A REVIEW ON TRANSDERMAL DRUG DELIVERY USING MICRONEEDLES: CURRENT RESEARCH AND PERSPECTIVE

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Abstract. *The paper presents a review on transdermal drug delivery using mechanical enhancer – microneedles. The paper overviews the delivery mechanism, the main delivery methods utilizing the microneedle array, the frequently used materials for the fabrication process, geometrical and shape considerations as well as the current pre-clinical and clinical applications of the microneedles array. Finally, we express our point of view regarding the perspectives in the field of transdermal drug delivery using mechanical enhancer.*

Keywords: transdermal drug delivery, microneedles, vaccine

1. Introduction

Currently medical professionals have a wide range of drug administration modalities to choose from and individualize the therapeutic schemes according to patients' needs. Oral route is one of the preferred methods of drug delivery owing to its non-invasive nature. However, the major disadvantage of this method is the impossibility of adequate peptide or protein delivery caused by the highly acidic gastric secretions, the first-pass effect of the liver or the intestine. These aspects, which contribute essentially to a lower bioavailability of the administered drugs are related to the alteration, destruction or reduced absorption of the macromolecular drug. [1, 2] One classical example is the one of Insulin, which cannot be administered orally leading to the need of a parenteral route and to negligence in treatment. [3, 4] Although the preferred delivery method is the oral route due to its non-invasiveness nature, the inability to achieve a maximum bioavailability of protein or peptide macromolecules has been considered when

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evaluating the diseases which depend on chronic medication. An alternative to orally administered drugs, the inhalable drugs, was considered despite the poor absorption of polar compounds. [5] Eventually, the parenteral route has been considered close to perfect pharmacologically, although disadvantaged by its association with pain and poor patients' compliance caused by the invasive character of the method. Out of the valid administration routes currently used in the medical field, the one transferring the medicine across the skin, transdermal drug delivery [6-9] is more and more investigated to be constantly improved. It is known that the application of preparations to the skin for medical purposes is as old as the history of medicine itself, with references to the use of ointments and salves found in the records of Babylonian and Egyptian medicine. [10] In modern times, Dimethyl sulfoxide was the first drug delivered through the skin in 1900, followed by nitroglycerine ointment introduced in 1954, for the management of angina. The process continues from one transdermal product approved by US Food and Drug Administration (FDA) in 1981 [11] to more than 20 in 2012. [12]

Since the process of approval is a complex and full of responsibility one, both advantages and disadvantages were continuously assessed so that a balanced system could be developed and used successfully. Consequently, microfabrication techniques emerged, and progressed rapidly and contributed actively to the development of novel and trustable drug-delivery systems. The outcomes of such technological evolution include micro-fabricated micro-particles, microchips, and microneedles, which have the potential to make significant contributions to the field of drug delivery through superior structural, mechanical and electronic properties. Micro- and Nano-technology started to have a huge impact on the development on new and sophisticated bio-medical devices [13-15], Micro-total-analysis-systems (μTAS) and laboratories-on-a-chip conferring advantages such as the small dimensions, easy to use, transportability and a little amount of sample required to be analyzed. In this respect, the "hot topics" were related to: manipulation and characterization of small quantities of biological samples [16-18] or to microfluidic approaches for tissue engineering. [19-22] These two targets represented the pivots of microfabrication domains, which will continuously support the drug screening viewed as a critical point in pharmaceutical industry. The microfabrication technology will contribute through the role it plays in reducing the costs and the time required from drug concept to drug marketing [23] their potential created further opportunities.

Consequently, administration of drugs started to be a new field where micro and nano-technologies confer modern alternatives. [24, 25] One significant example to highlight the technological advances are the microfabricated devices for transdermal drug delivery (TDD) which are recognized as a promising alternative to introduce medicine into the human body.[24, 26] This is possible due to distinct

advantages such as painless self-administration, high compliance (pediatric patients), gastrointestinal tract shortcut with no first-pass effect on the administered drug, uniform diffusion of delivered compound, controlled-release and cell-targeted delivery, low cost, and reduced risk of blood-borne diseases. [6, 27-30] Transdermal products, administered with or without incorporating permeation-enhancers [31-33] are already at diverse stages of formulation and clinical development with specific applications such as cardiovascular disease, Parkinson's disease, Alzheimer's disease, depression, anxiety, attention deficit hyperactivity disorder (ADHD), skin cancer, female sexual dysfunction, post-menopausal osteoporosis, and urinary incontinence were evaluated. In this direction, the pharmacokinetics principles were considered: the therapeutic efficacy of a locally applied drug mainly depends on its ability to penetrate and permeate the skin. [7, 31, 34-38] However, the technology is still to be perfected and limitations of the TDD to be addressed [7, 34, 38-42]:

- the low efficiency caused by the poor permeability of the SC for hydrophilic or large molecules [6, 7, 40, 43-45],
- the reduced diffusion rate and little bioavailability. It would require large-scale and longitudinal clinical studies to evaluate the significance and long-term consequences of the lack of interactions with the liver and other metabolic differences between the transdermal and the oral route in the cases of hormonal dosing [46-48] or new therapeutic options, which involve cell-therapy, gene therapy. [49-51]

The present review gives an overview of the current research in the field of transdermal drug delivery using microfabricated mechanical enhancers (microneedle array). The delivery mechanism, the delivery technics, the material used in the fabrication process as well as the preclinical and clinical applications are analyzed.

2. Delivery mechanism

As previously mentioned, the delivery through the skin presents multiple benefits over oral route or conventional injection. A number of methods have been developed in order to improve drug transport across the skin such as chemical enhancers, [52] iontophoresis [53], electroporation [54], sonophoresis [55], or mechanical enhancers (microneedles array). [56]

Microneedles are innovative systems in drug delivery as they proved to be one effective method of physically disrupting the stratum corneum barrier properties. MNs can be employed to deliver hydrophilic and other large molecular weight drugs. [6, 57] The use of microfabrication technology in drug delivery offers both the convenience of affordability and of mass production. The significant positive

aspect related to the MEMS products, particularly the microneedles is that they can be especially designed for a minimal invasiveness and for a specifically programmed drug release. Since it developed as an interdisciplinary area, the maximum exploitation of concepts from several disciplines offers the specific benefits of optimization of design and functional parameters to suit the clinical requisites. Micron-scaled needles, were first proposed in the 1976 by Alza Corporation, but conceptualized and realized only in the last decade by Henry et al. [57], when microfabrication techniques came to fore. The advancement in microfabrication techniques and application of technology from the semiconductor industry to the biomedical arena has led to the innovation of microneedles. MEMS have been used as one uniquely powerful platform for delivering potent therapeutic agents. [58]

Microneedles' primary function is to create a series of transient pores in the stratum corneum and to enable large molecules to be delivered across it (mechanism illustrated in Fig.1). Fig.1 depicts the reasons for which the technique eliminates pain and bleeding. The microneedle are long enough to perforate the stratum corneum and sufficiently short to reach the capillary blood vessels and nerves. Once the pores are generated in the stratum corneum the drug is applied and the drug molecules will diffuse towards the underlying subcutaneous tissues and eventually will be absorbed and distributed systemically. In this way, the microneedles are actually improving the pharmacokinetics of molecules for which absorption through passive diffusion is impossible. Consequently, encouraging results in delivering proteins, peptides and vaccines were reported. Since most novel therapeutic strategies/ agents are proteins or peptides based, there is a need for such minimally invasive yet efficient drug-delivery systems to increase the bioavailability of the drugs administered. However, the applicability of the drug-delivery systems like microneedles is only possible after the detailed research concludes their fabrication process and their physical properties, which is the actual stage in their development.

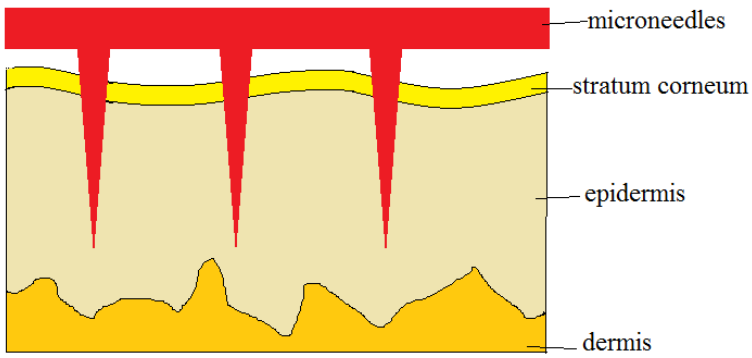

Fig. 1. Microneedles can create pathways into the skin for drug delivery and are painless due to their size.

Starting from the working principle previously mentioned four different strategies for TDD using a microneedles array were established: "poke and patch," "poke and released," "coat and poke" and "poke and flow." The first three methods involve solid needles (Figure 2a), while the last one make use of hollow microneedles (Figure 2b). We further analyzed the limitations, advantages and disadvantages of each method.

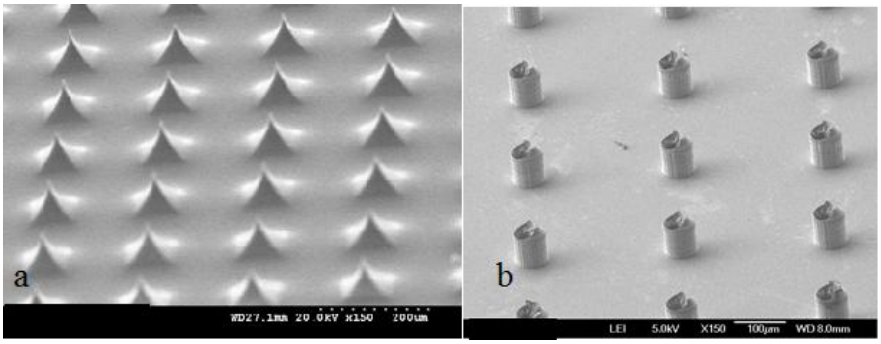

Fig. 2.(a) Solid microneedles and (b) hollow microneedles

The "Poke and patch" approach is depicted in Fig.3 and consists of two distinctive steps.

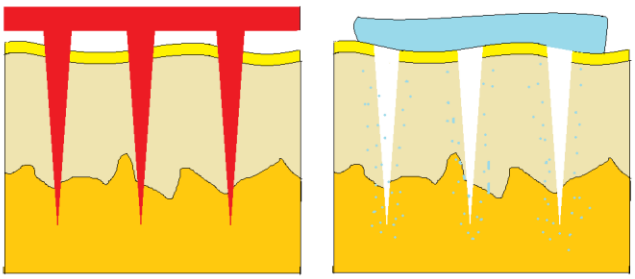

Fig. 3."Poke and Patch" method for transdermal drug delivery.

First, solid microneedles are inserted into the skin to pierce the stratum corneum. They create micro-conduits for the drug to reach the deeper layers of the epidermis. [57] Second, the drug is applied on the skin and diffuses through the generated channels. The drug-delivery rate is strongly dependent on the pore size generated into the skis as well as on the concentration of the drug. The method has the advantage of simplicity but is less efficient in terms of the percentage of the drug diffused into the skin, the dosage being difficult to be controlled. The micropores generated in the skin remain open from three days up to one week when they are exposed to occlusive conditions. [59, 60] The main disadvantage of this last approach is that longer opening time of the pore can cause infections. A variation of this method is "scrape and patch." In this case, the microneedles array is placed in contact with the skin and scraped multiple times against it. Thus, MNs produce microabbrasions before the patch is applied. [61]

Another strategy is called "Coat and poke" (Fig.4). It involves coating the surface of the microneedle with the drug to be delivered in a dip coating, roll coating, or spray coating manner. This approach allows the drug that coats the microneedles to dissolve, diffuse locally and to distribute into the systemic circulation after the MNs' insertion into the skin. [62] However, the rate of the drug delivery is limited by the thickness of the layer that covers the microneedle. A thick coating not only can decrease the sharpness (making the penetration process problematic), but it can reduce the adhesion of the drug on the needle surface. Thus, the coating layer detaches. The advantage of the method relies mainly in a more precise dosing of the drug, but the method requires a well establish coating procedure while the drug dose remains small.

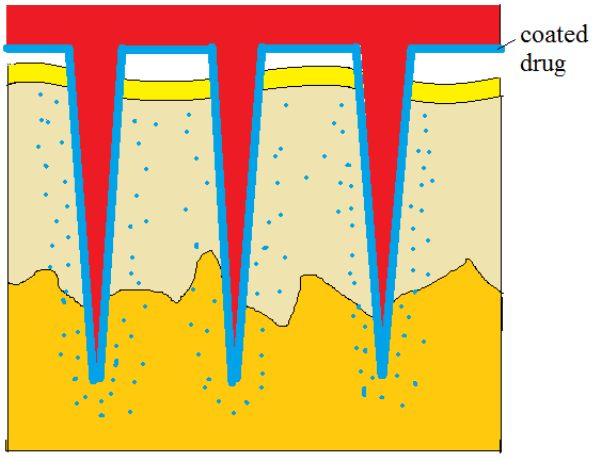

Fig. 4."Coat and poke" method for transdermal drug delivery.

The third method, "pokes and release," involved bulk "dissolving microneedles." [63] Dissolving microneedles are among the latest strategies of TDD developed in their attempt to reach the best possible delivery process [62] being facilitated by the variety of materials and design. This strategy allows a controlled release of the drug as the microneedles are fabricated from a biodegradable polymer and the drug itself in encapsulated with the polymer. Once the microneedles are inserted into the skin they dissolve and the drug is delivered and diffuses further. [64] The method is depicted in Fig.5. The advantage of the method consists in a precise dosing and a small quantity of drug that can be lost during the encapsulation process. The disadvantages are that the microneedles cannot be very sharp (a decrease penetration ability), and that the drug dose is little.

An alternative version of the method is the use of porous microneedles. The porous structure can absorb the drug solution and release it into the skin. [65] In this case the disadvantage arises from a relatively fragile structure of the microneedles.

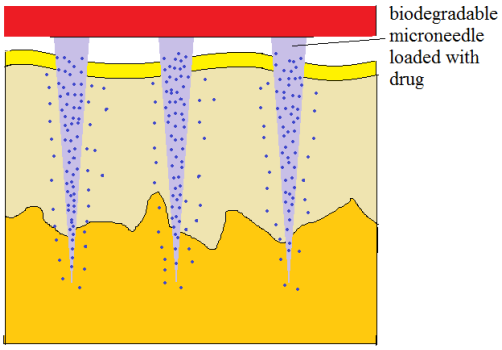

Fig. 5."Poke and release" method for transdermal drug delivery.

Last method known as "poke and flow" (Fig.6) is, in fact, a microinjection and was designed to deliver the drug through injection. It requires the design and fabrication of holes at the center or side of the needles to allow the drug molecules to be reach the inner layers of the skin. This method differs from the previous ones, which employed solid (non-hollowed) microneedles. [66] The delivery process is through the microneedle bore. The challenge is tuning between the applied pressure and the penetration depth, because once the microneedle array is removed there is the risk of back flow due to the insertion of the liquid very close to the skin surface. There is also a risk of clogging, and leakage, because such devices are more complex. A main advantage is that the diffusion point is inside the skin structure, closer to the capillary blood vessel, increasing drug absorption. The reported infusion rate was in the range of 50-300nL/min. [67]

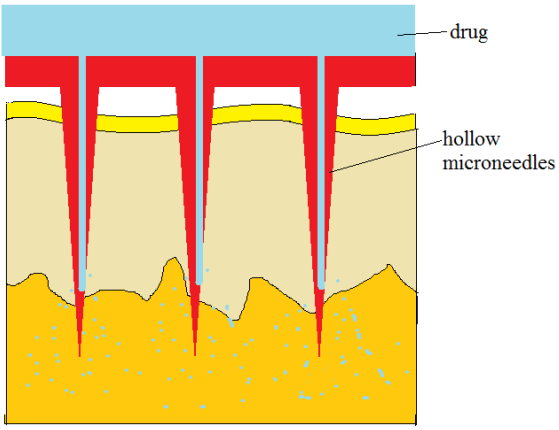

Fig. 6."Poke and flow" method for transdermal drug delivery.

Since these various methods employ the two major types of microneedles, the studies focused on the perfect geometry and mechanical characteristics [68], on the best material and on the cost-effective TDD process. [69-73]

3. Materials

Microneedles have been fabricated previously from silicon, metals, glass, zeolite, polymers and sugars. Studies research continuously the various possibilities to reduce the complexity of the fabrication processes, to increase the mechanical strength and to improve the geometry of the needles.

Based on the materials used in the fabrication process, microneedles can be categorized in two main groups: non-biodegradable and biodegradables microneedles. However, few reports are related to the combination of degradable and non-biodegradable materials. [74, 75]

Silicon is, in fact, the original material used in the fabrication of microneedles, Henry et al. reported the first silicon MN in 1998. [57] The main reason of choosing silicon was the developing of microfabrication techniques, which allowed precise and controllable 3-dimensional structures. These structures were manufactured by well-established and very well-controlled fabrication processes characteristic to microtechnology. Moreover, once these technologies move to mass production the total cost of the fabrication can drastically reduce at few cents per chip. Silicon has been widely used in TDD studies [71, 72, 76, 77] to fabricate microneedles of varying heights, shapes and densities using improved processing methods. [57, 69-71, 73, 74, 78, 79] Unfortunately, silicon is a brittle material and the risk of breaking during insertion into the skin is very high. An example is presented in Fig.7, which depicts one SEM image of a broken tip of a Silicon MN, after the insertion into the pig skin. Since the biocompatibility was addressed continuously to increase the employability of such microneedles, biodegradable porous silicon was used as it is well-known for its bioactive and bio-degradable properties. [65]

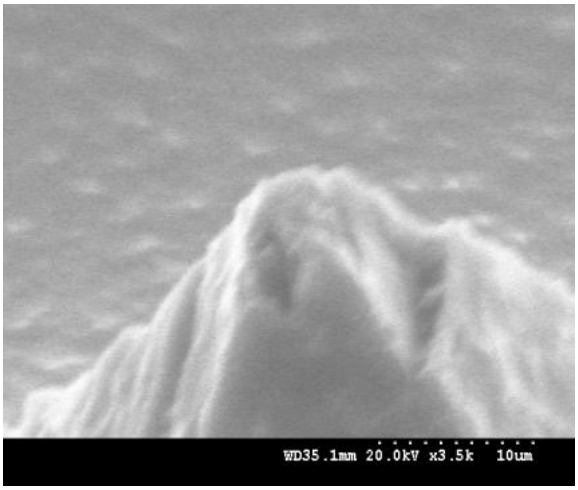

Fig. 7. SEM image presenting the tip of a broken microneedle after insertion into a pig skin.

Metal microneedles belong to the group of non-biodegradable MN. They are an option, which avoids brittleness of the silicon, keeps the scalability for mass fabrication given the microfabrication processes. Several metals have been used to fabricate MN from: stainless steel [62, 80], palladium [81], titanium [82], and nickel. [83, 84] Metals present the risk of generating bio-hazardous sharps whose disposal is a global alarm and houses the risk of transmission of diseases such as HIV AIDS, if the needles are accidentally or incidentally reused. Cases of immune-inflammatory responses have also been reported with stainless steel and titanium implants. To address these issues, ceramic and glass MNs have been fabricated. [85-87]

Diverse polymers have been used: polyvinyl acetate [88], carbomer [88], poly-etherimide [89], polycarbonate [90], polyethylene glycol [91], polyvinyl-pyrrolidone [92], poly (vinyl pyrrolidone) [93], its co-polymer with methacrylic acid [93], and poly-lactide-co-glycolide. [94] Sugars and sugar derivatives like dextrose [95], maltose [96], galactose [97], carboxymethyl-cellulose [64], and amylopectin [64] have also been used for fabricating microneedles. These materials are biocompatible, cost-effective and generate no bio-hazardous waste. Park et al. fabricated microneedles from poly (lactide-co-glycolide) incorporating calcein and Texas Red labeled albumin using a micro-moulding technique. [94] Lee et al. fabricated microneedles from carboxymethylcellulose, albumin and amylopectin. The model drugs sulforhodamine B, albumin and lysozyme were either incorporated in the needles or in the backing for bolus or sustained release respectively [64]. Sullivan et al. designed a novel room-temperature-moulding-technique, especially for biomolecules. [93] The process involved in-situ- polymerization of monomer vinyl pyrrolidone for encapsulation of albumin. Ito et al. used another simple process to fabricate microneedles from dextrin. [98] Kolli et al. used maltose microneedles to study the permeation characteristics of nicardipine hydrochloride across hairless rat skin and observed an increase as compared to passive diffusion. [99] Rapidly dissolving fibroin MNs, containing methylene blue as a drug, provided more benefit than conventional syringes for a painless transdermal-drug delivery. Polydimethyl-siloxane (PDMS) is often used in prototyping biomedical devices. [100] It is also utilized as a moulding material for MN. MN devices fabricated from these materials contributed to the progress in the field. However, limitations remain, such as high-temperature processing, quick dissolution of the MN resulting in less control over drug release, instability under humid conditions, UV cross-linking requirements, and drug materials/process incompatibility that can impact the potency of the compounds to be delivered. Various studies have shown the utilization of silk biomaterials for the release of different therapeutics in a controlled manner from the silk matrix. Utilization of silk as a biomaterial for transdermal drug delivery, with adjustable mechanical properties and drug release, adds control to this drug-delivery technology. [101, 102] Silk is a remarkable protein biopolymer that has gained attention for biomedical devices, biological implants, drug-delivery systems, cell cultivation substrates, gels, and

photonics materials [103-106] due to its robust mechanical features, biocompatibility, controllable processing in aqueous conditions, as well as its ability to be shaped into various formats. [107] To evaluate the MN from mechanic point of view, laser-engineered-dissolving-microneedle (DMN) arrays fabricated from aqueous blends of 15% w/w poly(methylvinylether-co-maleic anhydride) were used for the first time by Gomaa et al. They wished to demonstrate the array's mechanical strength and drug bioactivity. [108] In the meanwhile biodegradable sugar glass microneedles were fabricated following optimization of a simple and novel low-temperature-vacuum-deposition-micro-moulding methodology. [109]

With a plethora of materials to choose from the research in the field progresses towards the balance between the factors that influence the success of the MN as TDD. The next aspect to be discussed is the design of the devices as MN fabrication may vary according to the clinical purpose.

4. Microneedle's dimensions, shape and geometry

Since 1976, when the concept of micron scale arrays that transiently breach the stratum corneum emerged, the fabrication evolved. The MN progressed from the model manufactured by Henry et al. as extremely sharp tips 150 μm long silicon microneedles [57] to the hollow out-of-plane silicon microneedles presented by Stoeber and Liepmann. [110] Presently, there is a multitude of geometries to address specific applications. Therefore, the shape, geometry and the dimensions of the microneedles need to be address.

As Fig.1 depicts, the microneedles exhibit certain geometry: length, tip diameter, base diameter, needle-to-needle spacing and array dimensions. In practice, the variations in the microneedles' geometrical aspects have been implemented during fabrication to perfect the outcome and the TDD efficiency. Microneedle length can be tuned to deliver the drug to specific sites in the skin, especially vaccines that utilize skin's immune system, which houses a large population of Langerhans cells and dermal dendritic cells. [111] As such, they can be tailor-made: they can be long enough to breach the stratum corneum, but short enough not to stimulate the nerves in the underlying dermis. Such geometry had direct applications when MNs were a proven painless drug administration device in human volunteers [27] and when other studies showed that MN had higher immunogenicity than intramuscular injection at a lower immunogenic dose of the influenza vaccine. [111]

Beveled tip microneedles [112], side-opened out-of plane microneedles [66], hollow-out-of-plane microneedles [110], sharp-tipped-hollow microneedles [113] have been fabricated and their mechanics assessed. Generally, two main types of MN are used: solid (non-hollow) and hollow microneedles. Another classification can be from manufacturing perspective: in-plane (Figure 2a and 2b) or out-of-plane (Fig.8).

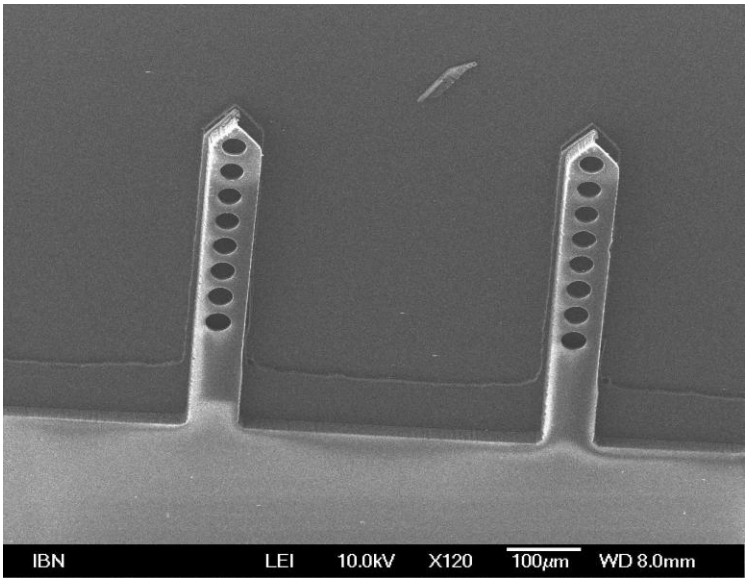

Fig. 8. SEM image out-of-plane microneedles.

Solid microneedles, (Fig.2a), especially metal ones, are easier fabricated, they are stronger and sharper than the hollow microneedles. Solid MNs have been fabricated from several materials such as stainless steel [62, 80], palladium [81], titanium [82], and nickel. [83, 84] When using the solid MN, specific strategies may be approached: "Poke with patch," "Coat and poke," "Dip and scrape," or "Dissolving microneedles," to increase their versatility.

Hollow microneedles (Fig.2b) as compared to the solid ones are used in microinjection method and require the design and fabrication of holes at the center or side of the needles to allow the drug molecules to be reach the inner layers of the skin. [66] Furthermore, another device, which incorporated polycarbonate hollow MNs array attached to a drug reservoir demonstrated the sustained release of small-molecule drugs and proteins during animal tests and discussed the opportunity for drug-self-application devices. [90] As observed in the Fig.2b, the design of the hollow microneedles allows the delivery of the drug. However, it reduces the sharpness and affects the penetration through skin's SC. Such observation was at the center of the ongoing research studies that addressed this imperfection and led to the new design of microneedles with the openings at the side of the needles. [84] Once the delivery-related aspect was solved, new ideas came across to address the ability of the needles to penetrate the SC: the initially closed tips could be designed and fabricated to open upon insertion into the skin if they are made of polymers, which can dissolve or if the necessary pressure is exerted to make this fracture possible. Wang et al. proposed the use of mechanical vibration to enhance the insertion of hollow microneedles. [85]

The skin reaction to MN has also been evaluated to consolidate the side effects besides the irritation or allergic reaction. Gupta et al indicated that all microneedle treated sites recovered barrier properties within 2-40 h, depending on microneedle geometry, presence or absence or subsequent removal of occlusion. [114] The results showed that in the absence of occlusion of the application site, skin reseals relatively rapidly within a variable time frame of 15 min to 4 hrs [114, 115], if compared to the plastic film- or solution-related occluded application site, which reseal slower. In such cases, the life time of the microchannels is much longer, 3-40 hrs, and it depends on the needles' geometry. However, there are still limited studies available on the mechanisms of restoring the skin barrier function following microneedles treatment.

In conclusion, the microneedles' versatility consists in their ability to be tailored for particular applications in association with other TDD enhancers [116, 117] to be used to control drug's pharmacokinetics [102] thus they a bring significant benefit as a platform material for transdermal drug delivery. [118] The direct application of the MN geometry was demonstrated by Kaushik et al. when they associated the MN geometry to the skin geometry and proved MN as painless drug delivery devices in human volunteers. [27] Other studies showed that MN had higher immunogenicity than intramuscular injection at a lower immunogenic dose of the influenza vaccine. [111]

5. Preclinical applications

Since their design and inception as viable medicine administration tools, the use of microneedles was explored intensively, both in vitro and in vivo. Recently, the use of micron-scale needles in increasing skin permeability has been proposed and shown to dramatically increase transdermal delivery, especially for macromolecules. There is a positive believe that MN will add to the existing commercially available transdermally delivered testosterone, estrogen, fentanyl, scopolamine, clonidine, nicotine, and oxybutynin. [119-121]

Since the efficacy of a drug is essentially influenced by the drug concentration reaching the targets, one important step is to analyze the permeability to various molecules to understand the range of drugs, which can be easily transported. In this direction, arrays of polymer microneedles were shown to increase permeability of human cadaver skin to a low-molecular weight tracer, calcein, and a macromolecular protein, bovine serum albumin, by up to three orders of magnitude. Altogether, these results indicate that biodegradable polymer microneedles can be fabricated with an appropriate geometry and sufficient strength to insert into skin, and thereby dramatically increase transdermal transport of molecules. [112] the investigations focused on both the ability to transport across SC and on the speed of delivery. Gill and Prausnitz reported one micron-scale dip-coating process and a coating

formulation designed to produce uniform coatings of compounds reliably. They proved that calcein, vitamin B, bovine serum albumin and plasmid DNA (microparticles of 1 to 20μm) dissolve rapidly (within 20sec) without being wiped off during insertion in porcine cadaver skin. [62]

Furthermore, dissolving microneedles were designed to encapsulate molecules such as sulforhodamine B, bovine serum albumin, and lysozyme, These molecules dissolve within the skin for bolus or sustained delivery and leave behind no biohazardous sharp medical waste. This type of MN facilitated the molecules' diffusion into the skin via channels formed by the dissolved microneedles. [64] The safety aspect is one important factor that maintains the capacity of microneedles as TDD systems and was considered when vaccines were delivered through coated microneedles. Safety meant the long-term stability of influenza vaccine-coated microneedles and the immunogenicity induced and maintained. These two aspects were evaluated and the coating process proved as important factor influencing long-run stability of influenza vaccine-coated microneedles [122] and implicitly the vaccine immunogenicity which eventually is the most important outcome in case of vaccines. Moreover, when compared the kinetics of TDD with the IM injection of the influenza vaccine *in vitro*, using densely packed microprojection arrays, valuable inputs regarding the peak-serum-antibody-response were reported. The results showed that TDD matches the kinetics achieved by intramuscular injection of liquid vaccine while not interfering with the long-lasting antibody response. [123] In conclusion, the *in vitro* experiments open the clear pathways for the next steps of the preclinical evaluation of the MN. *In vivo* studies are essential as preclinical evaluation of drug delivery.

In vivo-studies have demonstrated delivery of oligonucleotides, reduction of blood - glucose level by insulin, and induction of immune responses from protein and DNA vaccines either by the microneedles alone or in only few studies association with other enhancers. [40, 124]

One of the most used and valuable polypeptide is Insulin. [80, 125, 126] To assess the potential of transdermal delivery in its case, two-layered dissolving microneedles containing an intermediate-acting insulin (protamine sulfate insulin) were ready for a pharmacodynamics study in rats and compared with subcutaneous injection preparations. Since no significant difference of hypoglycemic curves was found between dissolving microneedles and injection solutions, the study suggested the usefulness of two-layered dissolving microneedles of protamine sulfate insulin for the displacement therapy of subcutaneous injection preparation. [127] Other in vivo studies focused on the developed hollow microneedles insulin to microinject the drug to diabetic rats to evaluate the efficacy of the method itself. [124] Besides diabetes, immunization plays an important role in epidemiology with the scope of prevention infectious diseases through health promotion programs around the world, especially

in the underdeveloped or developing counties. Since vaccine delivery was already demonstrated in vitro, the similarity between the kinetics of TDD through MN and intramuscular injection of liquid vaccine were brought forward to the in-vivo studies. The scope was to make possible a rapid vaccination with an adequate immunizations because the time window between the availability of appropriate antigen and the start of the seasonal epidemic is very short. [123] Another time-dependent procedure is renal dialysis, and microneedles have been fabricated for micro-dialysis. Other experiments besides microdialysis were conducted to obserce the delivery of fluorescein, fluorescently tagged dextrans (40 and 250 kDa), bevacizumab, and polymeric particles (20 nm to 10μm in diameter) through MN. These substances were administered into the suprachoroidal space of New Zealand white rabbits. The results presented this method as a simple and minimally invasive way to target the delivery of drugs to the choroid and retina for macular degeneration. [128] Therefore, the observations will contribute to the new approaches to these two seriously debilitating medical conditions.

One more way to demonstrate the usefulness of the microneedles is to evaluate their ability in diagnoses medical conditions for a better understanding of the pathological and physiological conditions of human subjects. A first technological assessment of a novel type of microneedles-based dry electrodes proved that these electrodes are a promising alternative to standard-wet-electrodes for the recording of biosignals in clinical examinations because they:

- seem to allow a better electro-mechanical interface with human skin,
- have performance comparable to wet electrodes in recording EEG, EMG and static ECG signals, showing an improvement in the monitoring of ECG signal in dynamic conditions, and
- do not need the long-lasting skin preparation as wet electrodes for EEG applications, appearing easy to use and to administer. [129]

This adds to the reports that show the successfully used microneedles to extract biological fluids for diagnosis of diabetes. The efforts converge towards a better medical approach for diabetes because of MNs' flexibility in fabrication and administration. The versatility of MN as TDD system, was once more proven when a modulation of low molecular weight heparin (LMWH) multidose therapy with single-step 'poke and release' approachlaser-engineered dissolving microneedle (DMN) arrays was demonstrated. Such technique allowed the control of the DMN array variables (MN length, array density, and application force) to meet various clinical requirements including adjustment for body mass and renal function. [108] Another example to sustain the above versatility of microneedles was the development of a microneedle-based multiplexed drug delivery actuator that enables the controlled, alternate and rapid delivery of multiple therapeutic agents through the corresponding microneedle channels. This application aids in the rapid administration of multiple

therapeutic agents and indicates the potential to counteract diverse and concomitant biomedical conditions. [130]

To further demonstrate the applicability of microneedles to protein delivery a transdermal patch containing microneedles coated with salmon calcitonin (a peptide drug) was used in vitro and in vivo as an alternative to traditional subcutaneous and nasal delivery routes. Results of approximately 13 times higher AUC for the microneedles-administered-protein compared with the nasal regimen showed that with a optimization of the coating process microneedles may enable administration of salmon calcitonin and other peptides without the need for hypodermic injections. [131] Phenylephrine as a local application to the anal sphincter was evaluated as a novel treatment for fecal incontinence. [132] BD Medical-Pharmaceutical conducted a clinical trial on the use of the rabies vaccine delivered through BD 34G hollow microneedles. [133]

Various other molecules have been used as drug models in the studies: sex-steroids for hormone replacement therapy and contraception [134] desmopressin [135], 20-merphosphoro-thioated oligodeoxynucleotides [136], ovalbumin coated onto a needle surface [137], DNA and small interfering RNA for genetic therapy, vaccines [138, 139], larger molecules and nanoparticles through solid microneedles for vaccines. [139] Moreover, Rhodamine B and black ink were delivered in *in vitro* and *ex vivo* models through 20 μm diameter microchannel integrated with a PDMS microfluidic chip [72], vitamin B and calcein, barium sulphate were administered without wiping off on the skin. [62] In the meanwhile, Collagen I which could permeate in significantly higher amounts and to greater depths in the skin [63] and could potentially provide a gateway for its enhanced efficacy as a cosmetic product to retard skin degradation in chronologically aged skin. [140, 141]

6. Clinical research and applications

MN have been proven to be painless in human volunteers by Kaushik et al. [27] and the preclinical studies consolidated the potential of MN as efficient TDD. Consequently, there are several commercial microneedles products, which confirm the ability of such devices both from the delivery and safety perspectives. Some are simple enhancers while others contain the active ingredients. There are products available for cosmetic purposes: Dermaroller the first released and marketed in Europe and used according to the length of the MN to treat a variety of skin problems from the simple increase in the skin texture to the scars and hyperpigmentation. As the name itself mentions it is a cylindrical roller to which an array of solid metal MN is attached. Besides this product, others have been promoted by various companies as cosmetics and medical applications. SPE^{TM} by Nanomed Skincare and LiteClearare some of them. The first one is a silicon-based microneedle device in form of an array of nanopins while the second is a microneedle-array-

based pen linked to the active ingredient's dispenser tube. However, both are working as SKIN Permeation Enhancers. [142] These devices are designed to be used in the treatment of acne or in the management of wrinkles or blemishes. Besides these products but also for cosmetic or dermatologic applications 3M™ Microchannel Skin System as a skin-pretreatment-device is used as it comprises polymeric MN and requires a little force to penetrate the skin up to 100µm. [143].

While non-medicated microneedles have been initially used, recently the technology answered to the clinical demands and introduced medicated microneedles for specific applications. In this direction, 3M reported the development of lidocaine coated microneedle product and showed that 3M's MTS successfully delivered drugs to the skin within seconds, and provided rapid onset of local analgesia (~ 1 minute) for routine or emergency procedures. The coating formulation developed achieved uniform lidocaine loading at target levels and the *in-vivo* application, has been followed by a readily release into intradermal space and a effective topical analgesia. Moreover when epinephrine bitartrate has been added as an adjuvant slowed the clearance of lidocaine from the skin, prolonged the local residence of lidocaine and maintained the rapid onset of action associated with lidocaine.

Patches of MN have been implemented in 2008 as medicated TDD devices for hyaluronic acid as MycroHyalaby Cosmed-pharm [144] applied as anti-wrinkle and whitening products. Also TDD devices for hyaluronic acid have been launched in 2011 by Sheiseido [145] as eye patch of 1200 MN. The product from BD, BD Soluvia™ as a prefillable microinjections system, comes more towards the medical application. This application consists of hollow MN attached to a syringe preloaded with vaccine or other medication intended for ID injection. This device is known already as the first Influenza vaccine approved TDD: Intanzaor IDfluby Sanofi-Pasteur. [146] in addition, FDA approved MicronJet600™ for vaccine administration. This system is a hollow silicon MN attachable to any conventional type of syringe preloaded with the vaccine. Consequently, MicronJet600™ is now the first TDD for ID H1N1 and which completed the worldwide flu vaccination study to demonstrate the superior immunogenicity to IM administration. [147] The first commercial MN product in the United States, an influenza vaccination for adults aged 18–64 [147] , was approved in 2011.

To support the fast progress in fabrication techniques for microneedles across academia and industry, their applicability in increasing permeability of skin, and their clinical usefulness, the approval process has to continue through clinical trials. At this point in time, some of the clinical trials have been completed some are in process. They have as scope to validate the MN as TDD to vaccines, proteins, or small molecules, to strengthen up MNs as TDD for diagnostic procedures or to monitor the compliance of the skin to the MN.

In this direction, the approved devices have been used to deliver the drugs models. Majority of the studies focused on vaccines like influenza and polio, on molecules that can be part of the osteoporosis treatment or actinic keratosis. [148] Specific examples of clinical trials include the already on the market TDD such as MicronJet600, BD Soluvia™ and 3M™ Microchannel Skin System. MicronJet600 has been further used and tested to deliver Insulin, Lidocaine, and influenza vaccine. BD Soluvia™ has been used in the diagnostic process of tuberculosis as a delivery mean of tuberculin intradermically. 3M™ Microchannel Skin System has been going through the 1st phase of the clinical trial to test for its irritation to the skin. Zosano Pharma conducted a phase I/II trial on the delivery of parathyroid hormone coated on Macrofluxmicroneedles. In a recent clinical study, microneedles were used for transcutaneous immunization and were shown to increase antibody titer levels without having any adverse effect on 20 healthy volunteers. [149]

The use of polymeric microneedles also provides an economical alternative to plastic or metallic microneedles. There are commercially available microneedle arrays from 3 MTM at approximately 1 USD per array while some newly proposed microneedles were evaluated at only 0.15 USD. Moreover, an important aspect of cost-benefit analysis is the flexibility of the fabrication so that it can be modified for a range of applications, both in drug delivery and bio sensing. [63]

7. Conclusions

The extensive body of research focused on microneedles both on fabrication and applications highlighted the main advantages of drug delivery using microneedles. We detail the advantages of microneedle-based transdermal drug delivery systems:

- They are painless alternative to hypodermic and intramuscular injections, known as invasive methods which stimulate the nociceptive receptors and sensory nerves fibers in the dermis. Microneedles deliver the drug to epidermis, which are not innervated. [27]
- They increase patient compliance as a consequence of painless administration of the medication. [27]
- The minimal patient education required, and the possibility of self-use increased the probability of successful immunization and vaccination in developing countries.
- Compared with the hypodermic or intramuscular injections the use of MNs prevents the risk of blood borne diseases: the real no-reuse of needles is possible since the polymeric microneedles are biocompatible and dissolve locally without generating hazardous biological waste.
- The risk of local infections is lower with the use of polymeric MNs, which are biocompatible and some are biodegradable.

- They allowed the topic administration of a large range of molecules, including hydrophilic such as proteins, peptides and the local or systemic distribution and the adequate therapeutic effects. [90, 150, 151].
- The versatility of application and administration as drug can be coated on the MN, encapsulated within the polymeric MN or be assisted with extra patches subsequently applied to increase permeation.
- They permit a targeted drug distribution to specific cells in the skin, e.g. vaccines delivered to antigen presenting cells like the Langerhans cells and dermal dendritic cells. [152]
- Dose delivered can be controlled easily since the administration can be terminated immediately by removing the microneedle patch from the skin.
- Dose administered can be reduced (increased drug potency) as the drug shortcuts the hepatic first-pass effect. MNs are associated with faster healing of the skin compared with hypodermic injection. [77, 114]
- They are related to an optimistic cost-benefit analysis based on the novel microfabrication techniques, with good reproducibility, high accuracy and moderate fabrication cost. [71]

However, the models are yet to be perfected as the fabrication is upon the type of application. Microneedles were produced using a variety of materials and their fabrication processes were based on a multitude of designed geometries. The result consisted in solid or hollow MN of different lengths, density in the array, or associated or not with applicators. However, will be difficult to be determining an „ideal microneedle" solution, the drug type and application will drive to correct answer to this problem.

8. Perspective

Successful transdermal drug delivery relies on the balance between efficiency of drug delivery and safety of the skin, coupled with the ease of application and economic nature of the system. This equilibrium can be achieved with a thorough design and fabrication and with comprehensive clinical research. Since it was evident that the factors that influence the success of MNs application consisted not only in the fabrication process but in the inter-variability and intra-variability caused by the intact or pathological skin and in the proprieties of the drug themselves, the body of research focused consistently on addressing the latest aspects towards strong preclinical result and further clinical trials. Generally, the challenges during clinical applications were considered in terms of low diffusion rate and low bioavailability. [7, 138, 153] Therefore, the efforts concentrated on the strategies to increase the range of successfully delivered beyond the small doses (<10 mg/day) of lipophilic and low-molecular weight drugs (< 500 Da) [45] and the approved drugs for TDD. [6, 154]

The optimistic perspectives of synergistically integrating MN with other permeation enhancers has been addressed only for few combinations such as MN and iontophoresis [116, 155] MNs and electroporation and iontophoresis, as well as with different drug carriers (e.g., lipid vesicles, micro- and nanoparticles). [156] One promising combination of permeation enhancers is sonophoresis enhanced microneedle array (SEMA) [15, 157] which integrated MNs and low frequency ultrasound and proved significant increase of drug models' delivery across skin *in vitro*. The optimistic results of focused studies on microneedles as TDD increased the confidence it their clinical potential. Microneedles-coupled-iontophoresis method was used to deliver theophylline, methylene blue, and fluorescein sodium across neonatal porcine skin *in vitro*. The optimized MNs array evaluated this combination's potential in the electrically facilitated delivery of peptide (bovine insulin) and protein macromolecules (fluorescein isothiocyanate—labelled bovine serum albumin). [116]

Nerveless, combination of hollow microneedles with "lab-on a chip" devices can be promising tools for the future. These devices developed for diabetics, for example, can achieve in „real time" the determination of the glucose level in blood (using hollow microneedles and glucose sensor) and, in the same time, can deliver insulin (using microfluidic pump and hollow microneedles) all in a „close-loop" circuit having an active control of drug delivered.

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