible solution may be the production of sterile MN products. Nevertheless, the sterilisation methods should be considered carefully to avoid modifying the original nature of the product and increasing manufacturing costs. For example, aseptic manufacturing will be expensive, terminal sterilisation using gamma irradiation, moist heat or microwave heating could damage the MNs or their cargos. Some of these issues were addressed by McCrudden et al. [280]. In this work, the effects of different sterilisation methods on dissolving and swelling MN products were evaluated. Another key aspect, as discussed previously, is the biocompatibility of the materials selected for MN manufacture (see Section 3.2). It will be obligatory that no local or systemic reactions to the materials used to fabricate MNs occur. Biocompatibility and safety studies can be performed for different materials. Nevertheless, to date little is known about long-term effects of repeatedly penetrating the skin.

When using polymeric dissolving MN systems, an additional safety consideration arises: polymer deposition in the skin. Typically selected polymers for MN manufacture have been previously classified as biocompatible and safe. However, due to the novelty of MN systems, normally these polymers have never been used before intradermally. This question should be addressed, especially for dissolving MN systems designed for regular use.

5.2.2. Microneedle application and patient acceptability

In order to increase patient and prescriber compliance, MN patches must be viewed as an easy-to-use alternative to oral and parenteral delivery. Additionally, the FDA draft guidance 'Applying Human Factors and Usability Engineering to Optimise Medical Device Design' highlights the importance of an easy-to-use device that can be use successfully with minimal chance of error prior to marketing [281].

A common way to insert MN arrays is by using applicators. A wide variety of these devices can be found in the literature, especially in patents [282]. However, only some of them are commercially available. These devices often use high-impact, velocity or rotary designs to apply MNs in a defined way, ensuring minimal inter-individual variability in skin insertion [282]. The majority of applicator devices are based on impact application. One of simplest approaches is to use a spring-loaded piston. Some examples of MN applicators using this principle were developed by Zosano Pharma (Fig. 17A) and 3M Innovative Properties Company [282]. Mainly, these types of device are based on a piston that is accelerated using a coilED compression spring towards the MN array that is placed on the surface of the skin. High impact insertion can be achieved using different approaches, such as using an elastic band rather than springs [282], as shown in Fig. 17B. In this design, prior to insertion of the MN device the band is strained by pulling the MN array away from the skin to a predetermined distance. Then, the connecting membrane detaches from the elastic band allowing the band to snap back towards the skin, inserting the MN. Such devices were able to successfully insert MN arrays. The previously-described applicators are easy-to-use but, due to their high impact mechanism, these types of devices may not be the best options since they may be perceived as painful by patients. Simpler applicator mechanisms can be found in the literature. These types of applicators are designed to use thumb pressure [282]. Corium International Ltd (Fig. 17D) and 3M (Fig. 17E) have designed applicators following this premise. These applicators should be placed on the surface of the skin and then pressed, allowing the MN arrays to be inserted. By using applicators, the insertion process is controlled. However, the application is more complex than that of conventional transdermal patches and may be perceived as overly complex by patients.

A different alternative to applicators is to use manual insertion. Manual insertion allows a simple and patient-friendly way to insert the arrays. On the other hand, insertion is not controlled as it is when applicators are used. Nevertheless, Donnelly et al. demonstrated that hydrogel-forming MNs can be manually self-applied by patients in a consistent way with no applicator when counselled by a pharmacist and having read a suitable patient information leaflet [11]. In a similar study, Norman et al. showed that the use of MN for vaccination, instead of hypodermic needle injection, lead to an increase in "vaccination intent" from 44% to 65% [283]. The same study showed that MN self-application with

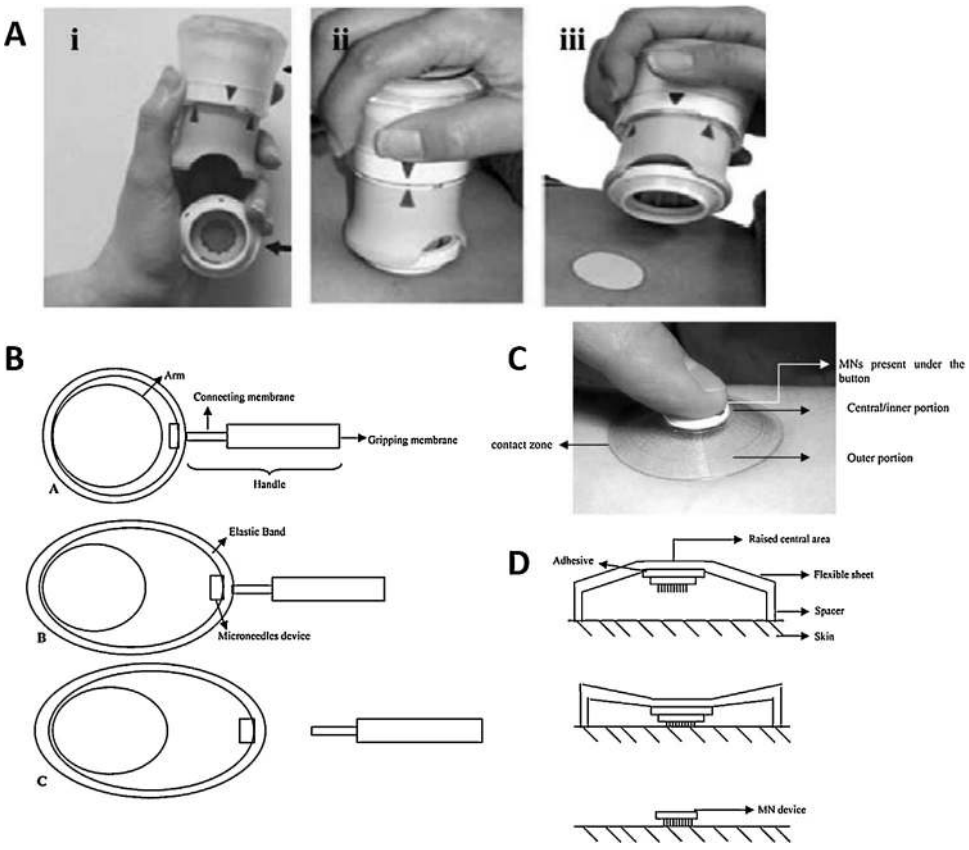

Fig. 17. (A) Zosano's Macroflux® applicator device at different stages of the insertion process. (B) Elastic band applicator device prior to insertion of MN device it is stretched so that the MN device is pulled away from the skin at predetermined level the connecting membrane detaches from elastic band, thereby allowing the band to snap back towards the arm subsequently causing the MN insertion into the skin. (C) MicroCorTM MN device. (D) 3M patented device.
Source: Reproduced with permission of: [282].

minimal volunteer training was successful, allowing at least 90% of MNs to penetrate the *stratum corneum.* Finally, Birchall et al. and Donnelly et al. pointed out that patients are likely to prefer MN with some feedback in response to correct application [11,284]. Additionally, Birchall et al. evaluated MN acceptability by healthcare professionals and members of the general public [284]. The main conclusion of this study was that 100% of the public and 74% of the healthcare professionals interviewed were positive in their feedback about MN technology. Moreover, in a recent study Mooney et al. evaluated the potential for use of MN in monitoring applications for children [285]. Overall, this study showed strong support for MN technology by the interviewed children in comparison with the unpopularity of traditional blood sampling methods. Nonetheless, further work in this area was recommended to evaluate the perspectives of children that require regular monitoring. Moreover, Norman et al. studied the delivery of insulin to children and adolescents suffering from type 1 diabetes using MNs [286]. The volunteers found MN insertion less painful than subcutaneous catheter insertion. All of these factors and considerations should be taken into account for the design of future MN-based devices. Additionally, aspects such as the necessity of an applicator or the suitability of manual insertion should be evaluated by regulatory authorities.

5.2.3. Scale-up and manufacturing considerations

Manufacturing MN aseptically, in low bioburden environments or employing terminal sterilisation procedures are likely to increase cost considerably. Scaling up MN production will require considerable thought. This is especially true given the plethora of small-scale production methods described in the literature. Very often a number of steps are required, especially for coated MN. Silicon MN requires clean room conditions. Overall, it is likely that any manufacturer wishing to develop MN products will need to make a substantial initial capital investment, given that equivalent manufacturing technologies are not currently available. Similarly, a range of new quality control tests will now also become necessary. It is likely that the regulatory requirements set for the first MN products to be approved for human use will set the standards for follow-on products. Packaging will be important in protecting MN from moisture and microbial ingress and suitable advice will need to be provided to avoid damage during patient handling and insertion.

5.2.4. Regulatory considerations

To the date there are no regulatory requirements defined for MN array-based products, due mainly to the innovative nature of this technology. This issue will have to be addressed in the coming years when companies intending to commercialise MN patches apply for marketing authorisation. Conventional transdermal patch systems are only applied to the surface of the skin. However, MN breach the *stratum corneum* barrier and often penetrate into the viable epidermis and dermis. Breaching the outermost protective layer of the skin generates a series of novel scientific/regulatory questions. Therefore, the totally different mechanism of action suggest that, from a regulatory perspective, MN will be defined as a new dosage form instead of a special type of the pre-existing transdermal patch systems. Additionally, specifications for these new products should be defined. The International

Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use provided guideline to assist the establishment of global specifications for new drug substances and drug products which have not been registered previously in the United States, the European Union or Japan [287]. These guidelines are known as Q6A. According to these guidelines, specifications are defined as follows: "A list of tests, references to analytical procedures, and appropriate acceptance criteria, which are numerical limits, ranges, or other criteria for the tests described. It establishes the set of criteria to which a drug substance or drug product should conform to be considered acceptable for its intended use". Specifications are an important part of the quality assurance system and are necessary to guarantee consistent production of drug substances and drug products of high quality [287].

In summary, the key regulatory questions that may need to be addressed for the establishment of MN product specifications are as follows:

1. *Microbiological standards of the MN dosage form*. MN dosage forms pierce the *stratum corneum*, reaching the dermis in some cases. Therefore, microbiological standards will be an important regulatory consideration. It may be acceptable for a product to have a low bioburden if it also has an inherent and proven antimicrobial activity.
2. *Uniformity of content*. This is a common pharmacopoeial requirement that should be applied to MN systems. However, depending on system design, this requirement can be applied either for the system as a whole or to individual drug-loaded MN within an array.
3. *Manufacturing aspects*. The normal aspects of quality applied to other dosage forms. Additionally, security packaging should be applied.
4. *Potential for re-use by patients or others*. Many MN systems, especially those made of silicon, metals and ceramic, can be removed intact from the skin after use. Thus, they could be re-used by the same patient or a different one. Due to safety reasons, a self-disabling system, guaranteeing a single-use only, may be required.
5. *Disposal*. As described above, some of the MN devices are not dissolvable/biodegradable and can be easily reinserted. Therefore they can be hazardous and a safe disposal procedure should be required.
6. *Deposition of MN materials in the skin*. Dissolvable, polymeric MN could deposit in the skin the materials from which they were fabricated, leading to adverse skin effects, such as granuloma formation or local erythema. This could be mitigated by alternating the application site. This issue should be addressed, as mentioned previously, especially for long-term use MN products. This is unlikely to be a major problem for single use application MN products, such as vaccines.
7. *Ease and reliability of application by patients*. Patients should be able to use the products in the optimum way without complications.
8. *Proper insertion assuring delivery*. As MN insertion produces no pain or obvious sensation, there is no feedback for the patient. A system that indicates correct application may be required.
9. *Immunological effects due to repeated use*. The repeated insertion of MN in the skin could possibly lead to immunological reactions. Therefore, assurances regarding immunological safety should be provided to the regulators.
10. *Long-term safety profile*. To the date, there are short-term safety studies with human volunteers. However, long-term safety of MN application should be addressed from the perspectives of intermittent and frequent repeat applications.

5.2.5. Commercialization of MN arrays

MN present substantial advantages over conventional transdermal patches, especially in terms of the delivery of biopharmaceuticals. Therefore, during the past decade, there has been a significant increase in industrial activity in this area. Currently, a certain number of MN-based products are being developed by different companies, including: Zosano Pharma (USA), 3M (USA), Sanofi Pasteur MSD (USA), Becton-Dickinson (BD) Technologies (USA), Valeritas (USA), Nanopass Technologies (Israel), Microneedle Therapy System (USA) [9], Rodan+Fields (USA), Vaxxas (Australia), Corium (USA) and, more recently, Lohmann Therapie-Systeme AG (Germany/USA), the world's largest transdermal patch manufacturer [288]. It is notable that no MN array-based drug delivery product is yet marketed. Micronjet® (Nanopass) and Soluvia® (BD) are available, yet they are not truly MN arrays, rather very short hollow needles that allow successful ID injection from a conventional syringe barrel.

3M developed the Microstructured Transdermal System® (Fig. 18A), a device formed by coated MN arrays that allows improved delivery of some drugs and vaccines in a quicker way. This system showed a rapid delivery of lidocaine, with administration sustainable for up to 90 min [289].

A different type of device was developed by BD Technologies, the Microinfusor (Fig. 18B). This is an automated hands-free system designed for delivery of a wide range of drugs to the subcutaneous tissue over a period of time, ranging from a few seconds to several minutes. This hollow MN system has a capacity of 0.2–15 ml and allows the delivery of highly viscous biotech drugs. The delivery of influenza vaccine with the same effectiveness of a conventional intramuscular injection was shown in preclinical studies [186].

Alza developed Macroflux®. This system was designed for the enhanced delivery of biopharmaceuticals using coated titanium microprojections [290]. This device allows a reproducible control of skin penetration depth due to the incorporation of an applicator device system. Moreover, the system was tested successfully for ovalbumin delivery.

Microneedle rollers have been becoming commercially available during the last years. The MTS Roller™ (Fig. 18D) was approved by the FDA for cosmetic purposes [9]. Clinical studies showed that the use of this type of device is more effective than other conventional ablative and non-ablative treatments in stimulating the production of collagen and elastin to smooth scars and erase wrinkles [269].

Valeritas developed two different types of MN-based device [9]. The first one was the Micro-Trans™ Microneedle Array Patch technology, a device that allows painless delivery drug delivery into the dermis. The second one was the h-Patch™, a subcutaneous controlled drug delivery platform.

Another type of MN-based device that has been developed are Micro-injection systems. These systems work in a similar way to conventional syringes. Nanopass Technologies developed MicronJet®, a hollow microneedle system for intradermal injection (Fig. 18G). This device is composed of four hollow silicon needles shorter than 500 μm attached to a plastic device that can be connected to any conventional syringe. This system was used to deliver influenza vaccine, demonstrating at least equivalent immunogenicity with only 20% of the dose used for conventional vaccination [9]. Micronjet® received FDA clearance in 2010 [12]. A similar approach was carried out by the Swiss company Debiotech. They developed an injector system containing a single or multiple silicon MNs called DebioJect™ [291]. It can be used to inject 100 μL in less than 2 and up to 500 μL in less than 5 s. Additionally, Sanofi Pasteur MSD Limited developed an intradermal micro-injection system for influenza vaccine (Fig 18H). This

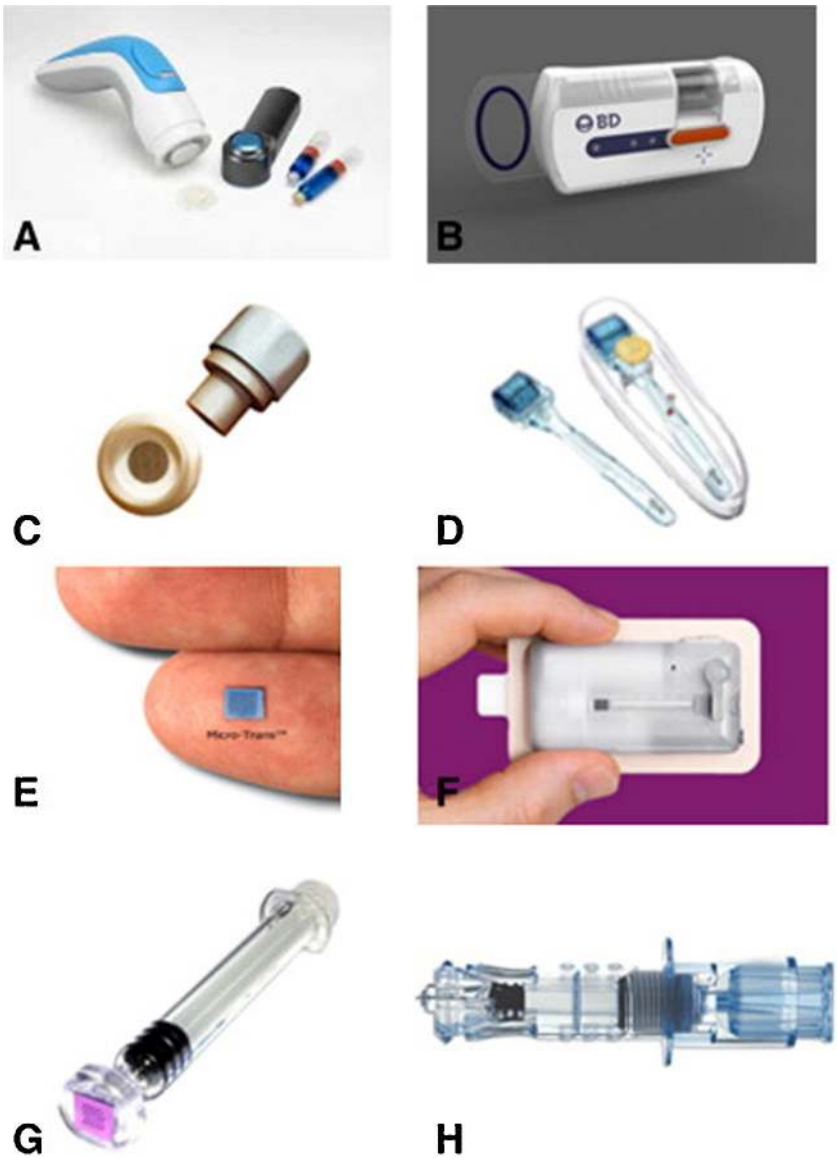

Fig. 18. Current microneedle devices. (A) Microstructured Transdermal System, (B) Microinfusor, (C) Macroflux®, (D) MTS Roller™, (E) Micro-trans™, (F) h-patch™, (G) MicronJet, (H) Intanza®.
Source: Reproduced with permission of: [9].

pioneering system was called Intanza® [9] and uses the Soluvia® injector developed by BD technologies. This device contains 1.5 mm long hypodermic needle attached to a syringe injector.

At this time Soluvia® and MicronJet® are the only MN-based medical systems available on the market for therapeutic applications. In addition to Intanza®, this injector is currently commercialised worldwide as IDflu® and Fluzone Intradermal® [12]. A cosmetic MN product available on the market was developed by Rodan+Fields Dermatologists. It is a dissolving MN array containing hydrolyzed hyaluronic acid for cosmetic application [292]. This product has been commercially available since October 2014.

The presence of the first MN devices in the market will undoubtedly encourage those working in this field to move forward to up-scaling manufacturing and marketing. Additionally, it serves to expand MN research, leading to the development of more varied and far-reaching MN products.

5.2.6. Microneedle in clinical trials

As can be seen in Table 2 to date, there have been a wide variety of completed clinical trials involving the use of MNs. All these trials are mainly focused on the following main topics: Influenza vaccine, delivery of insulin, delivery of parathyroid hormone, delivery of anaesthetics and dermabrasion. Dermabrasion has been described previously in Section 5.1.4.

Influenza intradermal vaccination has been extensively studied, due to the constant demand for a seasonal vaccine that is easy and safe to administer. The Soluvia® microinjection system has undergone extensive trials conducted around the world in thousands of volunteers. In a randomised, open-label Phase II clinical trial (978 healthy adults), the Soluvia® system showed equivalent (and superior in some cases) immune response to the IM vaccine [293]. This study was followed by a Phase III randomised, double-blind trial (2255 healthy adults), showing that the novel vaccine system and the IM vaccine induced similar post-vaccination antibody titres at 21 days post-immunisation [294]. Additionally, Phase II and Phase III clinical trials were conducted successfully to evaluate efficacy in elderly individuals (aged 60 years and older) [295]. Finally, a Phase II trial was conducted to examine the benefits in high-risk immunocompromised patients. For this purpose, 62 renal-transplant patients

Table 2
Completed clinical trials using MN.

Title	Year	Condition	Phase	Status
A study to assess the safety and efficacy of a microneedle device for local anaesthesia	2007	Local anaesthesia Intradermal Injections (MicronJet)	–	Completed
A pilot study to evaluate the safety and immunogenicity of low dose flu vaccines (NANOVAX)	2007	Influenza	–	Completed
A pilot study to assess the safety, PK and PD of insulin injected *via* MicronJet or conventional needle	2007	Intradermal injection	Phase 0	Completed
Insulin delivery using microneedles in type 1 diabetes	2009	Type 1 diabetes mellitus	Phase 2 Phase 3	Completed
Dose sparing intradermal S-OIV H1N1 influenza vaccination device	2010	Influenza	–	Completed
Pharmacokinetics/dynamics of basal (continuous) insulin infusion administered either intradermally or subcutaneously	2010	Diabetes mellitus, type 1 Diabetes mellitus, type 2	Phase 1 Phase 2	Completed
Tolerability study of the application of a 3M microstructure transdermal system	2010	Healthy	Phase 1	Completed
Optimisation of tuberculosis intradermal skin test	2011	Healthy Volunteers	Phase 1	Completed
2010/2011 Trivalent influenza vaccination	2011	Influenza	–	Completed
ADRN influenza vaccine pilot	2011	Atopic dermatitis	Phase 0	Completed
Comparison of 4 influenza vaccines in seniors (PCIRNRT09)	2011	Influenza vaccine	Phase 4	Completed
Physiological study to determine the allergic skin activity after different skin preparation	2012	Birch pollen allergy	Phase 1	Completed
Phase 2 study of BA058 (abaloparatide) transdermal delivery in postmenopausal women with osteoporosis	2012	Post-menopausal osteoporosis	Phase 2	Completed
Multi-day (3) in-patient evaluation of intradermal *versus* subcutaneous basal and bolus insulin infusion	2012	Type 1 diabetes	Phase 1 Phase 2	Completed
Routes of immunisation and flu immune responses (FLUWAY)	2012	Influenza	Phase 1 Phase 2	Completed
intradermal *versus* intramuscular polio vaccine booster in HIV-infected subjects (idipv)	2012	Polio immunity	Phase 2	Completed
Intradermal trivalent influenza vaccine with imiquimod	2012	Chronic illness	–	Completed
Atopic dermatitis research network (ADRN) influenza vaccine study	2012	Dermatitits Atopic	–	Completed
The use of microneedles in photodynamic therapy	2013	Actinic keratosis	–	Completed
Site selection for intracutaneous saline delivery	2013	Intracutaneous drug delivery	–	Completed
Intracutaneous delivery of varied dose volumes of saline	2013	Intracutaneous drug delivery	–	Completed
Safety study of suprachoroidal triamcinolone acetonide *via* microneedle to treat uveitis	2013	Uveitis Intermediate uveitis Posterior uveitis Panuveitis Noninfectious uveitis	Phase 1 Phase 2	Completed
Immunogenicity of inactivated and live polio vaccines	2013	Poliomyelitis	Phase 3	Completed
Comparison of mechanical penetration enhancers on metvixia skin penetration	2015	Healthy	Phase 1	Completed
Pretreatments of the skin prior to PDT	2015	Healthy	Phase 1	Completed

Data obtained from https://clinicaltrials.gov/.

(non-responders to conventional influenza vaccination) were randomised to receive ID and IM influenza vaccine. The results of this trial showed that the volunteers presented better immune responses when vaccinated ID. In addition to Soluvia®, the MicronJet® system has been studied for influenza vaccination (180 healthy adults), showing similar immunogenic responses to full-dose IM vaccination using lower doses.

The delivery of insulin using MN may be associated with increased patient compliance when compared with traditional subcutaneous injections. BD Technologies infusion system was tested in humans (10 healthy adults) for the delivery of a 10-IU standardised insulin lispro using stainless steel MNs of three different lengths (1.25, 1.50 and 1.75 mm) [296,297]. Alternative studies evaluated the pharmacokinetics and pharmacodynamics of insulin after administration to type 1 diabetes patients using different types of MNs [78,298]. The results of these studies have been consistent and demonstrated that the use of MN enables faster insulin uptake and equivalent bioavailability/blood-glucose effects when compared with subcutaneous administrations. Another study evaluated the patient preference of h-Patch over conventional needle and syringe systems as insulin administration devices (http://clinicaltrialsfeeds.org/clinical-trials/show/NCT00453934).

The Macroflux® device, originally developed by Alza, was conducted through Phase I and II clinical trials by Zosano Pharma for the delivery of parathyroid hormone to post-menopausal women [225]. The study was performed using 165 postmenopausal woman aged 50–81, whose last menstrual period was at least 1 year earlier. Volunteers were recruited in 13 centres in 3 different countries. The use of the Macroflux® device led to an increase in the total hip bone mineral density when compared to the placebo device and the commercial injection. These results, in combination with the positive feedback obtained in focus groups suggest that an upcoming Phase III study may well be successful. Focus groups (288 post-menopausal women with osteoporosis; aged 60–85 years) highlighted that more than 90% of the patients like the patch due to its ease of use. More than 80% of the interviewed patients were capable of applying the patch correctly without assistance.

The delivery of lidocaine and other local anaesthetics using solid and hollow MNs has been studied in several clinical trials. A randomised single blind study (15 volunteers) was conducted to compare time for anaesthesia onset and pain reduction after administration of lidocaine *via* hollow 500 μm MNs *versus* Mantoux injection with 26-Ga hypodermic needles [299]. Volunteers reported that MNs were less painful than hypodermic needles. The anaesthetic effect of lidocaine was equivalent for both

injection methods. Another study evaluated the effectiveness of MN arrays to facilitate the topical anaesthesia using a dyclonine cream (25 healthy volunteers) [300]. The use of MN reduced the time to meaningful pain reduction after a pain stimulus by a factor of 3.

It is noticeable that the number of MN clinical trials has markedly increased during the last 5 years. This can be explained mainly by the increasing interest in MN technology. Notably, the majority of these trials involve the use of MN injection systems, rather than MN array-based patches, and only a few conditions have been studied. Therefore, during the next decade additional clinical development will be required to prove the efficacy and the benefit of MN delivery systems *versus* traditional delivery systems.

6. Conclusions

Transdermal drug delivery has historically been limited to only those compounds that possess the appropriate physicochemical properties, which allow for movement across the *SC* and into the viable epidermis. The advancing nature of research into MN delivery systems shows continual improvement in transdermal delivery of therapeutics, which would otherwise never passively cross the *SC*. The initial MN concept has developed beyond the simplistic, solid MN design, into second and third generation technologies, formulated from a range of materials, in varying geometries and with diverse delivery briefs. The movement towards enhancing transdermal delivery of larger peptide, protein and vaccine components, as well as traditional molecules has led to MN product development and regulation becoming of key importance to researchers in this area. Clinical trials continue to be discussed by various research centres globally, and with the advent of Soluvia® and MicronJet®, the arrival of commercially available MN products is highly anticipated.

7. Expert opinion

Recent studies have shifted the original MN research paradigm to a more patient focussed one, which moving forward is an encouraging precedent. To proceed to the next step, we believe the priority lies with the regulatory questions and patient concerns as we examine what stands between our current MN knowledge and the availability of a MN product for the patient. Human factors concerning safety, efficacy and usability appear to be the key. Aligning engineering and product design, alongside the usability of the device will ultimately lead to an optimised product and faster progress through the relevant regulatory channels. The MN field is now at the stage where encompassing the end user in extensive clinical trials is important, while simultaneously making the necessary steps to scale up the production process.

A significant barrier remaining to a MN product being available relates to the regulation of MNs, primarily due to the innovative nature of the technology. Will drug delivery *via* MNs be classed as transdermal or intradermal? Will a MN patch be considered to be more akin to an injection or a transdermal patch? Manufacturers interested in the scale-up production of MNs await guidance relating to pharmacopoeial standards and appropriate quality control tests. Specific regulatory guidelines pertaining to patient use are also needed, concerning factors such as packaging, disposal, ease of use, assurance of correct use and also safety issues.

From a pharmaceutical viewpoint, it is evident that a number of questions relating to patient use of MNs remain unanswered, although it is noted that it may be some time until large population surveillance can be conducted and suitable answers obtained to all. Currently there are numerous studies which have involved human volunteers, both with placebo MNs and drug-loaded MNs. These investigations have formed the basis of an initial short-term safety profile, yielding promising results to date. The next step to contemplate is the long-term safety of MN application; from both the perspective of intermittent use and also of repeated application, for example when considering the number of doses required for insulin administration over a sustained period.

An example of a long-term issue worth further consideration is that of polymer deposition in the skin following MN removal. This is particularly likely to be of concern with dissolving MNs, suggesting that a dissolving MN platform is more suitable for "one-off" delivery, such as vaccination. If the deposited MN material is not efficiently removed and metabolised by the body, an as yet uncharacterised process; it is theorised that repeated application of this type of MN could lead to distribution and deposition of polymer throughout the body, hepatic accumulation and/or a build-up of polymer in the dermal tissue. The possibility of an immunological reaction is also worth noting, induced potentially by the barrier disruption caused upon MN insertion, thus stimulating the rich immune cell population of the skin.

Moving on from long-term safety to the patient use of the device, the pharmacist or healthcare provider would need to be convinced of the ability of the patient to use the device in their own home. Recent studies, as discussed previously, have provided some convincing evidence as to the validity of self-application. FDA draft guidance, 'Applying Human Factors and Usability Engineering to Optimise Medical Device Design,' stresses the importance of an easy-to-use device, with reduced need for training and reduced reliance on a user manual. Emphasis is also placed on the requirement for evidence to prove successful use of the device with minimal chance of error prior to marketing. It is thought manual application using only thumb pressure would be the preferred method of administration to maximise patient ease of us. In order to capitalise on preconceived positive beliefs relating to transdermal patches, MN patches must be viewed in the same way by both prescriber and patient, as a convenient alternative to oral and parenteral delivery. Furthermore, if used as a tool to increase medication adherence, MNs will have their greatest benefit if their application is a straightforward one-step process, with no additional devices.

MN knowledge has increased rapidly in recent years from the description of the first concept in 1976 to the present day, with a plethora of MN designs currently described, delivering a wide range of substrates. The bid to move towards MN commercialisation can only intensify as the research questions highlighted herein continue to be answered, in tandem with the publication of further studies, outlining the increased delivery capacities of MNs, with the ultimate endpoint of direct patient application. MN devices have the capacity to play a role in modern healthcare and, therefore it is now the researchers' role to ensure the present work is capitalised on and subsequently translates into patient benefit.

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