Available online on 15.03.2023 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Full Text Article

Review Article

Microneedles: Recent advances and development in the field of transdermal drug delivery technology

Monica Arora, Tanjima Tarique Laskar*

Department of Pharmaceutical Chemistry, Al-Ameen College of Pharmacy, Bengaluru-27, India. Affiliated to Rajiv Gandhi University of Health Sciences

Article Info:

Article History:

Received 07 Jan 2023
Reviewed 12 Feb 2023
Accepted 20 Feb 2023
Published 15 March 2023

Cite this article as:

Arora M, Laskar TT, Microneedles: Recent advances and development in the field of transdermal drug delivery technology, Journal of Drug Delivery and Therapeutics. 2023; 13(3):155-163

DOI: http://dx.doi.org/10.22270/jddt.v13i3.5967

*Address for Correspondence:

Tanjima Tarique Laskar, Department of Pharmaceutical Chemistry, Al-Ameen College of Pharmacy, Bengaluru-27, India

Abstract

Microneedles have emerged as a novel therapeutic modality in transdermal drug delivery system. The concept of microneedle has caught attention of the investigators owing to its immense utilities and unique promising features of painless drug delivery by crossing the impervious barrier layer of stratum corneum of skin efficiently in a non-invasive manner. It provides excellent therapeutic potency and bioavailability of drugs compared to most other conventional drug delivery routes. Microneedles have supremacy on avoidance of pre-systemic first pass metabolism of administered drugs, better patient compliance and avoidance of gastric irritation which outweighs the adherence of other conventional drug delivery system. As a trendy approach in the pharmaceutical and biomedical field, its applications are constantly evolving due to its ability to deliver large molecules with ionic and hydrophilic nature along with delivery of other products like insulin, vaccine, vitamins, peptides, oligonucleotides etc. The review provides an insight on recent advances of microneedles by emphasizing on various types, fabrication techniques, fabrication materials and various pharmaceutical and biomedical applications of microneedles along with some approved marketed microneedle products.

Keywords: Microneedles, types, fabrication techniques, commercial products

INTRODUCTION

The therapeutic efficacy, potency and bioavailability of pharmaceuticals rely not merely on the properties of drugs but on the mechanism underlying its delivery into the body as well[1]. Out of all the conventional drug delivery routes, oral route of administration is a simple and convenient drug delivery method because of effortless self-administration, lack of invasiveness and better patient convenience[2]. However pH changes and the enzymatic activities in the body causes drug degradation extensively in the liver and GIT before reaching into the systemic circulation thereby causing first-pass metabolism[3]. For achieving desired bioavailability (90-100%) drugs are administered through parenteral routes directly into blood stream using hypodermic injections. In this case the needle has to go deep into the dermis layer of skin where pain receptors are present so it has led to various issues like needle phobia, infection or skin injuries, scarring of tissues (Lipodystrophy) due to multiple needle administration, pain and bleeding at the site of injection and need for trained staff or expertise for drug administration[4,5].

In the Transdermal Drug Delivery System (TDDS), drugs are administered in the form of gels, creams, ointments or transdermal patches. This approach allows avoidance of first pass metabolism of drugs, prevents the exposure of drugs in acidic environment of GIT and allows for controlled and sustained release of drugs in a minimally invasive manner along with reduced fluctuations in peak plasma concentrations and reduced dosing frequency[6]. Despite having enormous advantages there are some limitations associated with this route due to outermost barrier layer of skin i.e. Stratum Corneum (SC) which prevents large molecules from bypassing the SC layer. Only drug with specific characteristics like low molecular weight (<500 Dalton), balanced lipophilicity (log P 1.5-3.5), low melting point (<200°C), low dose (<10 mg daily dose) can be able to make its way into the skin[7]. The limitations offered by the SC layer is due to its thickness along with 15-20 corneocyte layers which serves as a shield to protect the body against ingress of harmful chemicals, egress of water and other essential endogenous substances[8].

To overcome the aforesaid limitations, a refined technology called "Microneedles" have emerged as an advancement to TDDS which offers a unique clinical supremacy over the other conventional drug delivery system and helps to improve the various pitfalls of TDDS. It has promising features of non-invasive painless drug delivery by bypassing the SC layer efficiently and thereby providing enhanced therapeutic potency and bioavailability. This concept of miniature drug delivery had arrived after humongous research in 1960. It was first mentioned by Vander boot and Ludwig and the first patent was filed in 1971 in US by Martin Gestrel and Virgil A. Place who described their invention as "Puncturing Projections"[9]. The pioneer work on the utilization of microneedles for drug delivery via transdermal route was published in the late 1990s due to advancement in the field of Microelectromechanical system (MEMS Technology)[10-12]. The

first proof for the concept of "Microneedles" was emerged in the year 1998 by Henry et al. by using Silicon microneedles. In the year 2006, the first "Microneedle" product "Dermaroller ®" was developed by Dr. Desmond Fernandes[13].

Table-1: Table highlighting the fundamental findings of microneedles since the first conceptualization in the Year 1976 until the discovery of the last type of Microneedles in the Year 2012[14].

Year	Fundamental Findings
1976	Concept of Microneedles in "Drug Delivery Device" (Gestrel and Place)
1996	Hollow Needles with Drug Chamber (Gross et al.)
1997	Skin Perforating Device for transdermal delivery (Jung)
1998	Solid Microneedles (Henry et al.)
2000	Hollow Microneedles (Zahn et al.)
2004	Coated Microneedles (Cormier et al.)
2006	Dissolving Microneedles (Park et al.)
2007	Microneedles/ Delivery Device (Donnelly et al.)
2012	Hydrogel forming Microneedles (Donnelly et al.)

MECHANISM OF ACTION OF MICRONEEDLES

Microneedles with a precise geometry and appropriate physical properties can only be able to make its way to the skin. The force of insertion should not be too large otherwise bending or breaking of microneedles will occur before insertion. The margin of safety is determined by the ratio of fracture force to the insertion force. The value greater than 1 identifies the needles that can be inserted into the skin safely without bending or breaking. The largest margin of safety is achieved using needles of small tip radius (in order to facilitate the efficient needle insertion) and a larger thickness of the walls of microneedles (to provide sufficient strength)[15]. For transdermal delivery of drugs, the microneedles are first pierced into the surface of the skin which leads to temporary disruption of the barrier layer of Stratum Corneum. This leads to generation of micron sized pores or microchannels in the skin which facilitates the entry of drugs directly into the epidermis or upper dermis layer of skin. As the needles are micron sized, it does not affect the dermal nerves and blood vessels and so it is considered as non-invasive and there is no pain or bleeding associated with the insertion of microneedle patches[16,17].

DIMENSIONS OF MICRONEEDLES

Microneedles can be formulated in various sizes depending upon the types and the materials that are employed for its fabrication. Since the epidermis is having a thickness of 1500µm so the needle length of up to 1500µm is adequate enough to release the drug into the epidermal layer of skin. Mostly the typical geometries vary from 150-1500µm in length, a base width of 50-250µm and 1-25µm tip thickness or diameter. Microneedles tip can be fabricated in various shapes such as cylindrical, triangular, pointed, pentagonal, octagonal and are available in various other shapes as well. A microneedle device is made by arranging hundreds of micron sized needles in array which are attached to a small patch backing plate. Microneedles generally carry an array of 10^2-10^4 microneedles in an area of 1cm approximately[15,18].

MATERIALS MEANT FOR FABRICATION OF MICRONEEDLES

1. SILICON: Due to the highly flexible nature of silicon, a wide variety of shapes and sizes of microneedles can be manufactured[19]. It has a Young's modulus of 50-80Gpa and a tensile strength of 7000Mpa. They are precisely manufactured with small sharp tips with a length of 100µm or less. The main limitation associated with silicon microneedles are its time consuming multistep process of fabrication including a high cost of manufacture. Furthermore, there exists some issues concerning the biocompatibility of silicon as the needles may become brittle after fabrication and during piercing of the skin, there is a high risk of breaking or fracture of fragments which may remain inside the tissue and thereby causing various health hazards[20].

2. SILICA GLASS: Most glasses are brittle in nature with the exception of borosilicate glass which exhibit elastic properties due to the presence of a lower value of elastic moduli. It can be quickly prepared with a wide range of geometries and dimensions. It is physiologically inert and its transparency allows the ease of visualization of fluid flow. It exhibits biocompatibility and there is ease of sterilization because of its stability at high temperature and pressure. However it can get broken down easily under skin tissues causing inflammation or granulomas so they are not recommended for use in commercial purposes and its use are restricted to laboratory and experimental purposes only[21].

3. METAL: Metals have good mechanical properties, better biocompatibility and a high fracture force and as a result of which there is a less chance of breaking of needles beneath the skin tissues. The metals that are used for fabrication of microneedles are stainless steel, titanium, nickel, palladium and palladium cobalt alloy and among these stainless steel and titanium are mostly preferred. Titanium is a good alternative to stainless steel as it is less robust and suitable for biomedical applications. However it comparatively more costly and expensive[21].

5. CERAMICS: Ceramic materials that are employed for microneedles fabrication are Alumina, calcium phosphate dihydrate, calcium sulfate dihydrate and among these Alumina is widely used because of its resistance to corrosion, chemicals and adverse environmental conditions and it exhibits a good bioavailability. Recently an organically modified ceramic (hybrid) called Ormocer® has emerged which has a three-dimensional network of cross-linked co-polymers containing silicon alkoxides and organically modified organic monomers[22].

5. POLYMER: A wide variety of polymers like Carboxymethyl cellulose (CMC), Hydroxypropyl methylcellulose (HPMC), Polyvinylpyrrolidine (PVP), Polylactic-co-glycolic acid (PLGA), Poly-glycolic acid (PGA), Polymethyl methacrylate (PMMA), Polyvinyl alcohol (PVA), Polystyrene, amylopectin, dextrin, chitosan etc. are employed for fabrication of Microneedles. Polymeric microneedles exhibit a good biodegradability, biocompatibility, good strength/toughness value, low toxicity effects and a low cost of production. Polymers are a good alternative to various other materials employed for microneedles fabrication. It shows better toughness than ceramics or glass but exhibits lower strength than metal, ceramics, glass and silicon[22,23].

6. CARBOHYDRATE: Sugars such as raffinose, mannitol, trehalose, sucrose, maltose, xylitol and galactose are widely employed for fabrication of microneedles because they are cheap and safe for human health. Upon insertion into skin, the drug is released into the body by time-based dissolution of carbohydrates. However they have limitations like instability, need of high temperatures for processing, degradation and rapid pore resealing[19,22].

TYPES OF MICRONEEDLES:

Microneedles can be fabricated into various types because of their unique properties and each type of microneedles has its own way of drug delivery into the epidermis. Some microneedles are used for only creating pores in stratum corneum, some are precoated with drug solutions on their surface, some are dissolvable while some are prefilled with drug solution. Depending upon their unique features they are classified into:

1.SOLID MICRONEEDLES: Solid microneedles are fabricated using various metals like stainless steel, palladium, nickel, iron, cobalt alloy. Besides metal, various other materials like silicon, glass, maltose, sucrose, ceramic, biodegradable and non-biodegradable polymers can also be used. They have a length ranging from 150-300µm, a tapered tip angle of 15-20° and a wearing time ranging from 30 seconds to 10 minutes[24,25].

2. HOLLOW MICRONEEDLES: Hollow microneedles are employed for continuous administration of drugs. It is having a hollow bore at the centre of needle and upon insertion into skin it directly delivers drug solution into the lower layer of epidermis. It shows an enhanced drug infusion rate as pressure can be applied across the length of the hollow microneedles during administration. The pressure as well as rate of flow of liquid formulation can be modulated for the rapid bolus injection, slow rate infusion or for variable administration rate over time. Additionally, it has been employed to work as a conduit for drug diffusion into skin from a non-pressurized drug reservoir[26,27].

3. COATED MICRONEEDLES: Coated microneedles are solid arrays of microneedles fabricated using metal and silicon and it is further coated with drug solutions or dispersion layers to deliver drugs within the skin (sometimes within seconds). It is most widely employed for quick and instant bolus delivery of molecules. Furthermore, Coated microneedles are also employed for delivery of multiple drugs using the same formulation by enabling co-delivery of multiple agents with different properties[28].

4. DISSOLVING MICRONEEDLES: Dissolving microneedles are fabricated by encapsulating the drug into biodegradable/biocompatible polymers (PLA, PVA, CMC, PVP) Hyaluronic acid, Pullulan etc. Sugars like galactose, maltose and Dextrin are also used for fabrication. Following insertion, the dissolution takes place with release of drugs[29]. It is explored for delivery of water soluble and hydrophobic drugs like solid lipid nanoparticles and microparticles[30]. There is flexibility in drug loading and this type of microneedle can load large amount of drugs. The microneedles are fabricated using 15% PVA solution. Bubble microneedles can be able to achieve over 80% efficiency in drug delivery in near about 20 seconds which is only 10% for traditional solid microneedles[31]. It shows good performance in controlling drug distribution, quick dissolution, higher efficiency and a shorter administration time. It could be a progressive route for encapsulating vaccines and proteins[32].

5. HYDROGEL MICRONEEDLES: Due to the hydrophilic structure of hydrogel polymers it has the capability to take large amount of water into their 3D polymeric network[33]. Upon insertion into skin, it undergoes swelling by taking up the interstitial fluid present in the surroundings and as a result of which there is formation of channels between capillary circulation and the hydrogel microneedles patch. Prior to needling they are just used to disrupt the skin barrier and upon swelling up they serve as rate controlling membrane[34]. Hydrogel microneedles are considered to have high drug loading capacity, tuneable rate of drug release. Drug delivery using Hydrogel microneedles usually follows the mechanism of diffusion. They are considered as minimally invasive and can be easily sterilized. It can be detached easily from the skin with minimal damage to both microneedles and the skin[35].

MICRONEEDLES INSERTION TECHNIQUES

Various approaches have been adopted for releasing the drugs through microneedles. They are described as follows:

1. POKE AND PATCH: In this approach, the solid microneedles are used to poke the skin to create microchannels followed by application of transdermal patch which releases the drug into the micron sized pore channels by the mechanism of diffusion[36,37].

2. COAT AND POKE/ DIP AND SCRAPE: In this technique the needles are first coated with drugs to be diffused into the skin and then it is inserted into the skin for drug release. However an alternative technique to this is the method of 'Dip and scrape' where the microneedles are immersed into a drug containing solution and then the entire skin surface is scraped to administer the drugs into micro abrasions created by the microneedles[36,37].

3. POKE AND FLOW: This mechanism is employed for hollow microneedles where the microneedles are inserted into the skin and flow of drug takes place through microchannels[37].

4. POKE AND RELEASE: This mechanism is employed for dissolving and hydrogel forming microneedles where drug release takes place during dissolution of microneedles[37].

FABRICATION TECHNIQUES:

The selection of methods for microneedles fabrication depends solely on the geometry, kinds of microneedles and the type of materials employed for fabrication. The various fabrication techniques are applied for different types of microneedles which are described as follows:

Table 2: Representing the various methods of fabrication of different types of Microneedles:

Methods of fabrications	Type of Microneedles produced
1.Laser cutting	Solid metallic
2.Laser ablation	Solid metallic
3.Vapour deposition	Solid silicon
4.Dry etching	Solid silicon, hollow type
5.Wet etching	Solid silicon, solid metallic, hollow types
6. Metal electroplating	Solid metallic, hollow type.
7.Microstereolithography	Solid silicon, solid metallic.
8. Photolithography	Dissolvable/hydrogel forming, solid ceramic, hollow type
9. Deep X-ray lithography	Dissolvable/hydrogel forming, hollow type.
10. Drawing lithography	Dissolvable/hydrogel forming, hollow type.
11.Micromolding and melt casting	Dissolvable/hydrogel forming, solid ceramic
12. Droplet born air blowing	Dissolvable/hydrogel forming
13.Two photon polymerization	Dissolvable/hydrogel forming, hollow type, solid ceramic.
14. Pulling pipettes	Hollow glass.
15. Dipping	Coated type.
16.Spraying	Coated type
17. Continuous liquid interface production (CLIP)	Dissolving type

1. LASER CUTTING: This method is used primarily for manufacturing polymeric or metallic microneedles. The most frequently used material is stainless steel[38]. Herein flat metallic sheets are cut using infrared laser to generate 2D shape of micron sized needles and the shape, size and orientation of microneedles array are designed using computer aided software (Auto CAD). The microneedles are drawn in 2D form and then it a undergoes bending by 90° to generate 3D structure of microneedles. Final structure is generated by cleaning the tips or rough surface of needles by electropolishing which sharpens the tips of needles[39].

2. LASER ABLATION: Laser ablation is employed for fabrication of solid metallic array or polymeric microneedles. Along with stainless steel, other metals like tantalum are also fabricated by this technique. In this technique light is focused onto a 2D metallic or polymeric plate. This technique is based on the principle of "twisted light with spin" where in a protuberance appears on the surface being exposed after a single-shot. The centrally appeared protuberance can be given a proper shape by overlaying light pulses and after three pulses, the protuberance gets converted into needles having a height of around 10µm. The size of microneedles can be made narrow by depositing a few more twisted light pulses which results in a tip having diameter of less than 0.3µm[39].

3. VAPOUR DEPOSITION: The vapour deposition utilizes physical and chemicals techniques for fabrication. In the physical technique of vapour deposition, the raw materials are heated which causes release of vapours from the source of materials to be coated and the vapour gets deposited onto the surface of substrate. In chemical vapour deposition, thin films are generated in the chamber by a chemical reaction between inert gas carrier and the substrate. This technique is merely used to deposit a film of vapour materials onto solid silicon substrate wherein the films are then patterned using photolithographic and suitable etching techniques[39].

4. PHOTOLITHOGRAPHY: This method is used to fabricate hollow, polymeric and solid microneedles. In this process the substrate used is a silicon wafer and a photosensitive polymer called photoresist (hardened thermally) is coated onto the silicon wafer via a spin coating technique. Then through a mask, the photoresist is exposed to UV radiation where the UV rays manipulate " cross link" bonds of polymer i.e. either inhibits or initiates cross linking) and as a result the solubility of UV exposed polymeric portion differs drastically from that in the shade of mask. The exposed portion of the photoresist is then eliminated by dipping the treated substrate into developing solution. Subsequently the exposed substrate is etched through etching step (final step). Consequently, a desirable drawing pattern made of photoresist is then reproduced onto the silicon substrate with wet or dry chemical treatment[39].

5. DEEP X-RAY LITHOGRAPHY: This method is employed for fabrication of hollow microneedles with high precision utilizing the metallic, polymeric, ceramic and glass materials. In this technique, polymer is coated and exposed onto silicon wafer and then subjected to a vertical deep X-ray which creates an array of triangular columns along with a conduit. Subsequently the desired shape of the microneedles with the

conduit is provided to the developed arrays by exposure of it to inclined rays[39].

6. DRY ETCHING: This technique is utilized for fabrication of solid (silicon) or hollow microneedles. This process includes physical and chemical methods. Physical methods include ion milling and sputtering. In physical method an inert gas (e. g Argon) is ionized by unidirectional electrodes with high energy. As the ion strikes the silicon substrate at a high speed in a single direction, anisotropic etching is carried out. During the process of manufacturing, the area exposed on silicon is etched well and the area protected by oxide flow (sacrificial layer or photoresist) is etched only to a minimum extent. This method utilizes a low pressure of 10-4 Torr[39].

Chemical method includes reactive ion etching (RIE) which operates in the range of 10-3 and 10-1 Torr. It is similar to high pressure plasma etching process where a strong energy is utilized to generate a chemical reactive plasma gas. The plasma interacts with the surface of silicon substrate and gets converted into a volatile material which is subsequently blown away, thereby leading to isotropic etching of substrate. Operating conditions of plasma etching is between 0.1 and 0.5 Torr. RIE combines both physical and chemical methods. Both plasma and sputter etching can be utilized for controlling isotropic and anisotropic etching. By optimizing this process, a microneedle with precise sharp tip can be produced[39].

7. WET ETCHING: This technique is used to fabricate metal or silicon microneedles. This technique is used to produce microneedles with sharp shaped tips. In wet etching, a pattern is produced on the substrate using a chemical etchant. For a silicon wafer substrate, aqueous potassium hydroxide solution is used and a sharp tip of microneedle can be obtained by applying different etching rates depending on the direction of silicon crystals. Wet etching has a significantly faster etching rate than dry etching and it is primarily an isotropic etching process which occurs via a chemical reaction. Although the process of wet etching requires low cost but the poor accuracy of this method is a limitation for the fabrication of fine patterns[39].

8. PULLING PIPETTES: This method is employed for fabrication of hollow glass microneedles. The microneedle arrays are fabricated by utilizing a micropipette puller to pull out fire-polished type 1 borosilicate glass pipettes. Using a beveller, hollow microneedles having an oval shaped opening can be produced and the pulled needles are bevelled at a 30° angle[39].

9. METAL ELECTROPLATING: This technique is used to fabricate metallic microneedles (both solid and hollow microneedles). Using a constant current power supply and a nickel batch, nickel or suitable metal is electrodeposited over individual solid microneedle devices. The batch containing solutions are stirred constantly and after electrodeposition, the solution at the anode is replaced. Arrays are suspended into a solvent bath after completion of electrodeposition to dissolve it and the layers of polymers are disintegrated inside the cavity, thereby releasing the hollow metallic microneedles. The process of electrodeposition is carried out for a second time onto the sample microneedles so as to enhance the thickness of metals with no risk of occurrence of over deposition[39].

10. DIPPING: This method is employed for coating of a preformed solid microneedle with the desired drug formulation. The coating solution reservoir is held in such a way that it prevents the access of coating liquid only to microneedle shaft and doesn't cause contamination of base. Using micro positioners, the fixation and placing of reservoir are done which causes 3D alignment and dipping of microneedle rows or arrays into dip holes. The microneedle holder is positioned opposite to the solution reservoir such that the cover plate of coating solution reservoir contains dip holes corresponding to microneedles in the array or row. For coating microneedles, the microneedles array holder is allowed to slide down in order to dip the microneedles into the dip holes[39].

11. SPRAYING: This method is employed for coating microneedles by spray coating process. In this process, a nozzle is linked to a compressed air source and the coating solution is used to generate an atomized spray and the parameters like flow rate and pressure are adjusted. The microneedles are fixed using double-sided tape so as to fix the microneedles to an adjustable stage and it is then subjected to coating. Following the coating stage, it is subjected to drying at a room temperature for 12 hours prior to analysis[39].

12. MICROSTEREOLITHOGRAPHY (μSL): This technology is widely used for manufacturing of devices like tissue scaffolds, nerve guidance conduits cardiovascular stents in biomedical and tissue engineering. Using this method, the 3D objects are manufactured based on photopolymerization of a spatially controlled solidification of liquid resin by using a light source such as UV radiation. With the help of computer-controlled laser beam or digital light projector with a computer driven building stage, the light pattern is illuminated on the surface of resin. It leads to the solidification of the resin in the pattern to a desired depth and helps it for adhering to a supporting platform and thereby forms the desired structure. By this technology, a microneedle was prepared using poly propylene fumerate for treatment of skin cancer. This microneedle system enabled the administration of Dacarbazine (anti-cancer drug) for 5 weeks in a controlled release manner by modifying the drug dose and molecular weight of polymeric monomer[39].

13. DRAWING LITHOGRAPHY: This technique is used for fabrication of polymeric microneedles. In this method, liquid thermosetting polymer is drawn in a controlled manner into a desired shape and using thermal curing it is solidified. Thermosetting polymer also called as photoresist is employed for coating a glass substrate using spin-coating process. A patterned pillar is then allowed to contact with photoresist and is vertically drawn for development of axial strain and as a result the photoresist which lies in between the pillar and substrate acquires a wasp-waist shape. A constant and continued drawing of polymer produces conical shaped bridge. Upon generation of suitable shape and length the thermal curing completely cures the photoresists and this prevents relaxation of liquid from returning back to the substrate. Secondary aid of drawing helps in getting the final mould of microneedles and it separates the lower part of microstructure from the glass frame. Hollow microneedles can be fabricated using nickel electroplating and is followed by the solubilization of polymers[40].

14. MICROMOLDING AND MELT CASTING: This method is commonly utilized for manufacturing polymeric microneedles using natural polymers (pectin) and semi-synthetic polymers (PVA, PVP). By the use of polydimethyl siloxane a negative mold of microneedles array can be fabricated via this technique. Under vaccum condition the liquid mixture is degassed and the unpolymerized silicone elastomer is poured over the microneedle arrays (located at the centre of container) until the container is filled completely and is followed by exposure to vaccum again for removal of unwanted gas voids. Subsequently, it is then placed in an oven or onto a hot place in order to obtain cross-linking and when

polymerized mold cools down the master structure so formed is removed out from the mold (Molds can be obtained by application of UV lithography).Using an optimized concentration of polymer solution containing desired therapeutic medicaments, micromolds so formed are cast and allowed for centrifugation at high rpm so as to separate out the voids which was generated during water evaporation and it is then subjected to a temperature of 35° for drying. To fill the mold cavity completely and to eliminate the entrapped air bubbles in molds, vaccum is applied again when the centrifugation is unfeasible. If further cross-linking between the ingredients is required, then after application of vaccum or centrifugation, the polymeric solution containing molds can be heated further in order to induce cross-linking. In the final step, the micromolds are further dried to obtain the desired microneedles[39,40].

15. DROPLET BORN AIR BLOWING: This technique is employed for fabrication of polymeric microneedles without the utilization of silicon molds. The base array of microneedles can be prepared by dispensing a polymeric solution at a controlled rate using a dispenser solution. After the drying of polymeric solution containing drug, it is allowed to dispense on upper and lower plates and then it is followed by drying under controlled conditions by blowing air. At the final isolation step, the microneedles are separated out from each surface[39,40].

16. TWO PHOTON POLYMERIZATION (TPP): This technique is a sophisticated machinery method with a resolution of approximately 100nm. It is employed for fabrication of hollow microneedles using polymer and ceramics. TPP initiates polymerization of the resin via multiphoton absorption which takes place through the excitation of photo initiator which is located in a small area of polymer resin where laser is focused. The laser employed is a near infrared wavelength laser such as titanium sapphire laser. In TPP method, the curing reaction occurs only at a particular focal point. The substrate undergoes polymerization along with traces of laser and is subjected to scanning through resin. Upon completion of microneedle fabrication, using appropriate solvent the non-irradiated resin is washed away and is cured by the use of UV light. By this method it is possible to manufacture elaborate and complex 3D structures[39,41].

15. CONTINUOUS LIQUID INTERFACE PRODUCTION (CLIP): It is a novel additive manufacturing (3D printing) technique employed for fabrication of objects through Photopolymerization of a photoreactive resin by selectively targeting and solidifying the resin using UV light on a rising platform or with the light reflected from a general Digital Light Processing (DLP) chip[42]. This technique is extensively employed to rapidly prototype sharp microneedles with tuneable geometries (size, shape, aspect ratio, and spacing) and allows for mold-independent, one-step manufacturing of MN arrays of virtually any design in less than 10 minutes per patch[43]. Johnson et al. reported that using biocompatible polymers like Polyacrylic acid, Trimethylolpropane triacrylate, and photopolymerizable derivatives of Polyethylene glycol as well as Polycaprolactone, microneedles with 1000 mm height, 333 mm base width, and 2.3 mm tip radius can be produced by CLIP technique[44].

APPLICATIONS:

1. CANCER THERAPY: Microneedles have been investigated for delivery of various anti-cancer drugs. Dong et al. introduced Hyaluronic acid dissolving microneedles arrays containing anti-cancer drugs like Doxorubicin and gold nanocages to combine chemotherapy with photothermal therapy for treatment of superficial tumors in a synergistic manner. Wang et al. developed self-degradable microneedles for treatment of melanoma by loading Anti-PD-1 and glucose oxidase in pH sensitive Dextran nanoparticles. Bhatnagar et al. investigated microneedles for localized delivery of Tamoxifen and Gemcitabine for treatment of breast cancer with reduced side effects. Administration of solid microneedles loaded with 5-fluorouracil showed enhanced permeability up to 4.5 times than topical cream for treatment of basal cell carcinoma[45].

2. OCCULAR DRUG DELIVERY: Coated microneedles can be used to deliver drugs into anterior or posterior segment of eye via either intrascleral or intra corneal routes in a minimally invasive manner[45]. Coated microneedles are used for administration of Pilocarpine into the corneal stroma which causes improved bioavailability than topical formulation by 2 orders of magnitude. Hollow microneedles are also employed for fluid injection into targeted suprachoroidal space which enables flow of drug circumferentially around the eye and could reach the macula near limbus. An implantable microneedle was developed using biodegradable polymer and the drug Methotrexate was loaded into it for treatment of primary vitreo-retinal lymphoma[46].

3. VACCINES: Delivery of vaccines using microneedles shows improved immunogenicity as microneedle vaccinations are capable of targeting epidermal Langerhans cells and dermal dendritic cells. The first study for Vaccine delivery via microneedles was performed by Mikszta et al. [47]. Various types of microneedles have been investigated for in-vivo vaccination studies both in human and non-human primates for influenza, measles, polio virus, rotavirus, adenovirus, BCG, botulism, tetanus, anthrax, hepatitis B and C, HIV1, chikungunya, diphtheria, herpes simplex, human papilloma virus, poliomyelitis, rabies, plague, tuberculosis, West Nile fever etc [48,49].

Table 3: In-vivo studies with human and non-human primates for microneedles mediated vaccination:-

Subjects (in-vivo)	Types of Microneedles	Vaccines
1.Human	a) Hollow types b) Solid removable	Influenza, Polio-virus, Varicella-zoster. Rabies
2.Non-human primates	Hollow type	Japanese-encephalitis, Staphylococcus aureus, Botulism, Anthrax, Plague, Polio virus, Measles.

4. INSULIN DELIVERY: Traditional drug delivery methods for insulin doesn't closely match the physiological release of insulin and this imbalanced and inadequate release of insulin via other routes may induce kidney failures and blindness (in case of too low dose) or may cause hyperglycemia induced seizures, loss of consciousness and even death (in case of too high dose of insulin). Therefore microneedles serve as a better route which closely matches the dose needed by patients[50].

5. PROTEIN AND PEPTIDE DELIVERY: Coated microneedles are employed for delivery of proteins like serum albumin and ovalbumin. Peptides have poor skin penetrating property so less amount of drugs can be delivered by other routes.

Dissolving microneedles containing Cyclosporin A showed enhance bioavailability of drug. Coated microneedles were investigated for delivery of Desmopressin for treatment of diabetes insipidus and hemophilia A. Microneedles coated with Desmopressin acetate was inserted into hairless guinea pig and the result showed improved drug delivery within 15 minutes[51].

6. DELIVERY OF VITAMINS: For supplementation of vitamin D, a coated microneedle array loaded with PLGA nanoparticles was proposed by Kim et al. and it showed 5 folds improved delivery performance than other transdermal routes. Similarly, Vitamin K was also loaded in a dissolving microneedles and a positive in- vitro result was obtained[52].

7. LIDOCAINE DELIVERY: Microneedles such as coated and hollow microneedles are widely employed for delivery of Lidocaine. It is a viable alternative way to help patients overcome needle phobia specially in case of pediatric patients. Caffarel et al. introduced biodegradable microneedles loaded with Lidocaine for local anaesthetic effects and the results showed rapid dissolving property within the skin (approximately 15 minutes) upon application[53].

8. MICRONEEDLES IN COSMETICS AND DERMATOLOGY: Microneedles are widely used in the field of cosmetics and dermatology to treat different skin disorders. Solid removable microneedles were investigated for delivery of lysine-threonine-threonine-lysine-serine (KTTKS) and the results showed enhanced drug delivery using microneedles. Kumar et al. demonstrated the enhanced drug delivery of eflornithine hydrochloride using solid microneedles for treatment of facial hirsutism[54]. Microneedle rollers now-a-days are extensively used for treatment of psoriasis as microneedle, rollers can treat large areas of skin and causes improved drug delivery to large area of skin[55]. Currently microneedles are gaining importance in the field of cosmetics and dermatology for treatment of skin blemishes, rejuvenation, scars, wrinkles, acne vulgaris, androgenic alopecia, alopecia areata, melasma, periorbital hyper melanosis[56].

9. GENE THERAPY: Microneedles now a days are widely employed for the transdermal delivery of a broad range of low molecular weight drugs such as peptides, proteins, oligonucleotides, DNA and various other inactivated viruses. Moreover, substantial experiments with Bacillus Calmette–Guérin (BCG), influenza and other vaccines have proved that vaccine delivery into the skin is also promising by microneedle technique. Recently, microneedles are employed in gene therapy by combining it with siRNA. Microneedles are also reported for efficient and reproducible delivery of nucleic acids to the skin for the treatment of various wounds, cancers, genetic skin disorders and hyper proliferative diseases[57].

APPROVED COMMERCIAL MICRONEEDLE PRODUCTS

Table 4: List of marketed Microneedle Products with their applications in specific treatment [4,7,26,51,53, 58]:

Brand Names	Manufactured by	Description	Applications
1.Vax-Mat®	TheraJect Inc., USA	Dissolvable Microneedle patch	Delivery of macromolecules like proteins, peptides and vaccines.
2. Drugmat®	TheraJect Inc., USA	Dissolvable Microneedle patch	Rapidly delivers around 100mg of drugs through SC layer into epidermal tissue
3.Nanoject®	Debiotech, Switzerland	Microneedle array based device	Useful for interstitial fluid diagnostics and intradermal and hypodermic drug delivery
4. Dermaroller®	Derma spark, Canada	Metallic microneedle array	Treatment of acne, stretch mark, hair loss (enhances absorption of drugs such as Minodixil, Hyaluronic acid etc)
5.Soluvia®	Becton Dickinson, USA	Hollow microneedle array	It is a prefillable microinjection system used for accurate intradermal delivery of various drugs and Vaccines
6.Lite clear®	Nanomed skincare	Solid silicon microneedles	Treats blemishes and acne
7.h-patch	Valeritas	Small adhesive machine-like patch	Delivers Insulin in SC tissues
8.Microstructured transdermal patch	3M Corp., USA	Hollow microneedle array	Delivery of liquid formulations over a range of viscosities
9.MicroHyala®	CosMED Pharmaceutical Co. Ltd., Japan	Dissolvable microneedle patch	Contains Hyaluronic acid which is released into the skin for treatment of wrinkles
10.IDflu/Intanza	Sanofi Pasteur, Lyon, France	Intradermal microneedle injection	Prefilled with Influenza vaccine for intradermal vaccination
11.Micronject®	NanoPass Inc., Israel	Intradermal microneedle injection	Used along with any standard syringe for painless delivery of drugs, proteins and vaccines
12. Macroflux®	Zosano Pharma Inc., USA	Metallic microneedle array	Used for delivery of peptides and Vaccines
13. Microcore®	Corium International Inc., USA	Dissolvable peptide microneedle patch	Delivers small as well as large molecules like proteins, peptides and vaccines

CONCLUSION

Microneedle technology as a transdermal drug delivery system is rapidly growing in the field of research owing to its immense beneficial features. It is a unique technique because of its versatility and it is considered to be more proficient and a safer alternative to other conventional methods of drug delivery because of better patient compliance, possibility of self-administration, improved bioavailability and accuracy and precision in drug delivery. Microneedles have a great potential to provide improved therapeutic performance specially for the administration of macromolecules like Protein, Peptide, Oligonucleotide, Desmopressin, Vaccine, Insulin, Human growth hormone and various other products. Though microneedles serve as a better alternative to other approaches, it also suffers from some limitations like poor mechanical strength, limited amount of drug delivery and a high risk of microbial infection in case of delayed recovery of skin pores. Despite all the drawbacks, we believe that microneedles will resolve all hurdles and pitfalls as scientists are investigating on this technique to bring about revolutionary modifications. The global arrival of a variety of commercial microneedle products is highly anticipated as it holds great promise for pharmaceutical and biomedical applications.

Conflicts of Interest: There are no conflicts of interest or disclosure regarding the manuscript

Acknowledgement: Department of Pharmaceutical Chemistry, Al-Ameen College of Pharmacy, University Libraries and various other sources for their cooperation and guidance in writing this review

REFERENCES

1. Nagarkar R, Singh M, Nguyen HX, Jonnalagadda S. A review of recent advances in microneedle technology for transdermal drug delivery. Journal of Drug Delivery Science and Technology. 2020 Oct 1; 59:101923. https://doi.org/10.1016/j.jddst.2020.101923

2. Parhi R, Supriya ND. Review of microneedle based transdermal drug delivery systems. International Journal of Pharmaceutical Sciences and Nanotechnology. 2019 May 31; 12(3):4511-23. https://doi.org/10.37285/ijpsn.2019.12.3.1

3. Cheung K, Das DB. Microneedles for drug delivery: trends and progress. Drug delivery. 2016 Sep 1; 23(7):2338-54. https://doi.org/10.3109/10717544.2014.986309

4. Waghule T, Singhvi G, Dubey SK, Pandey MM, Gupta G, Singh M, Dua K. Microneedles: A smart approach and increasing potential for transdermal drug delivery system. Biomedicine & pharmacotherapy. 2019 Jan 1; 109:1249-58. https://doi.org/10.1016/j.biopha.2018.10.078

5. Ita K. Transdermal delivery of drugs with microneedles-potential and challenges. Pharmaceutics. 2015 Sep; 7(3):90-105. https://doi.org/10.3390/pharmaceutics7030090

6.Teo AL, Shearwood C, Ng KC, Lu J, Moochhala S. Transdermal microneedles for drug delivery applications. Materials Science and Engineering: B. 2006 Jul 25; 132(1-2):151-4. https://doi.org/10.1016/j.mseb.2006.02.008

7. Dugam S, Tade R, Dhole R, Nangare S. Emerging era of microneedle array for pharmaceutical and biomedical applications: recent advances and toxicological perspectives. Future Journal of Pharmaceutical Sciences. 2021 Dec; 7(1):1-26. https://doi.org/10.1186/s43094-020-00176-1

8.Mahmood TM, McCrudden MT, Torrisi BM, McAlister E, Donnelly RF. Microneedles for intradermal and transdermal delivery. Eur J Pharm Sci. 2013; 50:623-37. https://doi.org/10.1016/j.ejps.2013.05.005

9. Ogundele M, Okafor HK. Transdermal drug delivery: Microneedles, their fabrication and current trends in delivery methods. Journal of Pharmaceutical Research International. 2017 Sep 19:1-4. https://doi.org/10.9734/JPRI/2017/36164

10. Donnelly RF, Singh TR, Woolfson AD. Microneedle-based drug delivery systems: microfabrication, drug delivery, and safety. Drug delivery. 2010 May 1; 17(4):187-207. https://doi.org/10.3109/10717541003667798

11. Nagod S, Halse S.V. MEMS Based Microneedles in the Field of Drug Delivery. International Journal of Innovative Technology and Exploring Engineering. 2020 February: ISSN: 2278-3075, Volume-9 Issue-4 https://doi.org/10.35940/ijitee.D1761.029420

12. Mi O, Okafor HK. Microneedles: a potent modern tool in enhancing transdermal drug delivery. Current Trends in Biomedical Engineering & Biosciences. 2017; 10(1):1-9. https://doi.org/10.19080/CTBEB.2017.09.555776

13. Iriarte C, Awosika O, Rengifo-Pardo M, Ehrlich A. Review of applications of microneedling in dermatology. Clinical, cosmetic and investigational dermatology. 2017; 10:289. https://doi.org/10.2147/CCID.S142450

14. Ramadon D, McCrudden MT, Courtenay AJ, Donnelly RF. Enhancement strategies for transdermal drug delivery systems: Current trends and applications. Drug Delivery and Translational Research. 2021 Jan 20:1-34. https://doi.org/10.1007/s13346-021-00909-6

15. Sarang V, P.K M. Microneedles as a novel transdermal drug delivery. International Journal of Research and Analytical Reviews.2020; 7(3):943-956.

16. Larrañeta E, McCrudden MT, Courtenay AJ, Donnelly RF. Microneedles: a new frontier in nanomedicine delivery. Pharmaceutical research. 2016 May 1; 33(5):1055-73. https://doi.org/10.1007/s11095-016-1885-5

17. Thuy Trang Nguyen & Jung Hwan Park. Human studies with microneedles for evaluation of their efficacy and safety, Expert Opinion on Drug Delivery, 2018; 15(3):235-245 https://doi.org/10.1080/17425247.2018.1410138

18. Jeong Woo Lee & Mark R. Prausnitz. Drug delivery using microneedle patches: not just for skin, Expert Opinion on Drug Delivery, 2018; 15(6):541-543, https://doi.org/10.1080/17425247.2018.1471059

19. Serrano-Castañeda P, Escobar-Chavez JJ, Rodríguez-Cruz IM, Melgoza LM, Martinez-Hernandez J. Microneedles as enhancer of drug absorption through the skin and applications in medicine and cosmetology. Journal of Pharmacy & Pharmaceutical Sciences. 2018 Mar 30; 21:73-93. https://doi.org/10.18433/jpps29610

20. Kirkby M, Hutton AR, Donnelly RF. Microneedle mediated transdermal delivery of protein, peptide and antibody based therapeutics: current status and future considerations. Pharmaceutical research. 2020 Jun; 37:1-8. https://doi.org/10.1007/s11095-020-02844-6

21. Tucak A, Sirbubalo M, Hindija L, Rahić O, Hadžiabdić J, Muhamedagić K, Čekić A, Vranić E. Microneedles: Characteristics, materials, production methods and commercial development. Micromachines. 2020 Nov; 11(11):961 https://doi.org/10.3390/mi11110961

22. Makvandi P, Kirkby M, Hutton AR, Shabani M, Yiu CK, Baghbantaraghdari Z, Jamaledin R, Carlotti M, Mazzolai B, Mattoli V, Donnelly RF. Engineering Microneedle Patches for Improved Penetration: Analysis, Skin Models and Factors Affecting Needle Insertion. Nano-Micro Letters. 2021 Dec; 13(1):1-41. https://doi.org/10.1007/s40820-021-00611-9

23. Donnelly RF, Majithiya R, Singh TR, Morrow DI, Garland MJ, Demir YK, Migalska K, Ryan E, Gillen D, Scott CJ, Woolfson AD. Design, optimization and characterisation of polymeric microneedle arrays prepared by a novel laser-based micromoulding technique. Pharmaceutical research. 2011 Jan; 28(1):41-57 https://doi.org/10.1007/s11095-010-0169-8

24. Yang J, Liu X, Fu Y, Song Y. Recent advances of microneedles for biomedical applications: drug delivery and beyond. Acta Pharmaceutica Sinica B. 2019 May 1; 9(3):469-83. https://doi.org/10.1016/j.apsb.2019.03.007

25. Guillot AJ, Cordeiro AS, Donnelly RF, Montesinos MC, Garrigues TM, Melero A. Microneedle-based delivery: An overview of current applications and trends. Pharmaceutics. 2020 Jun; 12(6):569. https://doi.org/10.3390/pharmaceutics12060569

26. Nasrin N, C.K T, P.S S, M.K S. MICRONEEDLE BASED DRUG DELIVERY DEVICE.EUROPEAN JOURNAL OF PHARMACEUTICALAND MEDICAL RESEARCH.2020; 7(7):776-782. https://doi.org/10.1016/j.ijpharm.2021.120455

27. Bolton CJ, Howells O, Blayney GJ, Eng PF, Birchall JC, Gualeni B, Roberts K, Ashraf H, Guy OJ. Hollow silicon microneedle fabrication using advanced plasma etch technologies for applications in transdermal drug delivery. Lab on a Chip. 2020; 20(15):2788-95. https://doi.org/10.1039/D0LC00567C

28. Tarbox TN, Watts AB, Cui Z, Williams RO. An update on coating/manufacturing techniques of microneedles. Drug delivery and translational research. 2018 Dec 15; 8(6):1828-43. https://doi.org/10.1007/s13346-017-0466-4

29. Ye Y, Yu J, Wen D, Kahkoska AR, Gu Z. Polymeric microneedles for transdermal protein delivery. Advanced drug delivery reviews. 2018 Mar 1; 127:106-18. https://doi.org/10.1016/j.addr.2018.01.015

30. Zhang L, Guo R, Wang S, Yang X, Ling G, Zhang P. Fabrication, evaluation and applications of dissolving microneedles. International Journal of Pharmaceutics. 2021 Jul 15; 604:120749. https://doi.org/10.1016/j.ijpharm.2021.120749

31. Arya J, Henry S, Kalluri H, McAllister DV, Pewin WP, Prausnitz MR. Tolerability, usability and acceptability of dissolving microneedle patch administration in human subjects. Biomaterials. 2017 Jun 1; 128:1-7. https://doi.org/10.1016/j.biomaterials.2017.02.040

32. Wang QL, Zhu DD, Liu XB, Chen BZ, Guo XD. Microneedles with controlled bubble sizes and drug distributions for efficient transdermal drug delivery. Scientific reports. 2016 Dec 8; 6(1):1-1. https://doi.org/10.1038/srep38755

33. Peng K, Vora LK, Domínguez-Robles J, Naser YA, Li M, Larrañeta E, Donnelly RF. Hydrogel-forming microneedles for rapid and efficient skin deposition of controlled release tip-implants. Materials Science and Engineering: C. 2021 Aug 1; 127:112226. https://doi.org/10.1016/j.msec.2021.112226

34. Turner JG, White LR, Estrela P, Leese HS. Hydrogel-Forming Microneedles: Current Advancements and Future Trends. Macromolecular Bioscience. 2021 Feb; 21(2):2000307 https://doi.org/10.1002/mabi.202000307

35. Jamaledin R, Di Natale C, Onesto V, Taraghdari ZB, Zare EN, Makvandi P, Vecchione R, Netti PA. Progress in microneedle-mediated protein delivery. Journal of clinical medicine. 2020 Feb; 9 (2):542. https://doi.org/10.3390/jcm9020542

36. Tripathi A, Behera AK, Meshram DB. A novel approach towards drug delivery systems: Microneedles. RRBS.2013; 7(9):363-70

37. Agrawal S, Gandhi SN, Gurjar P, Saraswathy N. Microneedles: An advancement to transdermal drug delivery system approach. Journal of Applied Pharmaceutical Science. 2020 Mar; 10(03):149-59. https://doi.org/10.7324/JAPS.2020.103019

38. Adeyemi T. A Comprehensive Review on the Potential of Microfabricated Microneedles for Effective Systemic Delivery of Drugs. Reinvention: An International Journal of Undergraduate Research. 2019 Oct 31; 12(2). https://doi.org/10.31273/reinvention.v12i2.371

39. Dharadhar S, Majumdar A, Dhoble S, Patravale V. Microneedles for transdermal drug delivery: a systematic review. Drug development and industrial pharmacy. 2019 Feb 1; 45 (2):188-201. https://doi.org/10.1080/03639045.2018.1539497

40. Lee K, Lee HC, Lee DS, Jung H. Drawing lithography: three-dimensional fabrication of an ultrahigh-aspect-ratio microneedle. Advanced Materials. 2010 Jan 26; 22(4):483-6. https://doi.org/10.1002/adma.200902418

41. Jung JH, Jin SG. Microneedle for transdermal drug delivery: current trends and fabrication. Journal of pharmaceutical investigation. 2021; 4:1-5. https://doi.org/10.1007/s40005-021-00512-4

42. Caudill CL, Perry JL, Tian S, Luft JC, DeSimone JM. Spatially controlled coating of continuous liquid interface production microneedles for transdermal protein delivery. Journal of Controlled Release. 2018 Aug 28; 284:122-32. https://doi.org/10.1016/j.jconrel.2018.05.042

43. Johnson AR, Caudill CL, Tumbleston JR, Bloomquist CJ, Moga KA, Ermoshkin A, Shirvanyants D, Mecham SJ, Luft JC, DeSimone JM. Single-step fabrication of computationally designed microneedles by continuous liquid interface production. PloS one. 2016 Sep 8; 11(9): e0162518. https://doi.org/10.1371/journal.pone.0162518

44. Dabbagh SR, Sarabi MR, Rahbarghazi R, Sokullu E, Yetisen AK, Tasoglu S. 3D-printed microneedles in biomedical applications. Iscience. 2020 Dec 31:102012 https://doi.org/10.1016/j.isci.2020.102012

45. Du G, Sun X. Current advances in sustained release microneedles. Pharmaceutical Fronts. 2020 Mar; 2(01): e11-22. https://doi.org/10.1055/s-0040-1701435

46. Thakur Singh RR, Tekko I, McAvoy K, McMillan H, Jones D, Donnelly RF. Minimally invasive microneedles for ocular drug delivery. Expert opinion on drug delivery. 2017 Apr 3; 14(4):525-37. https://doi.org/10.1080/17425247.2016.1218460

47. Kim YC, Park JH, Prausnitz MR. Microneedles for drug and vaccine delivery. Advanced drug delivery reviews. 2012 Nov 1; 64(14):1547-68. https://doi.org/10.1016/j.addr.2012.04.005

48. Menon I, Bagwe P, Gomes KB, Bajaj L, Gala R, Uddin MN, D'souza MJ, Zughaier SM. Microneedles: A new generation vaccine delivery system. Micromachines. 2021 Apr; 12(4):435. https://doi.org/10.3390/mi12040435

49. Kwon KM, Lim SM, Choi S, Kim DH, Jin HE, Jee G, Hong KJ, Kim JY. Microneedles: quick and easy delivery methods of vaccines. Clinical and experimental vaccine research. 2017 Jul 1; 6(2):156-9. https://doi.org/10.7774/cevr.2017.6.2.156

50. Martanto W, Davis SP, Holiday NR, Wang J, Gill HS, Prausnitz MR. Transdermal delivery of insulin using microneedles in vivo. Pharmaceutical research. 2004 Jun; 21:947-52. https://doi.org/10.1023/B:PHAM.0000029282.44140.2e

51. Tabassum N, Sofi A, Khuroo T. Microneedle technology: a new drug delivery system. Int J Res Pharm Biomed Sci. 2011; 2(1):59-62.

52. Kim HG, Gater DL, Kim YC. Development of transdermal vitamin D3 (VD3) delivery system using combinations of PLGA nanoparticles and microneedles. Drug delivery and translational research. 2018 Feb; 8:281-90. https://doi.org/10.1007/s13346-017-0460-x

53. Zhang Y, Brown K, Siebenaler K, Determan A, Dohmeier D, Hansen K. Development of lidocaine-coated microneedle product for rapid, safe, and prolonged local analgesic action. Pharmaceutical research. 2012 Jan; 29:170-7. https://doi.org/10.1007/s11095-011-0524-4

54. Rzhevskiy AS, Singh TR, Donnelly RF, Anissimov YG. Microneedles as the technique of drug delivery enhancement in diverse organs and tissues. Journal of controlled release. 2018 Jan 28; 270:184-202. https://doi.org/10.1016/j.jconrel.2017.11.048

55. Park JH, Choi SO, Seo S, Choy YB, Prausnitz MR. A microneedle roller for transdermal drug delivery. European journal of pharmaceutics and biopharmaceutics.2010 Oct1; 76(2):282-9. https://doi.org/10.1016/j.ejpb.2010.07.001

56. Singh A, Yadav S. Microneedling: advances and widening horizons. Indian dermatology online journal. 2016 Jul; 7(4):244. https://doi.org/10.4103/2229-5178.185468

57. Chen W, Li H, Shi D, Liu Z, Yuan W. Microneedles as a delivery system for gene therapy. Frontiers in pharmacology. 2016 May 26; 7:137. https://doi.org/10.3389/fphar.2016.00137

58. Halder J, Gupta S, Kumari R, Gupta GD, Rai VK. Microneedle array: applications, recent advances, and clinical pertinence in transdermal drug delivery. Journal of Pharmaceutical Innovation. 2021 Sep; 16:558-65. https://doi.org/10.1007/s12247-020-09460-2