Chapter

Theranostic Microneedle Devices: Innovative Biosensing and Transdermal Drugs Administration

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Abstract

Biosensing systems based on microneedles can overcome the stratum corneum of the skin, i. e. the outer natural barrier of the human body, without any pain and detect the target analytes directly in the interstitial fluid. Moreover, microneedle-based devices (MNDs) can combine diagnostic sensing and therapeutic administration of drugs in one single tool. From this point of view, more than a painless door to the human body, a MND represents the a perfect example of theranostic instrument, since a single device could quantify the real value of a relevant biomolecule, such as glucose, and accurately deliver a drug, the insulin, if needed. MNDs could be integrated on printed circuit boards, flexible electronics and microfluidic channels, thus allowing a continuous monitoring of the physiological parameters with very low invasiveness, together with sustained and localized administration of drugs. MNDs can be designed for very specific applications, from the detection of skin cancer to the monitoring of metabolic pathways. Moreover, several fabrication approaches have been introduced, from laboratories to large-scale production. Finally MNDs can be properly functionalized to enhance analytical performances.

Keywords: microneedles, theranostics, biosensing, microfabrication, drug delivery

1. Introduction

A new type of biomedical devices was born when technologies and facilities for Micro and Nano Electro Mechanical Systems (MEMS and NEMS) fabrication have met medical and biological issues. These new kinds of biomedical devices are able to easily control physical and chemical parameters at a very small scale, down to nanomolar concentrations and nanometric sizes [1–3]. Moreover, the integration of such a device in wearable and/or mobile systems gave them popularity among commercial devices. In this technological frame, Microneedles based devices (MNDs) were born. Their height is sufficiently large to overcome the outer natural barrier of the human body, the stratum corneum of the skin, but not enough to reach the nerves, resulting in a lack of pain [4]. Usually, MN height is ranging from 10 to

1000 μm, depending on the application and how deep in the epidermis is the specific target analyte. Then, MNs based devices act as an interface between the body of the patient and a biomedical device, whose applications can range from fluid extraction for ex-situ analysis to drug and gene delivery, from *in situ* diagnostic tools to targeted cell therapy [1–4]. Material, length, shape of the body and the tip of the MNs drastically vary depending on the application [2, 3] and the fabrication technology, according to new needs and challenges. Biosensing systems based on MNs have to overcome the stratum corneum without pain and to detect the target

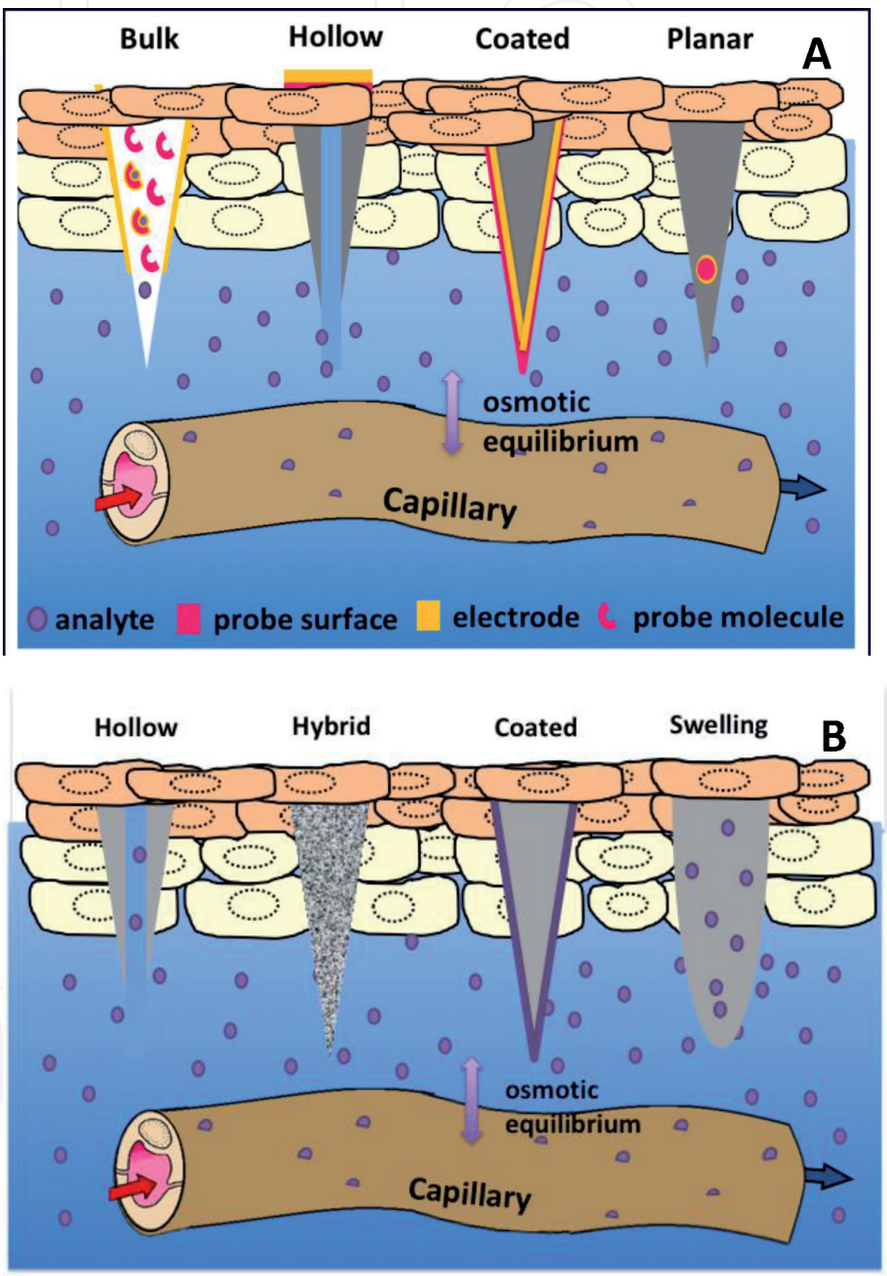

Figure 1.
(A) Sketch of different MNDs for sensing purposes together with their working conditions into the human skin. Starting from the right, they are characterized by the locus of probe-analyte interaction: Swelling bulk MNs sensors (BMNDs), where probe-analyte interaction is inside the volume of MN; hollow MNs sensors (HMNDs), where a small material sampling of ISF is analyzed on or offline; coated MNs sensors (CMNDs), whose surface is the locus of the interaction between analytes and bioprobes; planar MNs sensors (PMNDs), where the probe–analyte interaction is on a specific zone of a flat MNs surface. (B) some configurations for MNs for drugs delivery: (from left) hollow MNs present a inner cavities to immediately administration of high dose and high MW drugs; soluble and hybrid MNs for fast administration with a high doses and medium MW; coated MNs for fast administration of low doses and any MW; swelling MNs for very slow administration of high doses of smaller molecules. Reproduced with permission of Ref. [8].

analytes directly in the interstitial fluid. In those cases, more than a strategy could be used: the functionalization of the surface of MNs with a specific probe, realizing a coated MND [5], the trapping of probe molecules into a swelling material [6] or the extraction of fluids and the analysis into a microfluidic system [7] are only some examples. In **Figure 1A** a sketch of different MNDs for sensing purposes is shown.

Moreover, microneedle-based devices (MNDs) can combine diagnostic sensing and therapeutic administration of drugs in one single tool. From this point of view, more than a painless door to the human body, a MND represents the a perfect example of theranostic instrument, since a single device could quantify the real value of a relevant biomolecule, such as glucose, and accurately deliver a drug, the insulin, if needed. MNDs are particularly interesting as simple drug administration tools, too. In fact, the transdermal route for drug administration is a very fascinating way, not only for the very low invasiveness and the easiness of self-administration, but also for the absence of first pass metabolism. However, the intercellular lipid matrix of the epidermis consists of ceramides, free fatty acids, and cholesterol, a complex mixture of neutral lipids arranged as bilayers with hydrophobic chains facing each other (lipophilic bimolecular leaflet) [9]. Transdermal delivery works only for lipophilic uncharged drugs with low MW (<500 Da), which need low dose and continuous delivery. Moreover, components, formulations and drugs must be non-irritating and non-sensitizing. MNs can be used with both lipophilic and hydrophilic formulations, both charged and uncharged drugs, both small and oversized molecules.

For all these cases, MN configurations are illustrated in **Figure 1B**, where the possibility to use solving or hybrid soluble/insoluble MNs are considered.

MNDs could be integrated on printed circuit boards, flexible electronics and microfluidic channels, thus allowing a continuous monitoring of the physiological parameters with very low invasiveness, together with sustained and localized administration of drugs. MNDs can be designed for very specific applications, from the detection of skin cancer to the monitoring of metabolic pathways.

2. Microneedles array fabrication

Technologies, skills and facilities for Micro and Nano Electro Mechanical Systems (MEMS and NEMS) fabrication are the key elements for the development of new biomedical devices [1–3, 10]. Fabrication methods for Microneedles (MNs) strongly depend on the MNs shape, tip model, length, density of the MNs matrix, and the material of which they are made of.

Moreover, structural characteristics of the MNs matrix in turn depend on the specific application considered [11]. In fact, MNDs are exploited in fluid extraction [12] and *in-situ* diagnosis of diseases [13], in drug and gene delivery strategies [1, 11], in cell therapy [3] and so on.

At first, Silicon and silicon-based nanostructured materials, such as porous silicon, were largely employed in MNDs fabrication due to the well-established functionalization chemistry protocols and fabrication techniques, extenseively used in microelectronics, which simplified the integration into more complex systems [14]. However, silicon revealed to be a non-biocompatible material, due to its fragility and to the local inflammations (silicosis) it could provoke; for this reason its use has been limited in cell applications [15].

To overcome limitations on the use of silicon, polymers have been extensively proposed as alternative materials in many applications. Poly Dimethyl Siloxane

(PDMS) is one of the most used materials in microfluidics to design biomedical devices, due to its well-known biological compatibility [16]. Usually, PDMS is employed as mold to fabricate MNDs by replica molding (see **Figure 2**). In case of PDMS molding, the fabrication involves the following steps: female PDMS mold fabrication by means of standard photolithography or laser drilling; patterned MNs in PDMS mold filling with liquid polymers in vacuum conditions; curing of the polymers by temperature and/or UV exposure; mold removal; eventually, an additional curing step [16]. Biodegradable polymers have been largely employed in MNDs for drugs delivery application [20–24], but the biodegradability is not required for biosensing.

A direct method for MNDs fabrication is the so-called drawing lithography [19]. Drawing lithography is a fabrication method, which does not need light irradiation and a mask, since it is based on the use of a thermosetting polymer directly drawn from a 2D solid surface (see **Figure 2**). In drawing lithography, commercial photoresist is usually spin coated or drop casted onto the substrate and cooled down. Drills are fixed in an array on a PDMS frame and used as pillars contacted with the photoresist. Conical-shaped bridges between the substrate and the pillars appear when their relative distance is increased by drawing (elongation). The bridges are cured to generate a rigid structure. Finally, the separation of the bridges produces the desired MND.

However, drawing method lacks in flexibility and the curing at high temperature of the polymers encapsulating biopharmaceutical molecules can cause their denaturation or inactivation. In fact, MNDs encapsulating drugs or bioprobes must be fabricated in a controlled environment to preserve the biological activity.

The increasing demand for simple methods that preserve the biological activity by utilizing the natural properties of polymers has conducted to the idea of centrifugal lithography [17]. In [17], centrifugal lithography was used for the fabrication of MNDs in a single centrifugation, by exploiting the self-shaping properties

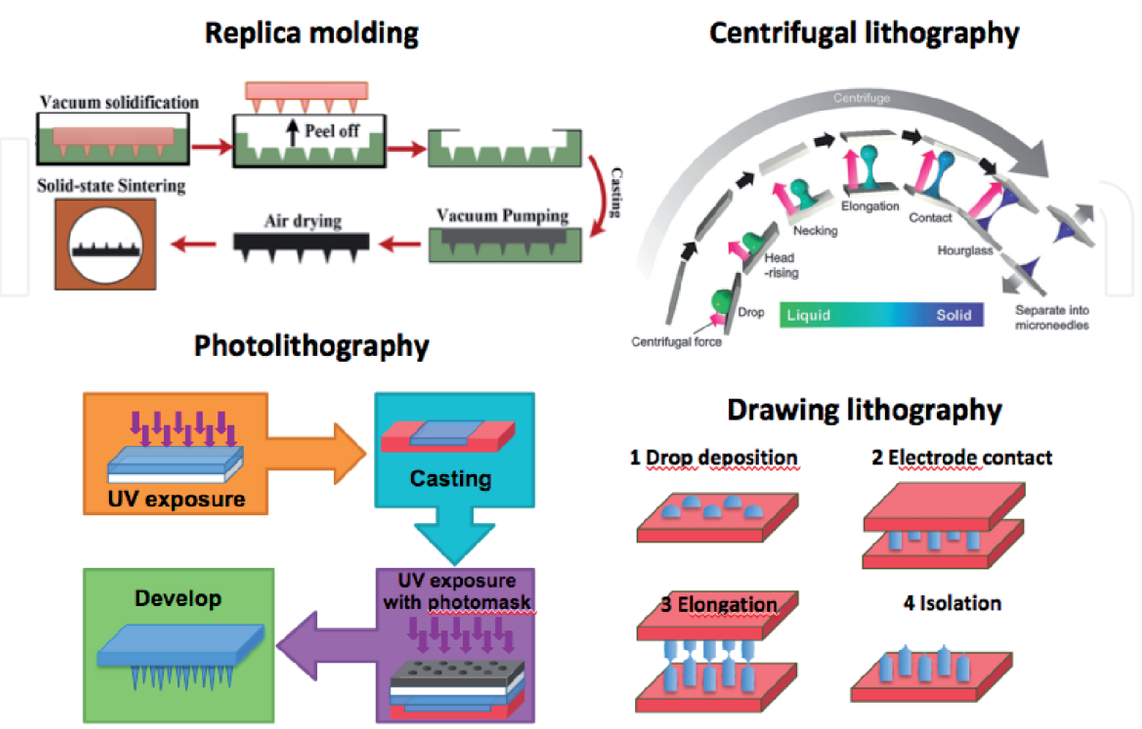

Figure 2.
Main fabrication strategies for MNs fabrication. Replica molding [16] centrifugal lithography [17] photolithography [18] drawing lithography [19].

of hyaluronic acid (HA). Briefly, fabrication involves the following steps: HA drops encapsulating drugs molecules are casted onto the substrate; centrifugal force is applied under refrigerated conditions (4°C) to the droplets in order to shape in hourglass microstructures; finally, the mirroring shapes are separated to form MNs. Also in the case of HA, drug delivery is successfully obtained, but biosensing is unavailable due to its biodegradability.

On the other hand, hydrogel polymers are very attractive materials for MNDs and, generally, for biomedical devices, since a hydrated gel provides near physiological conditions. These gels are excellent encapsulation matrices for biological probes, such as enzymes and peptides [18, 20, 25, 26]. Moreover, the standard photolithographic processes can be employed to fabricate micrometric devices based on polymeric hydrogels [materials] (**Figure 2**). In [6, 18, 27, 28], authors proposed procedures of standard direct photolithography, where a mixture of Poly(ethylene glycol) diacrylate (PEGDA) and a commercial photoinitiator were used as an ordinary photoresist, without any etching step being required. In fact, PEGDA is a biocompatible polymer that solidifies at room temperature in presence of a photoinitiator after exposure to ultraviolet (UV) light for few seconds. In case of photolithographic process, the fabrication involves the following steps: the liquid photosensitive polymeric mixture is casted onto a UV-transparent substrate and exposed to ultraviolet radiation, in order to fabricate the MNDs base; a vessel is fulfilled with a second quantity of liquid mixture and the MNDs base is put on; a second exposure through a mask, whose pattern is an array of holes, is applied; finally the structure is developed by simply washing in deionized water. The PEGDA mixture can be customized to encapsulate a variety of drugs or sensing probes as biological molecules or inorganic nanoparticles [29–31].

Comparing the fabrication methods, all produced MNs have demonstrated high quality in indentation proof and a good grade of reproducibility, with some critical issues during the mold removal step in replica molding method.

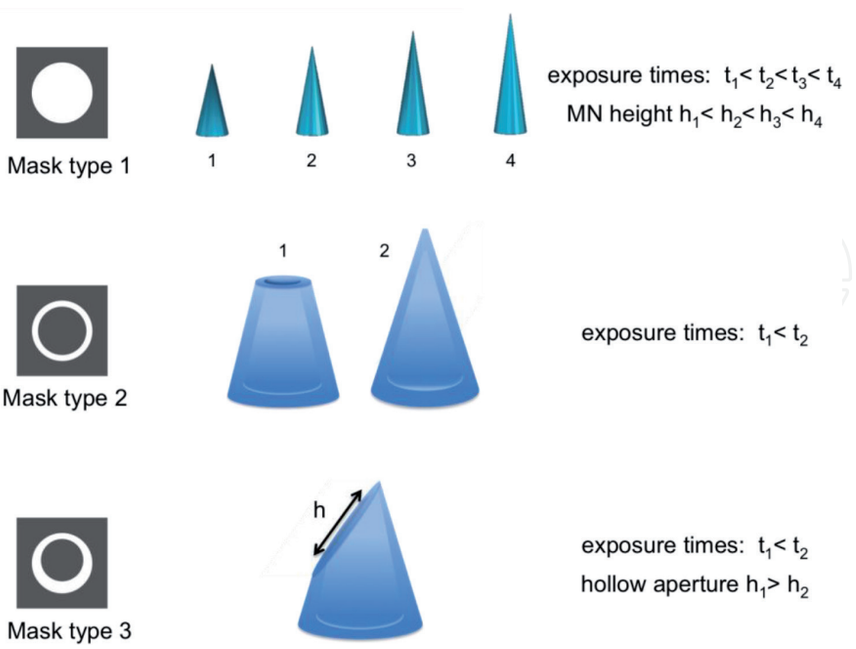

Figure 3.
The photolithographic methods offer a wide range of solutions for MNDs. Changing time exposure and/or photolithographic mask several configurations and arrays of MNs for both therapeutics and biosensing can be fabricated. From above: Mask type 1 (simple circle) enables MNs with several heights depending on time exposure; mask type 2 (ring) enables hollow MNs with height and closure depending on time exposure; mask type 3 (mismatched concentric ring) enables in one only exposure hollow MNs with a lateral oblique aperture, which is smaller as the exposure time increases.

Finally, we highlight that the photolithographic approach allows the fabrication of MNDs for a wide range of applications. In fact, this process allows the design of a wide range of MN types with different shape, length and tip, simply by adjusting the exposure parameters or shape photolithographic masks [18, 28]. In **Figure 3**, the whole range of possibilities enabled by photolithographic method are summarized: mask type 1 (simple circle) enables MNs with several heights depending on time exposure; mask type 2 (ring) enables hollow MNs with height and closure depending on time exposure; mask type 3 (mismatched concentric ring) enables in a single exposure the fabrication of hollow MNs with a lateral oblique aperture as in hypodermic syringes. Also in this case, lateral aperture is smaller as the exposure time increases.

3. Drug delivery patches

MNs represent actually a flexible technological platform, which enables innovative diagnostic solutions and breakthrough therapeutic issues in biomedicine [1–3]. First, finding a painless alternative to hypodermic injections has driven researchers to the development of MNDs. In fact, belonephobia, which is the unreasonable fear of needles, affects up to 10% of the population and has implications for treatment and follow up, especially in the pediatric patients [32]. In reverse, the sensation caused by MNs has proved to be statistically indistinguishable from a smooth surface and the pain caused by a hypodermic needle has been perceived substantially more than MNs [4]. Moreover, as previously stated, the transdermal route for drug administration is a very fascinating way, not only for the very low invasiveness and the easiness of self-administration, but also for the absence of first pass metabolism. However, the intercellular lipid matrix of the epidermis consists of ceramides, free fatty acids, and cholesterol, a complex mixture of neutral lipids arranged as bilayers with hydrophobic chains facing each other (lipophilic bimolecular leaflet) [9]. Transdermal delivery works only for lipophilic uncharged drugs with low MW (<500 Da), which need low dose and continuous delivery. Moreover, components, formulations and drugs must be non-irritating and non-sensitizing. MNs can be used with both lipophilic and hydrophilic formulations, both charged and uncharged drugs, both small and oversized molecules. In fact, currently, interesting MNDs are involved in clinical trials both for some topical applications, as analgesic compounds, anti-inflammatory or anesthetic drugs, and for some traditional systemic drugs, such as anticancer drugs, vaccines, insulin or hormones [33].

Among the topical applications, MNDs can replace very invasive methods for warts therapy, such as electrocautery and cryotherapy. A MND developed by Ryu et al. for warts treatment resulted to be innovative and effective [34]. In this study, quite 40 patients with wart lesions were enrolled and referred less pain than cryotherapy, as well as more tolerability with respect to electrocautery. Other skin diseases have been treated by means of MNDs, as melasma in [35], where authors fabricated biocompatible polymeric MNs based on methacrylic acid and polyvinyl pyrrolidone (PVP) to locally administer tranexamic acid, an innovative molecule that inhibits excessive melanin production by acting on melanocytes.

Acne vulgaris is another common inflammatory skin disease, affecting both physiologically and psychologically on patients. Barrier properties of skin strongly limit the usual antibiotic drug creams used to cure acne, but the use of MNs can overcome this limits, by using a reactive oxygen species–responsive [36]. In some *in-vivo* studies, MNDs for anti-acne therapy demonstrated bioresponsivity and efficiency to prevent bacterial growth. Finally, among the local administration taking advantage of MNs, the treatment of cornea diseases must be quoted. In particular,

using dissolving polymeric microneedles to deliver besifloxacin to the cornea, a significant improvement in besifloxacin deposition and permeation were proven after only 5 minutes of application [37].

On the other hand, also administration of systemic drugs by means of MNDs showed good results in effectiveness, safe and economic efficiency as disposal devices. A wide range of molecules has been proven to be compatible with MNDs and each category of drug showed specific advantages compared to the use of oral or hypodermic administration.

First of all, vaccine delivery is probably the most involved health issue in MN technology, due to the large number of people involved each year. Nguyen and Park recently reviewed MNDs enrolled in human studies and reported the progress of MNDs in the clinical trials [38]. Finally, the use of MNs in therapy for clinical vaccine was recognized as very important, but further tests are recommended.

When MNDs is used to deliver vaccines based on DNA, some studies show that the gene expression is improved with respect to the results of conventional hypodermic injection. Consequently, the use of MNs to administrate DNA based vaccine results in an improvement of the immune responses [38, 39]. In [39], Authors hypothesized that the improvement of the immune response by delivering DNA vaccine by means of MNs could be due to the enhancement of the protein expression of the encoded gene.

Another important issue of vaccine administration improved by MNDs is the stability of the active ingredients into dissolving or swellable MNs. Encapsulation of inactivated polio vaccine (IPV) into dissolving MNs gains a better thermal stability with respect to that of the conventional liquid formulation of IPV [40]. The greater thermostability of the MN patches can generally enable a mass distribution with less constrains on cold chain storage resulting in a great reduction of costs, since global vaccination strategies require large immunization coverage. Moreover, new MNDs have been proposed as an alternative solution to the standard needle injections, for the advantage of self-vaccination.

Further studies have been done to elucidate the interactions between polymers and vaccines, as in the case of hydrogel based MNs and dissolving MNs. In these cases, the antigen ovalbumin was used as a model protein interaction with polymers and the consequences on the immune response [40, 41].

Hollow MNs have the advantage of overcoming the skin barrier imposed by the stratum corneum and delivering bigger molecules, such as macromolecules or nanoparticle systems, in the fastest possible way. Polymeric nanoparticles encapsulating the model antigen ovalbumin have been intradermal delivered by means of hollow MNDs by Niu et al., reporting that this kind of delivery is a promising approach to improve the effectiveness of vaccine formulations [42]. Among the dissolving devices, MNs based on hyaluronic acid (HA) resulted a promising encapsulation method of high content of antigen molecules in intradermal vaccination [43].

Also anticancer drugs belong to an important field of application of MNDs: two research groups have investigated on DOX administration by means of MNs in [44] and in [45]. Nguyen et al. found *in vitro* studies that Polyvinyl Alcohol (PVA) MNs enhance the transdermal delivery of DOX. In an *in vivo* antitumor study of Hao et al., a near-infrared responsive PEGylated gold nanorod (GNR-PEG) and DOX-loaded dissolvable HA-based microneedle (GNR-PEG&DOX@HA MN) has been developed against cancer of epidermis. In the study, mice treated with GNR-PEG&DOX@HA MNs taken remarkable advantage in antitumor efficacy in only one treatment, such that all mice have been cured without recurrence.

Moreover, lipophilic drugs found a lot of benefits from the use of MNs: poorly soluble drugs were encapsulated and easily administrated by MNDs, as in the case

of the widely used specific 5-HT3 receptor antagonist, namely granisetron, that prevents nausea and vomiting during emetogenic chemotherapy in cancer patients [46]. *In vivo* results in [46] proved the evidence of controlled release systemic delivery.

An innovative pharmaceutical solution involving a MND in the field of HIV treatment has been proposed by Yavuz et al. in [47]. Also in this case, the self-injection route of administration represents the key issue for care improvement, since it limited the risks of contamination of the personnel involved in therapy and guaranteed a painless delivery for the patients via patches of microneedles.

New drugs and innovative therapies have been put in place with the help of MNDs. In particular, polymeric MNs have been widely exploited for their porous nature, which is expressed both by soluble MNs and by simply biocompatible ones. In [48, 49], anti-obesity substances have been successfully administered. These substances modified the metabolic process by increasing the energy consumed and

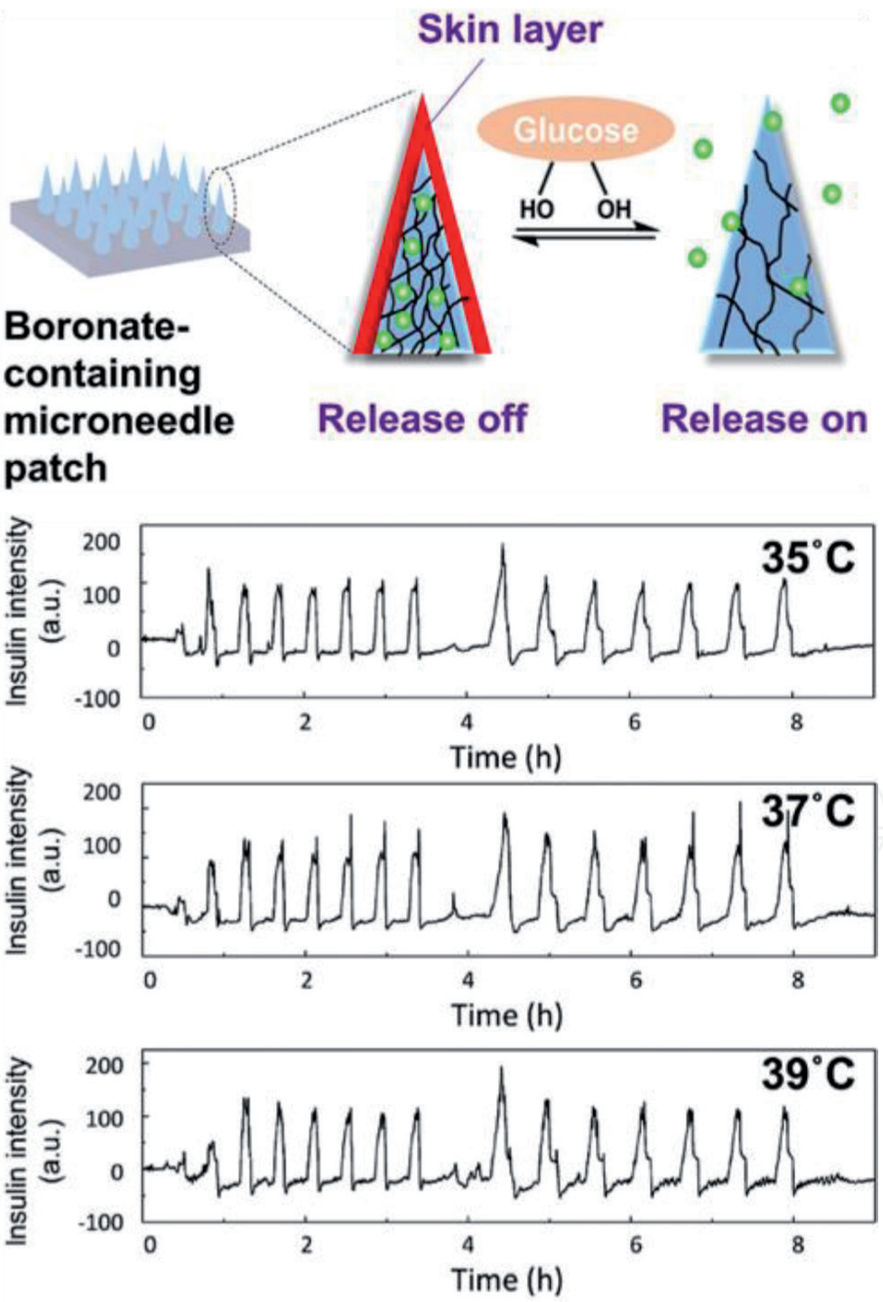

Figure 4.
Adapted with permission from ACS Appl. Polym. Mater. 2020, 2, 7, 2781–2790. Copyright (2020) American Chemical Society. Sketch of device and in vitro *FITC-labeled insulin release at various temperatures, pH 7.4.*

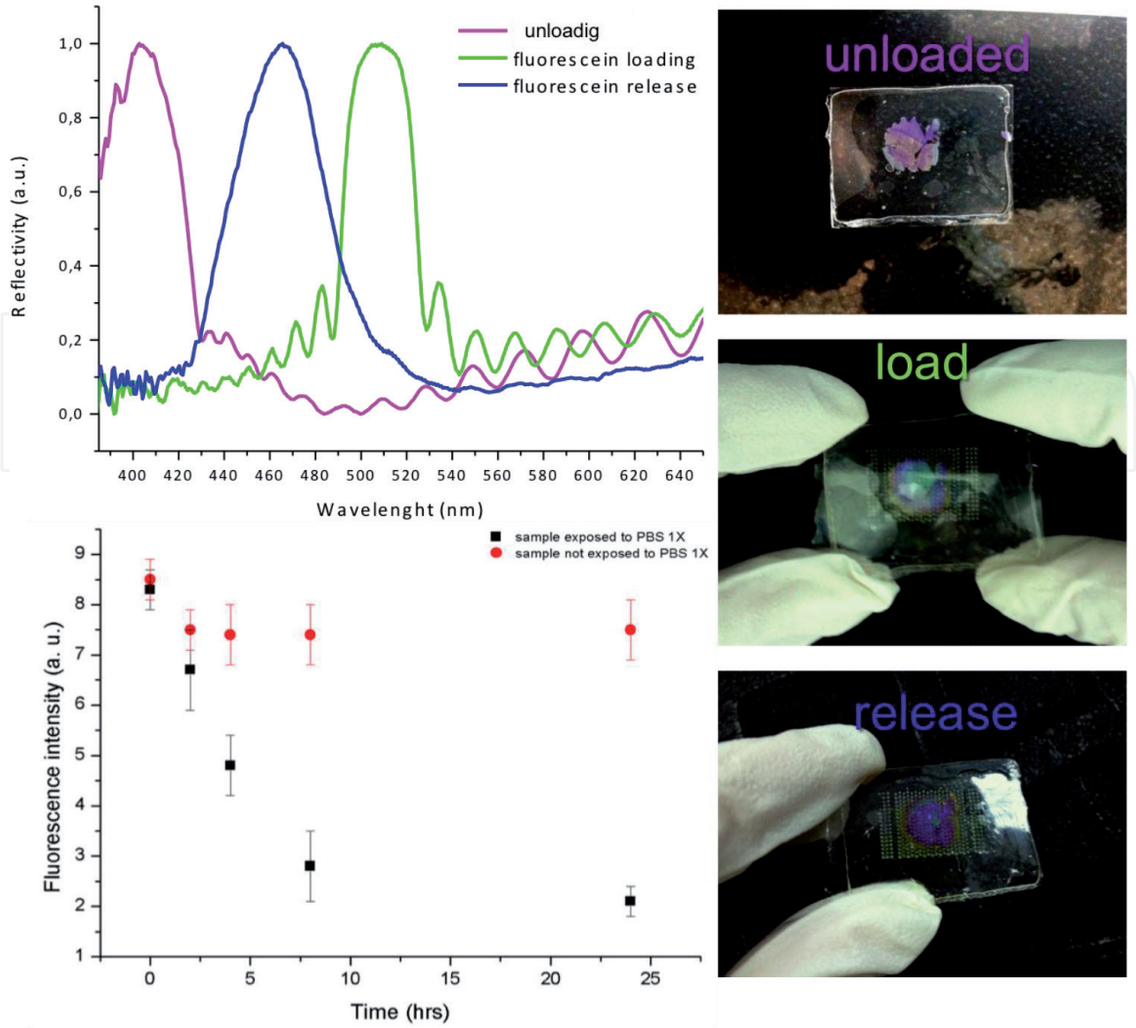

Figure 5.
The optical integrated MND presented in [6] have got a naked-eye monitor made up with a psi membrane to follow the release of a drug loaded in.

transforming the white fat that stores calories into brown fat that burns calories [48]. While in [49], gelatin MNDs were used to induce lipolysis and suppress adipocyte lipogenesis in fatty rats.

Particular attention has to be paid on insulin delivery, since diabetes is one of the most common diseases, not only in elder patients, but also in obesity-affected patients.

Avoiding use of enzymes, a polymeric MND has been developed for on-demand insulin delivery by Chen et al. [50]. Continuous and acute glycemic control was realized with a long-acting, safe, stable, economically efficient and on-demand insulin delivery by MND, without depending on patient compliance. Thus, this technology opens to next generation of diabetes therapies.

In the same field, the treatment of individuals with type II diabetes mellitus has been successfully obtained with metformin HCl, the most widely used drug for this disease, delivered by means of hydrogel MNs [51].

In [52], authors proposed a temperature-independent MND for glucose-responsive insulin release. The rapid and sustained regulation is enabled through a "skin layer" of Phenylboronic acid (PBA), formed on the surface of MNs. PBA is a synthetic hydrogel with reversible binding capability with glucose. Compared to other glucose-responsive MNDs based on nanoparticles or glucose oxidase, the proposed patch overcomes the safety concerns and provides a good sustainability for large-scale production. In **Figure 4**, a sketch of the proposed glucose-responsive

MN type	Disease	Experiments	Refs.
Swelling	Acne vulgaris	*In vivo* (mouse)	[36]
Swelling	Diabetes	*In vitro*	[50]
Swelling	Diabetes	*In vivo* (mouse)	[51]
Swelling	Immunity (vaccines)	*In vitro*	[40]
Swelling	Nausea and vomiting	*In vivo*	[46]
Swelling	Keloid scar	*Ex vivo*	[53]
Swelling/hybrid	—	*in vitro*	[6]
Hollow	Immunity (vaccines)	—	[42]
Dissolving	Melasma	*In vivo* (mouse)	[35]
Dissolving	Ocular infection	*Ex vivo* (cornea)	[37]
Dissolving	Cancer	*In vitro*	[44]
Dissolving	Cancer	*In vivo* (mouse)	[45, 54]
Dissolving	Immunity (vaccines)	*In vivo* (mouse)	[41, 43]
Dissolving	Obesity	*In vivo* (mouse)	[48, 49]
Dissolving	Vitamin K deficiency	*In vitro*	[55]
Coated	Warts	*In vivo* (human)	[34]
Coated	Immunity (vaccines)	*In vivo* (mouse)	[39]
hybrid	Diabetes	*In vivo* (mouse)	[52]

Table 1.
Main studies on therapeutic delivery with MNDs. Adapted from [56].

insulin dispensing MND is presented together with main results in on-demand insulin release at physiological temperature.

Finally, we cite the engage of MNDs in effective administration of small peptides, vitamin K and mRNA administered, both *in vitro* and *in vivo* studies [53–56].

In **Table 1**, main studies on therapeutic delivery with MNDs are summarized. Another important issue is the integration of MNs in optical, microelectronic or microfluidic devices. In [6], authors present the proof-of-concept of an optical integrated MNDs based on polymeric MNs and porous silicon (PSi) for transdermal drug delivery (**Figure 5**). Since its surface can be chemically modified, PSi is one of the most popular porous material used in drug administration [57]. Moreover, PSi structures have a tunable refractive index that depends on their porosity [58]. The MND presented in [6] is based on PEGDA hydrogel MNs and includes a PSi free-standing membrane with a Bragg mirror optical structure, i.e. an optical structure that reflects a specific wavelength (color) in the visible spectrum. Furthermore, the Psi membrane not only acts as a drug/biomolecules reservoir, but also it can be used to optically monitor the released drug, since the reflected wavelength changes with the emptying of pores (**Figure 5**). In [6], the integrated-chip optical device guarantees the optimum disposable MND, which can be self-administrated and self-wasted, once the drug has been all delivered by only looking at the color variation at naked-eye.

4. Biosensing with microneedles

Human interstitial fluid (ISF) is on average between 9 and 13.5 L [59, 60]. Fluid moves from the lymphatic vasculature into the interstitium, among the endothelial

walls of cells, then to the blood plasma, and finally returns to the lymphatic vasculature. Analytes enter into the ISF through three paths: first, by transcellular path, through the capillaries; secondly, by paracellular path, through the cell walls; finally, by vesicular path, from the cells to the ISF [61, 62]. ISF moves within a network of glycosaminoglycans, elastin, and collagen and transports electrolytes and metabolites to muscle cells, bone cells, cartilage, tissues, organs and so on [60, 63].

Dermal ISF is localized in the extracellular spaces between the vasculature, connective tissues and the cells. A lot of research efforts have been done to develop extraction methods of ISF in order to obtain an analytical composition and understand the relationship between plasma and ISF. **Table 2** summarizes the main ISF constituents, measured concentrations, and typical concentration ranges for healthy people [63].

Since its location just under human skin (the largest human organ) and its relationship with the vasculature system, analysis of ISF has received interest for the realization of new wearable devices.

On the other hand, new diagnostic methods can sensitively, rapidly and accurately detect, analyze and monitor relevant diseases of social interest, and can lead to an effective management of healthcare. Biomarkers and biosensors research receive, then, a constantly increasing thrust.

Despite the transduction method used, innovation in standard sensing technologies is continuously pursued. Although several optical techniques, such as fluorescence, surface plasmon resonance and surface enhanced Raman spectroscopy have been exploited, electrochemical methods, based either on voltammetry or impedance spectroscopy, have been demonstrated to quantify analytes in ISF with high sensitivity and easily integration into a MND [8].

Standard electrochemical sensors are realized confining bioprobes onto an electrode surface directly immersed in a solution, as the ISF. A key issue in the innovation of electrochemical devices is the design of the so-called working electrode, that can increase the performance of the whole biosensor. The development of electrochemical engineered biosensors has been recently the focus of many research groups, which provided several fabrication strategies [64].

Electrochemical sensors based on MNs can analytically monitor biomarkers, drug release, metabolites, electrolytes and other chemical species present in dermal ISF and involved in biological functions. Recently, in [65] authors gave a proteomic characterization of the dermal ISF, extracted by means of a hollow MND. In this work, 407 proteins have been found and quantified [65]. Moreover, less than 1% of these proteins have been identified only into the ISF, confirming that the ISF is strictly connected to both plasma and serum. Then, the MNDs can be minimally

ISF constituent	Measured concentration	Typical concentration ranges
Glucose	4–8 mM	4.5–8 mM
Cortisol	24–40 nM	Morning: 1–50 nM Afternoon: 27–42 nM
Lactate	1.17 ± 0.23 mM	1–2 mM
Lipids	1.5 ± 0.3 μM	Not reported
Na+	141 mM	135–150 mM
K+	4.4 mM	3.8–4.9 mM
Cl–	110 mM	99–117 mM

Table 2.
Main ISF constituents, measured concentrations and typical concentration ranges for healthy people [63].

invasive alternative devices to blood-derived fluids sensors with potential for real-time monitoring applications. In addition, in [66] an extremely small quantity (<1 nL) of the ISF was extracted by means of a hollow MN to measure drug concentrations and the typically painful blood drawn was avoided. In [66], the inner cavity of a hollow MN was derivatized to bind vancomycin. Optical absorbance is used as off-line transducer method, after extracting ISF with an integrated optofluidic device. The optofluidic MND detected the vancomycin in a sample volume of 0.6 nL with a limit of detection (LoD) of less than 100 nM.

Before being widely adopted into clinical practice, MNDs used as biosensors have to pursuit some general issues: a low cost fabrication; continuous monitoring and/or long-lasting working time; the possibly of integration in MEMS; the protection of the bioprobe, critical in enzyme-based detection; a good electrical conductivity (EC) for electrochemical sensing [67]. Moreover, the biofouling at the tissue–device interface must be avoided to successfully realize a wearable MND sensor [68]. According to Da Silva et al., currently, wearable sensors are still not yet ready for commercial develop, but within a few years MNDs biosensors will conquer the market [69].

In the field of MNDs for diagnostics, as well as for therapy, the approach can drastically vary with shapes and materials; **Figure 1A** shows a sketch of different MNDs for sensing purposes together with their working conditions into the human skin. Starting from the right, **Figure 6** reports: swelling bulk MNs sensors (BMNDs), whose diagnostic approach includes a volume effect in the probe-analyte interaction that will be considered separately; hollow MNs sensors (HMNDs), where a small material sampling of ISF is analyzed on or offline [70–72]; coated MNs sensors (CMNDs), whose surface is the locus of the interaction between analytes and bioprobes [73–81]; planar MNs sensors (PMNDs), where the probe–analyte interaction is on a specific zone of a flat MNs surface [82].

The bulk volume of solid MNs (BMNDs) is often exploited in electrochemical biosensing. Usually, hydrogels and swelling polymers are employed in the fabrication of BMN. Examples are polyethylene glycol diacrylate (PEGDA), polyvinyl pyrrolidone (PVP), polyvinyl alcohol, poly(acrylic acid), poly-l-lactide,

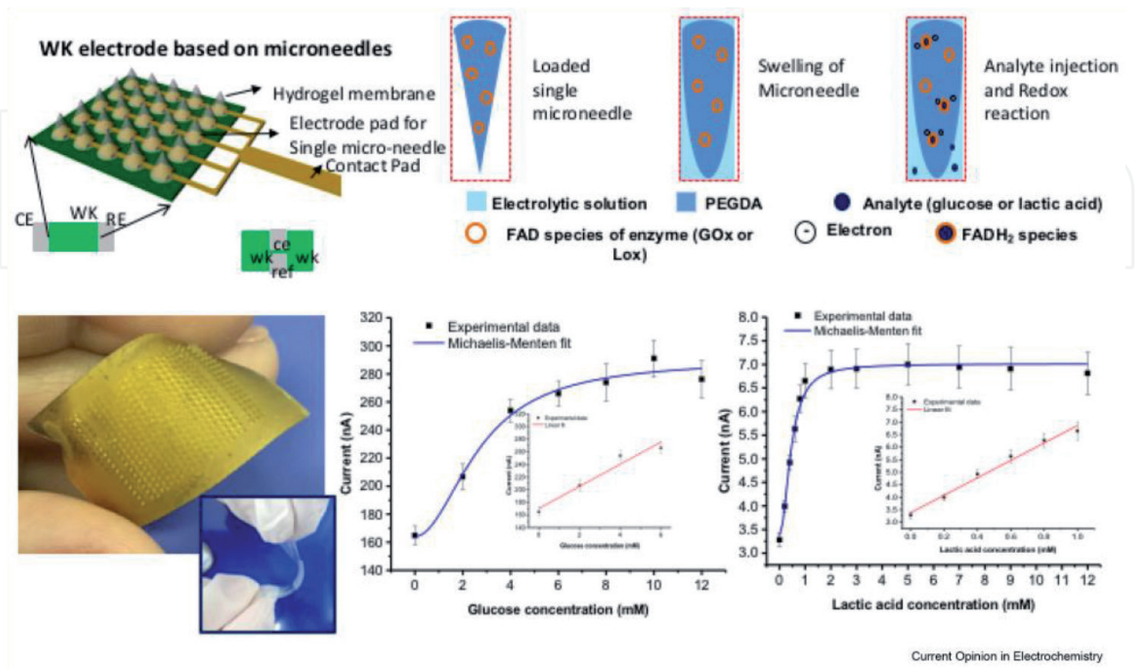

Figure 6.
Design of the working electrode, optical images with and without metal coating and sketch of working of the MNDs. Experimental data for glucose and lactate acid dose–response. Reproduced with permission of Ref. [8]. PEGDA, polyethylene glycol diacrylate; FAD, flavin adenine dinucleotide.

poly(lactide-coglycolide acid) and poly-N-isopropylacrylamide [83, 84]. These types of polymers can be processed by several fabrication techniques, such as replica molding, photolithography, drawing lithography and more [85]. Usually, probes and enhancers of transduction mechanisms are directly embedded in the porous polymer matrix during the fabrication. This environment protects probes without avoiding interaction between target analytes and bioprobes.

Caliò et al. trapped enzymes with vinyl-ferrocene mediator into a polymeric matrix of PEGDA in order to detect glucose and lactate exploiting the volume effect of the hydrogel matrix [27]. After being in contact with the ISF, the PEGDA matrix swells and the analytes solved into ISF enter the volume of the MNs, where a large number of probe molecules (enzymes) can be stored. The redox reaction takes place inside the volume and is transmitted to the electrode. The fabrication of the electrochemical MN biosensor only required a single further step (metal coating) in addition to the direct photolithographic process. The hybrid device traps GOx and LOX enzymes to enable the electrochemical detection of glucose and lactic acid, respectively, in physiological solution. The sensing MND showed a linear response from 0 to 4 mM for glucose, and from 0 to 1 mM for lactic acid (**Figure 6**) and a LoD of about 1 μM was found for both cases. **Figure 6** shows design of the MN based working electrode, optical images with and without metal coating and a sketch of the working principle of the swelling MNDs. Moreover, experimental data for glucose and lactate acid dose–response are reported.

5. Conclusions

Appeared on scene as a painless alternative to syringes, MNDs have conquered the biomedicine. The flexibility of these innovative devices makes these technological platforms really attractive for even new fields of application. Almost all materials can be used in the fabrication of MNDs: noble metals (gold and silver), semiconductors (silicon), plastics (polymers and hydrogels), amorphous materials (ceramics) and artificial nanostructured materials (porous silicon). MNDs have been used for drug delivery, cosmetic industry or biosensing, where the MN microstructures have been used as electrodes for electrochemical transduction. For biosensing systems, pros and cons have been highlighted for each device type in terms of analytical performances such as LoD, detection time, sensitivity and so on. In all the application cases, considerations about the safety of MNDs is due, since MNDs are conceived for being in contact with the human body. Then, inert, biocompatible, or physiologically dissolvable materials have to be engaged for device fabrication, even if they show lower analytical or delivery performances. After the overcoming of the skin natural barrier, MNs are directly in contact with human ISF. Hollow, coated, and swelling MNDs are all used in two ways: sensing of analytes and delivery of drugs; biosensing and administration; therapy and diagnosis.

Conflict of interest

The authors declare no conflict of interest.

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