Microneedles as an Alternative Strategy for Drug Delivery

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ABSTRACT -- The transdermal route has been widely studied in the last decade due to its multiple advantages, where one of the most promising transdermal systems are microneedles, these allow the delivery of drugs in a painless way and with easy application, being very attractive for patients with chronic treatments. This review highlights the new research that develops this approach to transdermal therapies, including examples of materials and methods used for their manufacture and presenting an overview of the clinical trials currently available in Cochrane in a demonstrative way to understand the growing popularity of this strategy.

INTRODUCTION

Conventional methods for oral drug administration are characterized by numerous drawbacks, including poor drug solubility, unfavorable pharmacokinetics, and lack of selectivity, leading to an inefficient therapy and high incidence of serious adverse effects, for these reasons, various strategies have been developed to improve the pharmacotherapy of patients. Various approaches have been used to modifying the physicochemical properties of drugs such as the use of prodrugs, complex formation, solid dispersions among others, in addition, delivery technologies have been modified (1) to avoid problems of low or variable bioavailability due to first-pass liver metabolism (2), drug-drug and/or drug-food interactions, making increasing use of the transdermal route. Transdermal drug delivery is a painless method that suggests applying a formulation to healthy skin. The drug initially crosses the stratum corneum, reaching the deepest dermis through the epidermis, without accumulation of the drug in the dermal layer where it will be available for systemic absorption (3-5). For this reason, a review of the use of microneedles (MN) as an alternative strategy for drug delivery is presented below, including the most used materials, manufacturing methods and the most recent applications.

THE TRANSDERMAL ROUTE OF ADMINISTRATION

The transdermal route allows the permeation of drugs through the skin towards the capillary bed, which implies diffusion through the intact epidermis. Human skin is the largest organ in our body with a surface area of 1.8-2.0 m^2, which is very convenient for self-administration (6). It is composed of three main layers: The epidermis, dermis, and hypodermis (subcutaneous layer), (Figure 1).

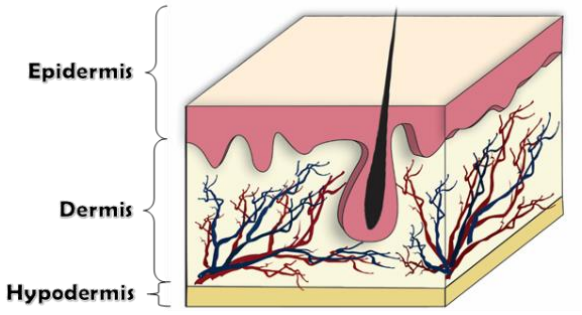

Figure 1. The general structure of the skin.

The skin is a potentially useful interface for the administration of therapeutic agents with systemic and/or local effects. On the contrary, the inherent function of the outermost layer of this tissue, the stratum corneum (SC) which is made up of corneocytes and an intercellular lipid matrix, forming

a "brick and mortar" structure, is to provide a barrier for the entry of exogenous material and the invasion of microorganisms (7).

The transdermal route of administration has many advantages over other conventional routes of drug delivery (8-9). It can provide a non-invasive alternative to parenteral routes, thus avoiding problems such as needle phobia. Furthermore, it allows achieving a sustained release with more uniform pharmacokinetic profiles, which minimizes the risk of toxic side effects and ensures serum drug levels above the therapeutic minimum. It can improve patient compliance due to the reduced dosage, typically 24-72 h, and is also suitable for patients who are unconscious, vomiting, or for those who depend on someone else's administration. The transdermal route of administration avoids pre-systemic metabolism, thus improving bioavailability (10). However, the clinical utility of conventional transdermal drugs such as creams or patches is limited to drugs capable of being passively absorbed by the SC so that only reasonably low dose lipid and relatively low molecular weight drugs are suitable candidates (11).

Traditionally, transdermal delivery has been restricted to novel formulation strategies to enhance the delivery of potent lipophilic molecules having a molecular weight of less than 600 Da (12). However, in the last decade, several delivery devices have been designed to enhance the delivery of macromolecules through the skin (13), where the rate of diffusion depends on the molecular weight and the concentration gradient, which makes the controlled administration of large molecules difficult because they diffuse slowly due to their limited solubility in aqueous media (9).

To successfully develop transdermal systems, it is necessary to understand the kinetics of skin permeation. Percutaneous absorption is the penetration of substances into various layers of the skin and the penetration through the skin into the systemic circulation (14-15). The percutaneous absorption of molecules is a gradual process that involves: (i) Penetration of a substance into a particular layer of the skin; (ii) Partition of the stratum corneum in the viable aqueous epidermis; (iii) Diffusion through the viable epidermis and into the upper dermis; (iv) Permeation of molecules from one layer to another, which is different both functionally and structurally from the first layer; (v.) Absorption of a substance into the systemic circulation (11).

Despite the multiple advantages of this route, problems of poor permeability and low bioavailability have also been reported; therefore, to date, approximately only 20 drugs have been delivered transdermally (such as: buprenorphine-Transtec® (16), capsaicin-Qutenza® (17), clonidine-Catapres-TTS® (18), ethinyloestradiol/norelgestromin -Ortho-Evra ® (19), fentanyl-Duragesic® (20), nicotine-NiQuitin® (21), rivastigmine-Exelon® (22), rotigotine-Neupro® (23), estradiol-Evopad®, nitroglycerin-Nitro-Dur®, oestradiol -Climara®, testosterone-Androderm®, oxybutynin-Oxytrol®, scopolamine-Scopoderm® (24)) mainly in the form of commercially available patches (25). Therefore, different approaches have been studied to increase permeability through the skin, using penetration enhancers, electric fields, and pressure waves generated by ultrasound or photoacoustic effects, although the mechanisms are all different (Figure 2).

These methods share the common goal of interrupting the SC structure to create the "holes" large enough for the molecules to pass through. The disruptions generated by each one of these methods are believed to be nanosized, which are large enough to allow the transport of low molecular weight drugs and, in some cases, macromolecules, but probably small enough to avoid damage of clinical importance (26). The technologies used by transdermal devices can be divided into passive or active methods depending on the source of energy that is required to improve skin penetration. Passive methods include the use of chemical enhancers, emulsions, and lipid pools, as well as biological methods such as peptides (27-30). Chemical methods are relatively easy to incorporate into transdermal patches and can be used to deliver variable doses by changing the area of application. However, these methods can have a delay of up to hours and, therefore, the effectiveness of the therapy is compromised in drugs that require rapid onset of activity, such as insulin (31).

The primary goal of novel drug delivery systems is to ensure safety and improve the efficacy of drugs, as well as patient compliance. Some of these new advanced transdermal technologies include (14) the application of microneedles, matrices that were first proposed by Vandervoort and Ludwig (32). Worldwide, it is estimated that approximately 10% of the population suffers from needle phobia, which is a disadvantage among the different pharmaceutical forms. Fortunately, needles of micrometer dimensions (generally ranging from

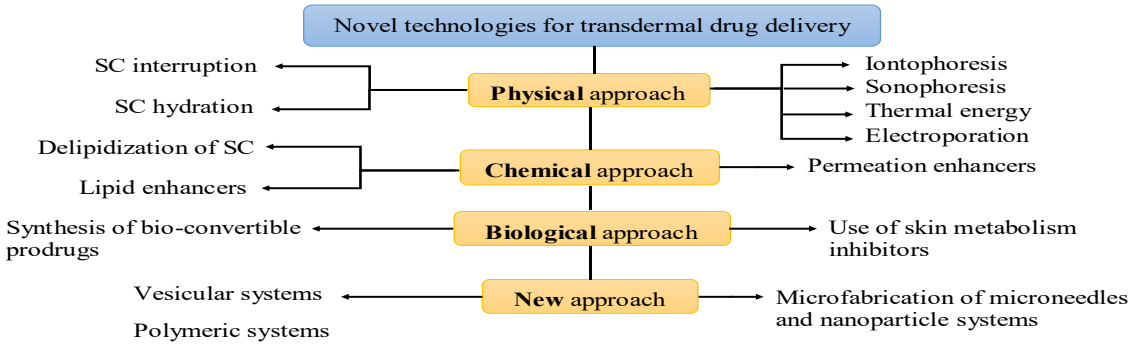

Figure 2. Novel technologies for transdermal drug delivery.

25 to 2000 µm in height) have emerged as minimally invasive devices creating transport pathways large enough for small drugs, macromolecules, nanoparticles, and fluid flow (33), but sufficiently small to avoid stimulating or damaging dermal nerve fibers or dermal blood vessels and therefore avoid causing pain. While avoiding these unwanted effects, microneedles facilitate specific localization and even intracellular targeting that can be applied for the detection, sampling, and delivery of molecules (34).

The penetration capacity of nanoparticles through human skin is complex and is determined by the material properties of the nanoparticles, the size of the individual particles, their shape, and other physicochemical factors such as non-covalent interaction with the skin surface, formulation instability/flocculation, steric hindrances, and degradation of the therapeutic formulation within the biological environment (35).

MICRONEEDLES AS A DELIVERY SYSTEM FOR DRUG DELIVERY

The preliminary study on MN began in 1976 but was not widely exploited until 1998, when Henry et al. studied the use of microneedles as a painless system capable of increased permeability in human skin by up to four orders of magnitude, using calcein as a model drug (36-37).

Microneedles are composed of several neatly arranged needles capable of creating temporary channels on the skin's surface by penetrating the SC barrier and delivering the drug into the epidermis and superficial dermis, where the drug diffuses rapidly and due to the short length of the Individual needles are considered painless and minimally invasive (25,38). Following these objectives, a series of specific strategies have been developed for the use of transdermal microneedles. The "poke with patch" method uses microneedles to pierce the SC and then apply a transdermal patch to the surface of the skin. Transport can occur by diffusion or by iontophoresis when an electric field is applied.

Another approach is “coat and poke,” where the needles are first covered with the drug and then inserted into the skin, there is no deposit of drug on the skin surface; rather, all drug that will be delivered is in the needle. A variation of this second approach is “dip and scrape,” where the surface of the skin is scraped with microneedles previously dipped in a drug solution, allowing it to be deposited within the micro-abrasions created by the needles (39).

In the literature, five main types of microneedle designs have been described: solid, coated, dissolvable, hollow, and inflatable or hydrogel-forming microneedles, see Figure 3.

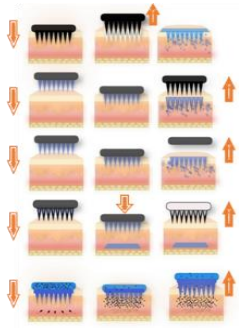

Figure 3. Types of microneedles: solid MN (I) the microneedles are inserted and subsequently withdrawn to give way to the placement of the drug-loaded patch that will permeate through the created channels; Coated MN (II) solid matrix coated with the drug; Dissolvable MNs (III) soluble/biodegradable matrix that includes the active principle; Hollow MNs (IV) Insertion of hollow microneedles on the skin followed by infusion of liquid and hydrogel-forming MNs (V) swellable material with a drug reservoir attached to the matrix base plate. After insertion, the matrix absorbs the interstitial fluid leading to drug diffusion (40).

Microneedles are a promising system for transporting large therapeutic agents through the skin

(7). The advantages and disadvantages of using microneedles as transdermal delivery systems are listed below in Table 1.

MATERIALS AND METHODS FOR PREPARING MICRONEEDLES

Materials

The microneedles have been manufactured from metals (3,49), silicon (37,50), and polymers (51-52) as shown in Figure 4. Some metals have been approved by the FDA for biomedical products, which can be used for the manufacture of microneedles and although they are robust, they present potential risks of infection in the reuse, they break in the skin and generate biohazard sharp objects, consequently, a search has been carried out for more viable substitutes for the manufacture of microneedles.

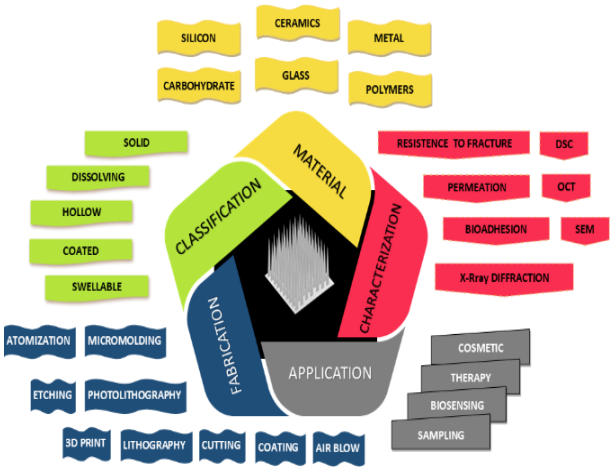

Figure 4. General diagram of the classification, manufacture, characterization, materials, and application of the microneedles.

Dissolvable MNs have been manufactured from polymers and biopolymers that can incorporate small molecules or biomolecules, such materials include carboxymethylcellulose (CMC), amylopectin (45), polyvinylpyrrolidone (PVP), polyvinyl alcohol (PVA) (53), sodium hyaluronate (54-55), sucrose, polyglycolic acid (PGA), poly (vinylpyrrolidone-comethacrylic) (PVPMAA) (56), polylactic-co-glycolic acid (PLGA) (57), poly (methyl vinyl ether maleic anhydride), chitosan, dextran (58), chondroitin sulfate and low molecular weight sugars such as maltose (59), galactose (60) and trehalose (61-62). However, these types of MN must have sufficient mechanical strength to create the microchannels in the skin. Heat-resistant polymers provide in situ forming gels after injection, formed from poloxamers that have been widely used due to their sol-gel transition, both methods for controlled drug release (25).

Methods

Microneedle manufacturing methods integrate techniques such as deep radiography, lithography, ultraviolet lithography, wet silicon etching, and reactive ion etching, lens-based lithographic patterns, photopolymerization with increased UV exposure, micro-molding, atomized spraying, printing by inkjet, drawing lithography, air blowing, continuous liquid interface production, 3D printing, multi-layer polyelectrolyte coating, photolithography, laser cutting, and metallic electropolishing as shown in Figure 4. However, manufacturing processes using light and laser involves sophisticated equipment that accumulates the total cost of the process and makes it potentially inaccessible for many researchers (63). Likewise, there are drawbacks involved in these methods, which include inter- and intra-batch variability, potential deterioration to the stability of drugs caused by radiation, high temperature and polymerization reagents used in these processes (38).

Polymeric microneedles can be made in several ways, but the most common, inexpensive, and commercially viable, involves first creating molds to form the microneedles inside. These molds are typically made via photolithography with optically curable polymers, such as ultraviolet (UV) curable SU-8. After this, the reverse molds are usually made with silicone polymers, such as polydimethylsiloxane (PDMS), pouring them into the master mold and curing them. Aluminum, PVA, and silicon have also been used to make molds, but PDMS is the most common and cheapest (64). Finally, the polymer solution is emptied into a mold, followed by the solidification and demoulding process sequentially. During the micro-molding process, centrifugation or aspiration is a commonly used technique to remove bubbles and later fill the microholes (47,65-66). However, centrifugation is time-consuming with high labor intensity and less controllability (65).

One option to eliminate trapped air and avoid the need for centrifugation is to fill the molds by the atomized spray method which could improve continuous manufacturing to reduce mechanical failures due to voids within individual MNs, and is also scalable. McGrath et al. hypothesized that the atomization of aqueous solutions from a nozzle could interrupt the cohesive forces and wet the surface of the MNs mold, reducing voids (62).

Table 1. Advantages and disadvantages of microneedles as transdermal delivery systems.

ADVANTAGES	DISADVANTAGES
Increased patient compliance (41).	Risk of accumulation of non-biodegradable materials within the skin (42).
Potentially hazardous sharps waste reduction (42).	Risk of involuntary MN fracture within the skin (42).
Elimination of the need for a trained physician (43).	They can cause skin irritation and in some cases allergy (44).
Reduced pain, tissue damage, and risk of infection transmission compared to conventional injections (43).	Studies are still lacking to ensure the reproducible delivery of a sufficient number of doses to elicit the necessary immune response (45).
Controllable dose delivery (43) Decreased anxiety and stress in patients with needle phobia (46)	
Avoid first-pass liver metabolism (38). They can be formulated with polymers that provide controlled release systems (47). Minimize side effects (48). It increases the distribution and amount of drug that reaches the deeper regions of tumors while minimizing therapeutic leakage to adjacent tissues, in cancer therapy (48).	
Nanoparticles can be incorporated that allow better permeability and/or improve the immune response in the case of vaccines (45).	

They demonstrated this by manufacturing dissolvable MNs by the atomized spray method at room temperature in PDMS molds, using an external two-fluid mixing nozzle capable of producing 10–50 μm droplets with a 0.25 bar compressed air feed and an aqueous feed of deionized water at 1.5 mL/min with 5% w / v of dissolved solids. Various materials were investigated, including trehalose, fructose, raffinose, PVA, PVP, CMC with glycerol, hydroxypropylmethylcellulose (HPMC), and sodium alginate, and although the viscosity of the materials in solution varied between 1 and 22 mPas, the changes in viscosity did not prevent enough mold filling by this atomized spraying process. Yet, the MN material affected the physical penetration of the skin, more often in those made of trehalose and fructose. In general, the micro-molding technique is capable of continuous manufacturing under moderate processing conditions and could be useful for active ingredients that are sensitive to high temperatures, viscosity, or concentration. The main obstacles to increasing the production of MNs made with this process include sterilization and possible safety problems related to the use and repeated application of non-therapeutic materials that would dissolve and accumulate within the skin (64).

Piezo dispensing technology is a 'drop-on-demand' inkjet printing technology that enables the production of drop sizes in the low picoliter range (1-70 pl). High-density drop patterns can be accurately dispensed using robotic systems. Piezoelectric dispensing is based on the application of voltage pulses to a piezoelectric material that surrounds a reservoir of liquid. The resulting electric fields cause the piezoelectric material to deform creating a pressure wave within the reservoir and resulting in a drop that is dispensed from a jet or nozzle located at one end of the reservoir. The number of drops that can be dispensed per second is controlled by the frequency setting, with the potential to dispense tens of thousands of drops per second (67). Again, the main obstacles to scaling up to include sterilization and potential safety concerns related to the use of non-therapeutic materials that would dissolve and could persist on the skin (42).

On the other hand, electrohydrodynamic atomization (EHDA) is used to produce particles by applying electrical energy to polymer solutions with which Donnelly et al. obtained in 2020 a prolonged release for approx. 28 days with PLGA nanoparticles loaded with ovalbumin (OVA) in hydrogel-forming MN matrices. Furthermore, they conclude that EHDA could generate a uniform particle coating in MN, with approx. 30% coating efficiency, that the coating process does not modify the mechanical characteristics of the MN, that the coated MN does not lead to an increase in the specific anti-OVA responses in an *in vivo* murine model. Therefore, they are not considered immunogenic, which is why a coating technique could have significant potential as a new non-invasive strategy to achieve a prolonged release of biologics (68).

In 2020, Uddin et al. recently studied another technique, 3D printing of microneedles. This technique focused on manufactured new polymeric matrices to improve the administration of cisplatin to

epidermoid tumors of the skin A-431 for the treatment of cancer. The microneedles were made from selective photopolymerization of consecutive layers of a biocompatible photopolymer resin using stereolithography, followed by a cisplatin coating using inkjet on the surface of the needle. Franz cell diffusion studies showed rapid cisplatin release rates of 80-90% in 1 hour and *in vivo* evaluation with Balb/c nude mice showed enough cisplatin permeabilization with high anticancer activity and tumor regression, glimpsing the high potential of 3D-printed microneedles in transdermal delivery of antineoplastic drugs (69).

Mechanical properties of microneedles.
Microneedle-based devices must be sharp and thin enough to easily penetrate the skin, but strong enough to ensure that the needles do not break or lodge in the skin. Two biomechanical parameters that apply to the safe and efficient design of microneedles are the force at which the structural integrity of the microneedle fails or resistance to fracture and the force necessary to insert the microneedle into the skin also called the effective penetration force. The ratio of these two forces is commonly referred to as the "factor of safety" or "margin of safety" (64) and microneedle designers aim to have this ratio as high as possible to allow for variations in user application methods.

The mechanical properties of microneedles can be determined using a texture analyzer by pressing the devices against a rigid surface, evaluating failure due to fracture, or buckling along their center axis, or bending and shearing due to forces acting in a lateral direction. (70).

The structure of the individual needles mainly defines the properties of the system as a dosage form, eg silicon dioxide is brittle; metal strength is much better, but thin films of metal formed by sputtering or evaporation are smooth, impacting microneedle insertion mechanics. Obviously, only microneedles with the proper geometry and proper physical properties can penetrate the skin without breaking or bending during insertion.

Zhang et al. considered that the fracture force increases with increasing thickness of the needle wall and decreases with increasing wall angle, but it is independent of the radius of the tip (64) and thus, needles with a radius of a small tip and a large wall thickness are considered the best option, so they designed an array of hollow metallic microneedles using a double mask technique, generating a cylindrical body with lateral openings, a very sharp submicron diameter tip, and three edges. In the shape of a knife, with this, they improved the characteristics of conventional metallic microneedles obtaining high needle density; relatively easy manufacturing; single-crystal silicon manufacturing; high structural strength; providing a large area of fluid tissue exposure and precise control over needle length for different penetration requirements (71).

Effective penetration force. Effective penetration force refers to the force necessary to allow 100% penetration of a microneedle array into the skin, that is, the force necessary for 100 microneedles to form 100 microchannels (72).

Nguyen et al. determined an effective force of (41.04 ± 18.33 N) to achieve a penetration efficiency of 100% of microneedles with 1.0 mm inside the skin. They used a stainless-steel microneedle array, which was firmly attached to the shaft of the texture analyzer with the needles facing down. Porcine ear skin (freshly dermatomized on five Parafilm sheets) was secured. The needles moved downward at a speed of 0.5 mm/s to reach the skin surface with an activating force of 0.049 N, then continued to descend (0.5 mm/s) at a predetermined distance on the skin. After a second embedding in the skin, the needles were withdrawn from the skin tissue (0.5 mm/s). The microneedle-treated skin was covered with 1.0 µm FluoSpheres® aldehyde sulfate microspheres and recorded with a fluorescent camera to count the number of channels created by each insert. Finally, they observed a positive correlation between the distance and pressure of the microneedle insertion, being related to the inherent viscoelasticity of the skin tissue (70)

Scanning electron microscopy (SEM). Scanning electron microscopy (SEM) is a technique capable of producing images that allow us to measure the length of the needle, the dimension of the base, size of the tip, distance from needle to needle, and morphology of the surface of the microneedle structure (before treatment, after two minutes of insertion in the skin and after deformation with an axial load) also, it is a reliable method to measure the dimensions of microchannels in the skin (70,73). Figure 5 shows the image of a polymeric microneedle obtained by SEM.

Microneedle mechanical uniformity. The uniformity of the mechanical resistance of the microneedles in the matrix is an essential indicator of uniform channels created in the skin, for which eight layers of stacked Parafilm M® can be taken, where

the microneedles are pressed in the center of the area with a force of 2N for two minutes with moderate pressure of a thumb, after removing the needles, each layer of parafilm is manually separated and viewed under an optical microscope to measure the dimensions of some randomly selected pores in the first layer, it is calculated the standard deviation of these dimensions demonstrating the uniformity of the needles. Finally, the number of pores that appeared in the last layer of Parafilm is evaluated to or show the uniformity of the length of the needles (38,70). The penetration rate is calculated using the following equation: % penetration rate = (number of pores on the surface) / (total number of microneedles) x 100 (1)

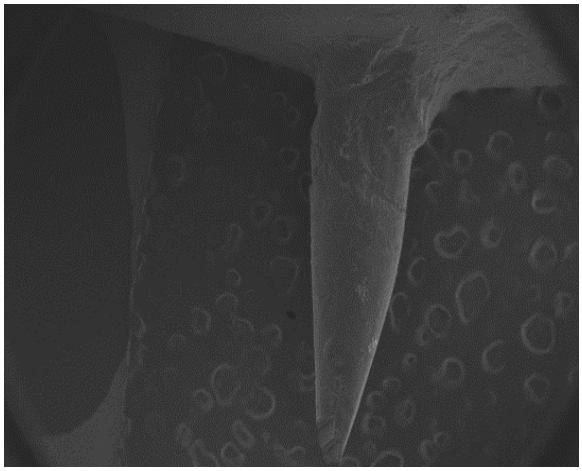

Figure 5. Image of a polymeric microneedle obtained by SEM.

Histology studies. The histological images show the different layers of the skin with different colors, easily distinguishable between each one. For this, the area of skin tissue is stained with methylene blue dye (1% w / v), freezing the optimal section, and vertically sectioning 10 μm thick. Subsequently, the sections are stained with hematoxylin and eosin (standard protocol) and viewed under a microscope. This to prove the efficacy of microneedles to penetrate the SC and the epidermis to reach the superficial layer of the dermis (74).

Pore uniformity studies. The conventional method for studying the uniformity of microchannels is evaluated by imaging of calcein, a fluorescent dye detected by fluorescence microscopy. Fluorescent images of the skin surface are captured and analyzed to report the pattern of the fluorescent intensity distribution in and around individual pores, known as the pore permeability index (PPI), which indicates the uniformity of the flux of calcein within the channels. Besides, the depth of the channels and the calcein dye distribution pattern within the pores can be evaluated using confocal laser microscopy or optical coherence tomography (25,70).

In vitro permeation studies. *In vitro* transdermal permeation studies are performed using Franz diffusion cells. The diffusion cell is a system composed of two chambers, a donor and an acceptor, separated by skin or an artificial membrane. The SC is oriented towards the donor chamber through which the systems to be studied are applied. The diffusion cells are kept at 32-35ºC (70).

Incorporation of NP into microneedles

Combinatorial strategies have recently emerged as a technological alternative to soluble and / or coated matrices that allow the administration of NP to the skin. The soluble MNs contain the nanoparticles in the microneedle matrix, which are inserted into the skin allowing the microneedles to dissolve and release the drug NPs into the epidermis and superficial dermis. In addition, hollow biodegradable polymeric microneedles can be used for injection of NP suspensions (45).

The first report of penetration of NP through the skin using MNs was published in 2003 by McAllister et al. Where the ability to improve the permeation of compounds with different molecular radius (insulin, calcein, BSA, and NP) using solid matrices was evaluated. As a model for NPs, two types of polystyrene latex nanospheres (25 and 50 nm radius) were used. They observed that the permeability of the compounds used is a function of the radius of their molecules (75).

Similarly, Coulman et al. Discussed the contribution of physicochemical factors on the speed and extent of delivery of nanoparticles through microchannels created in biological tissue (35). Although it can be assumed that the microchannels created in the SC will aid nanoparticulate systems in intraepidermal penetration, the movement of a charged colloidal system through micron-sized ducts within skin tissue is a complex and poorly understood process that has received limited research (76-79). For example, predicted impediments to the success of intradermal delivery of macromolecular and nanoparticle formulations include (i) non-covalent interaction with the skin surface and other tissue components, (ii) unstable/flocculating formulation, (iii) steric obstacles, and (iv) degradation of the

therapeutic formulation within the biological environment, yet, further studies are required to prove the complete relationship of these factors with the therapeutic utility of nanoparticle-based formulations that are intrinsically related to an effective delivery to the target tissue (35).

Polymeric nanoparticle drug delivery systems have proven to be a vehicle for targeting specific sites and controlling the delivery of many therapeutic agents. Besides their ability to control drug release, these drug carriers are also capable of improving water-insoluble drug delivery, reducing drug-associated side effects, protecting compounds from inactivating, and increasing intracellular penetration (80).

A wide variety of materials derived from natural products have been explored to synthesize nanoparticles, due to their properties such as biocompatibility, biodegradability, and ease of processing, however, attention must be paid to the toxicity that these could cause (81).

Pamornpathomkul et al. observed that when using hollow MNs combined with nanocarriers for the administration of plasmid DNA encoding ovalbumin (pOVA), skin permeation was greater than for the administration of naked pOVA, likewise, pOVA in the skin of mice induced an immune response of IgG stronger than conventional subcutaneous (SC) injections giving a promising approach to deliver pOVA complexes to the skin promoting successful immunization (82).

Likewise, Li et al developed PVP-based fast-dissolving MN matrices where the tips of the needles were loaded with chitosan nanoparticles, which encapsulated OVA as model antigen and oligodeoxynucleotides (CpG) as adjuvants to evoke humoral and immune responses. Their results demonstrated that positively charged chitosan is an excellent carrier for the negative charge of OVA and CpG, which formed nanocomplexes through simple electrostatic interactions and greatly improved the absorption efficiency of OVA (7).

Pawar and Shende synthesized artemether co-charged lumefantrine nanoparticles by ionotropic gelling method encapsulated in dissolvable microneedle matrices (PVA and PVP K-12) for long-lasting action as first-line treatment for P. falciparum malaria. Their system showed a biphasic release profile with an initial burst followed by a controlled release profile (up to 24h) for artemether and lumefantrine. However, pharmacokinetic studies are still required to estimate bioavailability and calculate the dose for MN matrices (83).

Finally, and more recently Pineda-Álvarez et al. developed and compared polymeric microneedles of starch-gelatin loaded with either losartan powder or lecithin-gelatin nanoparticles loaded with losartan. Their results show that both systems meet the necessary mechanical properties to break SC, yet the nanoparticles accelerated the permeation process of the drug through the skin (84).

Functionalization of nanoparticles by modifying their surface

The functionalization of nanocarriers is one of the most important challenges in the formulation of these systems since their efficacy and specificity must be ensured, there are many ways to functionalize nanoparticles which can be classified into active mechanisms through targeted ligands such as specific peptides, aptamers, antibodies, and small molecules (85) that drive the drug to the target organ or passive (EPR effect) which depends on its size and surface. For example, the size must be established to avoid rapid absorption by the reticulum system endothelial cells and thus avoid filtration through the liver and spleen (86). Therefore, nanostructures of less than 100 nm with a hydrophilic surface have the greatest ability to evade the molecular phagocytic system (87) by prolonging the circulation time and increasing the probability that NPs will encounter the target organ (88). Both mechanisms increase the concentration of the drug available, and therefore the pharmacological response, on the contrary, reduces systemic side effects (89).

If the nanoparticles are not modified, they are rapidly absorbed, mainly by opsonins, and are eliminated from the body by macrophages of the reticuloendothelial system before they can exert their therapeutic effects (90-91). The drug molecule is conjugated to the surface of the nanoparticles or becomes trapped and protected within the nucleus where it is necessary to modify the surface of the nanoparticles to evade the body's natural defense systems when drugs are transported into the bloodstream. (92). These modified nanoparticles can be administered for transdermal drug delivery by using microneedle polymeric arrays.

To increase circulation time, the particles can be coated with molecules that provide a hydrophilic protective layer, such as PEG, PVP, human serum albumin, poloxamers, polysorbate 80, polysorbate 20, Vitamin E TPGS, polysaccharides (eg, dextran), and different types of copolymers (93-97).

Microneedle application

MNs have been established as promising medical devices since they are minimally invasive, painless and favor self-administration by patients, thus improving their adherence to treatment and, their efficacy. In the last decades, there has been a rapid development in MN for transdermal systems for cosmetic, therapeutic, monitoring, and diagnostic applications (98) as summarized in Figure 4.

a) Cosmetic Use. In the cosmetic area, the application of microneedles to human skin improves conditions such as seborrheic keratosis (99), scars (100), stretch marks, anti-aging, wrinkles, or depigmentation (101). The original instrument used is the "dermaroller," which consists of a handle with a cylinder with stainless steel needles (0.5-2 mm in length).

b) Antineoplastics. Despite all efforts, cancer remains one of the main problems affecting the world population, it is estimated that the number of cases will exceed 20 million by 2025, according to the World Cancer Report of the World Organization of the Health (WHO).

Numerous studies have been carried out on the use of microneedles in oncology, doxorubicin treatments (74), methotrexate (70), paclitaxel (102), cisplatin (103), 5-fluorouracil (104), tamoxifen and gemcitabine (105), docetaxel (106) and 5-aminolevulinic acid (107) have been tested in polymeric matrices of PVA, PLGA, hyaluronic acid (108), polycaprolactone, PVP, carboxymethyl chitosan (109), maltose (61), among others. Techniques such as micromolding or polymerization (108) show beneficial results for the treatment of different types of cancer. In addition, vaccines have been studied as one of the first lines for cancer control. Antigen-presenting cells (APC), such as dendritic cells (DC), macrophages, and B cells, are known to play a central role in the effective induction of antitumor responses after vaccination. For APC maturation and activation, efficient delivery of antigens and immunomodulators called adjuvants is critical (110).

Zaric et al. administered antigen-encapsulated PGLA nanoparticles through microneedles which generated robust antigen-specific cellular immune responses in mice, providing complete *in vivo* protection against the development of antigen-expressing B16 melanoma tumors and a murine parainfluenza model. Using the activation of antigen specific cytotoxic CD8 (+) T cells resulting inefficient elimination of tumors and viruses (111). Similarly, Kim et al. administered resiquimod and tumor antigens through a microneedle patch composed of polyethylene glycol and Pluronic F-127 which could not only activate the skin's (APC) but also migrate to the lymph nodes. In addition, the authors highlighted an improvement in the immune response compared to hypodermic needle-based treatment resulting in obstruction of tumor growth (110).

c) Metabolic diseases. **Diabetes.** Diabetes is part of a group of metabolic diseases characterized by hyperglycemia that currently affects 422 million people in the world and is estimated to affect 642 million by 2040 (112).

In this area, patches capable of monitoring and treating diabetes have been developed using an array of microneedles. MN-based glucose sensors use amperometric measurement with immobilized glucose oxidase (GOx) for the detection of H_2O_2 generated from the following reaction: Glucose + O_2 GOX Gluconic acid + H_2O_2 (2) The H_2O_2 generated is detected by a working electrode, following the reaction indicated below: H_2O_2 + 700 mV O_2 + 2H + + 2e- (3). These studies are rapidly emerging from the laboratory level to clinical testing (113).

GhavamiNejad et al. developed in 2019 a "smart" patch consisting of a microneedle array of photo-cross-linked methacrylated hyaluronic acid with integrated multifunctional microgels. The microgels incorporate zwitterionic moieties that stabilize the charged glucagon and phenylboronic acid moieties that provide a glucose-dependent volume change to ease glucagon release. The hypoglycemic-triggered release of structurally unaltered glucagon from the patch is demonstrated *in vitro* and in a rat model of type 1 diabetes. The results they obtained were the prevention of insulin-induced hypoglycemia in diabetic rats after the transdermal application of the patch (114).

In another study, Yu et al. developed a removable (non-degradable) transdermal patch, which carries insulin-loaded microneedles in a glucose-sensitive polymeric matrix, manufactured by photopolymerization *in situ*, capable of regulating blood glucose in insulin-deficient minipigs, demonstrating that the patch could keep the minipig glucose levels in a near normal range for more than 20 hours under normal feeding conditions. Their results may aid the development of other microneedle patches that respond to translational stimuli for drug delivery (115).

Hypercholesterolemia. In 2020 Castañeda et al. presented a study where they develop microneedles of poly (methyl vinyl ether-alt-maleic acid) loaded with atorvastatin calcium by micromolding method for the treatment of hypercholesterolemia, MN were evaluated through studies of DSC, SEM, bioadhesion, post-wetting bioadhesion, resistance to the breakdown, drug release kinetics, and *in vitro* percutaneous absorption studies, resulting in a system with excellent mechanical properties capable of releasing atorvastatin for 21 days by the transdermal route, being a new alternative to the oral route for the treatment of hypercholesterolemia (116, 117).

d) Infectious diseases. Infectious diseases are caused by pathogenic microorganisms such as bacteria, viruses, parasites, or fungi. These diseases can be transmitted, directly or indirectly, from one person to another, and for that, many vaccines are availably capable of generating immunity against disease by stimulating the production of antibodies, these solutions require storage in cold chain and systems of transport. Therefore, the development of vaccines, which are easy to use in administration and superior in the stability of formulations, is critically important.

In 2015 Hirobe et al. developed a soluble microneedle patch based on sodium hyaluronate manufactured by micro-molding techniques, capable of administering a "single application" transdermal flu vaccine that, when compared with subcutaneous administration, it was observed that microneedles induce levels higher immunity (55). Similarly, NPs have been prepared from poly (lactic-co-glycolic acid) and poly (Ɛ-caprolactone) coated with chitosan for the administration of antibiotics such as doxycycline. Permana et al. reported that the incorporation of these NP's in the MN solution significantly improved the dermatokinetic profiles of doxycycline, referred to by a longer retention time compared to patches without needle (118).

In 2017 Yang et al developed a system capable of increasing the stability of the vaccine during its storage, this being a device with low production cost and easy self-administration. The researchers concluded that the formulation of EboDNA with PLGA-PLL / YPGA nanoparticles and its delivery using MN patches induced a stronger immune response after immunization (119-120).

Despite the studies carried out on the potential usefulness of MNs in the administration of biological materials, a better understanding of the immunology of the skin is still necessary to generate reliable immune responses and thus get the greatest benefit for the patient (121).

Available clinical trials

A Cochrane search was performed with the keyword "microneedles", 307 clinical trials related to the use of microneedles were found from 2005, observing an increase in the use of these systems, particularly in 2018.

The clinical trials based on the use of microneedles are in greater proportion for therapeutic applications, among which the use of insulin, vaccines against flu, influenza and rabies, lidocaine, tacrolimus, adalimumab, among others, stand out, however, the application of microneedles in the use cosmetic/dermatological has also had great growth in recent years, as shown in Figure 6, implementing new strategies in the management of acne scars, melasma, wrinkles, and keratosis, mainly with the management of fractional radiofrequency of microneedles.

It should be noted that in May 2021, a double-blind, randomized, actively controlled, phase 1/2 age reduction study was initiated to test the safety and immunogenicity of a microneedle patch of measles vaccine and rubella in adults and young children. The study concludes in June 2022 and its results are not yet available (122).

Commercially available drugs

Despite the promising release studies involving microneedles, it is still a new field, and, combined with the lengthy approval process by the US Food and Drug Administration (FDA) associated with the delivery of therapeutic products, this translates into a limited total number of microneedles marketed. However, those devices currently approved by the FDA are reviewed below (100).

The FDA approved Fluzone ® intradermal flu vaccine on May 9, 2011, the first vaccine against influenza caused by influenza virus subtypes A and B licensed in the US, which it uses a new microinjection system for intradermal administration of vaccines (Soluvia ™, Becton Dickinson). Its antigen content is lower (9 µg of haemagglutinin per strain) than the conventional intramuscular vaccine (15 µg) and is indicated for the active immunization of adults between 18 and 64 years of age (123) Sanofi Pasteur had before authorized intradermal

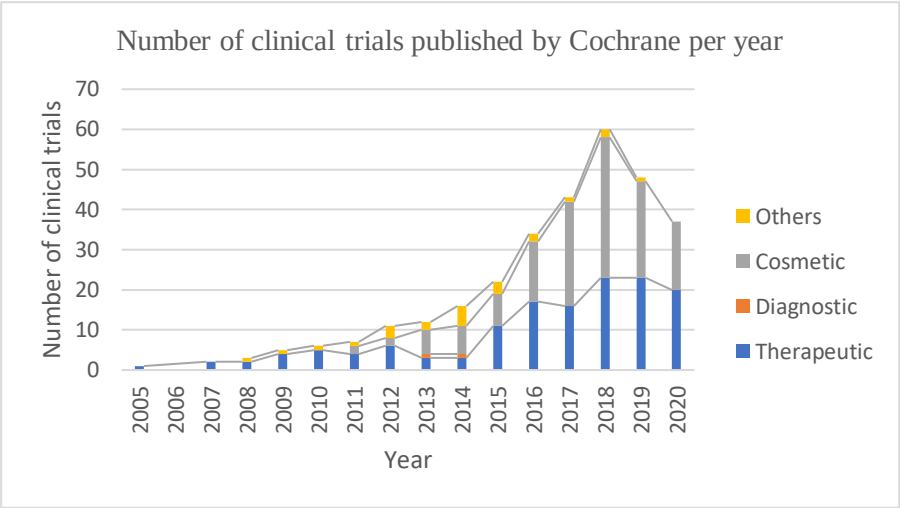

Figure 6. Graphical representation of the number of clinical trials currently available in Cochrane, classified by year and by area of interest, where the blue color represents clinical trials focused on therapeutics, the orange color the clinical trials related to the diagnosis and / or monitoring of diseases , the gray represents the clinical trials of the use of microneedles in the cosmetic area and the yellow color other types of clinical trials related to the use and characterization of microneedles.

microinjection vaccines against influenza, marketed as Intanza® (licensed February 26, 2009) or IDflu®, in more than 40 countries, including Australia, Canada, and countries in Europe. Also, it completed a phase II trial evaluating the non-inferiority of fractional doses of IMOVAX® Polio administered intradermally versus full doses of IMOVAX Polio administered intramuscularly, the results are not yet available.

Finally, on March 4, 2020, Zosano Pharma announces the FDA's acceptance of the application for Qtrypta ™ (zolmitriptan intracutaneous microneedle system) for the acute treatment of migraine with or without aura in adult patients. Expecting Qtrypta to be available, if approved, in 2021.

CONCLUSION

The large number of research published in recent decades on the advantages of using dissolvable biodegradable polymer microneedles has shown a prolonged-release application in a wide variety of drugs, the PLGA is the most popular polymer because it is reabsorbed and minimizes the risks of accumulation at the application site, likewise, the molding method is still the most common manufacturing process due to its cost, handling, and easy scaling. Further research and knowledge on the potential for polymer build-up within the skin after repeated application of the dissolving and coated microneedles is still needed, combined with the lack of human safety data, there is a great need for further clinical research. In addition, safety and efficacy evaluation are required for future international regulation of these novel delivery systems.

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