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REVIEW ARTICLE

MICRONEEDLE TECHNOLOGY FOR TRANSDERMAL DRUG DELIVERY: APPLICATIONS AND COMBINATION WITH OTHER ENHANCING TECHNIQUES***Nayak Smita, Suryawanshi Sanidhya, Vaidhun Bhaskar**

Department of Quality Assurance, Gahlot Institute of Pharmacy, Koparkhairane, Navi Mumbai-400709, Maharashtra, India

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*** Corresponding Author****Smita Nayak**, Department of Quality Assurance, Gahlot Institute of PharmacyE-mail: smitanayak125@yahoo.com, Tel: 022-27550816**ABSTRACT**

Transdermal delivery system has been extensively employed over the years owing to its advantages over the conventional delivery systems. However, the stratum corneum hinders the penetration of substances through the skin. Microneedle technology is the latest advancement and has gained tremendous attention in transdermal delivery system, overcoming the obstacles of transdermal delivery system and providing advantages over the conventional delivery systems. Numerous studies have been carried out for exploring microneedles. This review article covers the types of microneedles, mechanism of action of microneedles, methodology of drug delivery via microneedles while mainly focusing on the applications of microneedles and their combination with other enhancing techniques in transdermal delivery of drug and other molecules.

Keywords: Transdermal delivery, Microneedles, Stratum corneum, Iontophoresis, Electroporation, Sonophoresis, Vibratory actuation, Immunologicals, Biopharmaceuticals, Phlebotomy, Diagnosis.

DOI: <http://dx.doi.org/10.22270/jddt.v6i5.1285>**URI:** <http://jddtonline.info/index.php/jddt/article/view/1285>**1. INTRODUCTION**

Administration of drugs can be achieved through various possible delivery routes available including the most common routes like the oral, parenteral, ophthalmic and transdermal route, as well as less explored routes such as nasal, pulmonary and buccal¹. Each of these routes has specific merits and demerits. Over the years transdermal drug delivery systems have shown a potential alternative for the oral and parenteral routes of drug delivery systems overcoming their demerits of drug degradation in the gastrointestinal tract, first-pass metabolism, poor absorption, local irritation and variability in absorption (due to factors like pH, motility, food, mucus layer, etc.), in case of oral drug delivery systems^{2,3} and pain associated with the injections, expertise required to deliver the drug, risk of infection and difficulty in obtaining sustained drug delivery in case of parenteral drug delivery systems⁴. However transdermal technology has limitations due to the inability of a large majority of drugs to cross the skin at the desired therapeutic rates because of the presence of a relatively impermeable thick outer stratum corneum layer. This barrier posed by human skin limits transdermal

delivery only to lipophilic, low molecular weight potent drugs⁵. Different strategies, including chemical and physical enhancers have shown, in many cases, a limited impact to overcome this disadvantage. In recent years, great attention has been paid to microneedles, a hybrid between hypodermic needles and transdermal patches to overcome the individual limitations of both the injections as well as patches. These are micron scale needles assembled on a transdermal patch, able to pierce and create micron sized pores in the skin to effectively deliver drugs in a painless manner through the stratum corneum, thus bypassing the most important permeability barrier of the skin. Microneedles have found application in the pharmaceutical field, not only to facilitate the administration of drugs with minimal invasion, but also for the extraction of biological fluids. Numerous studies have found a marked increase in drug transport through the skin when using microneedle arrays alone, in combination with other enhancers (e.g., iontophoresis or electroporation) or even when including more sophisticated devices such as micropumps. Microneedles have shown great

effectiveness in facilitating the passage of macromolecules, hydrophilic substances, and different carriers such as lipid vesicles and nanoparticles.

2. CONCEPT OF MICRONEEDLES

Microneedles are microstructured transdermal systems, consisting of an array of microstructured projections coated with a drug or vaccine that is applied to the skin to provide intradermal delivery of active agents, which otherwise would not cross the stratum corneum⁶. Microneedles are somewhat like traditional needles, but are fabricated on the micro scale. They are smaller than hypodermic needle, generally one micron in diameter and ranges from 1-100 microns in length.

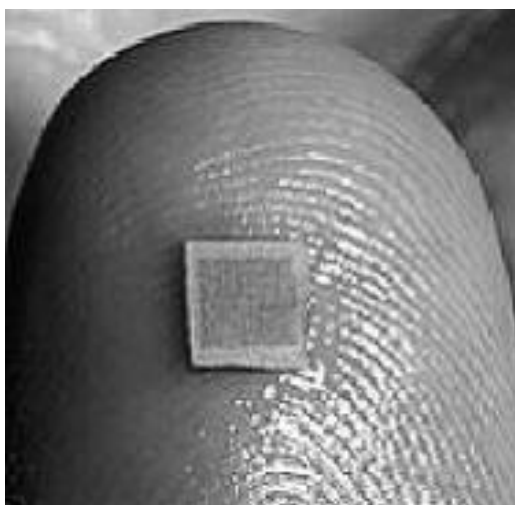


Figure 1: Experimental microneedle array consisting 400 microneedles.

Advantages of Microneedles⁷⁻¹⁰:

- rapid onset of action
- painless administration of the active pharmaceutical ingredient
- Large molecules can be administered
- first-pass metabolism is avoided
- faster healing at injection site than with a hypodermic needle
- patient compliance due to ease of administration
- decreased microbial penetration as compared with a hypodermic needle, the microneedle bypasses the stratum corneum and punctures only the epidermis
- specific skin area can be targeted for desired drug delivery
- Drug can be administered at constant rate for a longer period
- good reproducibility
- good stability and enhanced drug efficacy may result in dose reduction

- good tolerability without long-term oedema or erythema

Disadvantages of Microneedles⁷:

- skin irritation may result because of allergy or sensitive skin
- local inflammation may result if the concentration of drug is high under the skin
- careful use of the device may be needed to avoid particles ‘bouncing off’ the skin surface; if the device is not held vertically, the dose may escape or can penetrate the skin to differing degrees
- the thickness of the stratum corneum and other skin layers varies between individuals and so penetration depth of particles could vary too
- external environment, like hydration of the skin, could affect delivery
- tip of the microneedle may break off and remain within the skin on removal of the patch
- Compressed dermal tissue can block hollow microneedles

Selection of the material for constitution of the microneedle should be based on criteria such as gentle fabrication without damage to sensitive biomolecules, sufficient mechanical strength for insertion into skin and controlled or rapid drug release as per the requirement. Considering the requirement of microneedle to be long enough to penetrate the stratum corneum and at the same time should be short enough to be painless, they have been manufactured in a variety of shapes and sizes, as required for different applications using different materials as mentioned below¹¹. Most microneedle fabrication methods are based on the conventional microfabrication techniques of replicating microstructures using micromoulds, utilizing photolithographic processes, silicon etching, laser cutting, metal electroplating, metal electropolishing etc.

3. MECHANISM OF ACTION

The mechanism of action depends on the type of microneedle design. The general mechanism of delivery via microneedles is based on mechanical disruption of the skin and application of the drug or vaccine within the epidermis, from where it can more readily reach its targeted site of action. The drug is entrapped within the microneedles, which when inserted into the skin and releases the drug into the layers of skin which are highly vascularized. In some cases the needles dissolve within minutes, releasing the entrapped drug at the intended site of delivery from where they reach the target site.

Table 1: Material of construction for microneedles

Material of construction	Example	Type of microneedle produced	Merits	Demerits
Metal	Nickel-iron ¹¹ , stainless steel ¹² , titanium ¹³ ,	Hollow/solid/coated	Good mechanical strength, cost effective	Non-biodegradable, expensive, brittle
Silicon	Silicon dioxide ¹⁴	Solid/hollow	Greater mechanical strength	Expensive, brittle
Glass	-----	Hollow	High drug loading capacity	Non-biodegradable
Biodegradable Polymers	Polylactic acid ¹⁵ , Polyglycolic acid ¹⁵ , Polylactide-co-glycolic acid (PLGA) ¹⁵ , polycarbonate ¹⁶ , Polyvinylpyrrolidone (PVP) ¹⁷	Solid	Cost effective, good resistance, biocompatible	Thermal instability
Non-biodegradable polymers	Polyvinyl acetate (PVA) ¹⁸ , Alginate acid ¹⁸ , Gantrez AN-139, a copolymer of methylvinylether and maleic anhydride (PMVE/MA) ¹⁸ , Carbopol 971 P-NF ¹⁸ , Polyetherimide ¹⁹	Solid	Cost effective, good resistance	Non-biodegradable
Polysaccharides	carboxymethylcellulose ²⁰ , Amylopectin ²⁰ , Maltose ²¹ , Dextran ²² , Galactose ²² , Chondroitin Sulfate ²² , Thermoplastic starch ²³	Solid, dissolving	Rapid drug delivery, biodegradable	Hygroscopic, caramelization

4. TYPES OF MICRONEEDLES

Classification for microneedles usually used in literature is based on the fabrication process: in-plane or out-of-plane microneedles.

Another useful point of distinction is whether the microneedles are solid, hollow, coated or dissolving. The following sections will give an overview of these microneedles.

4.1 Solid microneedles

Solid micro needles are defined as the arrays of projections that are employed for creating holes in stratum corneum and are applied before the application of a drug and then removed afterwards. These can essentially create micron scale holes in the skin, through which drug molecules can easily enter²⁵. These needles are inserted into the skin for specified time period. The micro channels developed by the insertion of micro needles promote the drug transport in to the viable epidermis²⁶.

4.2 Coated microneedles

Coated microneedles are solid microneedles that act as vehicles to carry and deposit drug within the skin or other tissue. This includes coating microneedles with a drug in a formulation suitable for coating and subsequent dissolution. In this way, the desired dose of the drug is delivered into tissue quickly upon insertion of the microneedles. The drug dose that can be administered this way is limited to the amount that can be coated onto the tip and shaft of the microneedles, which is typically less than 1 mg for small microneedle arrays²⁷.

4.3. Dissolving microneedles

Unlike coated microneedles, dissolving microneedles completely dissolve in the skin and thus leave no bio hazardous waste behind after use. These microneedles typically constitute of water-soluble materials such as polymers and sugars that are safe and inert and will dissolve in the skin after insertion. While dissolving microneedles can be used as a skin pretreatment to increase permeability, drugs are often encapsulated inside the microneedle for release into the skin²⁸.

4.4 Hollow microneedles

Hollow microneedles contain a hollow bore in the center of the needle. When inserted into the skin, the hollow bore present bypasses the stratum corneum layer of the skin and produces a direct channel into the other lower layers of the epidermis²⁶. These microneedles are mainly employed to inject the drug solutions directly into the skin²⁹, to carry the drug into the body by diffusion³⁰. Similar to hypodermic injection, hollow microneedles enable pressure-driven flow of a liquid formulation. Pressure, and thereby flow rate, can be modulated for a rapid bolus injection, a slow infusion or a time-varying delivery rate. The apparent advantage of this is that a considerably larger amount of drug can be delivered for a given time, thus opening for applications where relatively large amounts are needed to obtain a therapeutic effect. The liquid formulation may simplify use of existing injectable formulations for delivery using microneedles, but misses the opportunity of solid microneedle delivery methods to administer dry-state drug formulations without reconstitution to improve drug stability and the patient convenience of a patch-based delivery method. Hollow microneedles are also used to remove the fluid from the body for analysis³⁰.

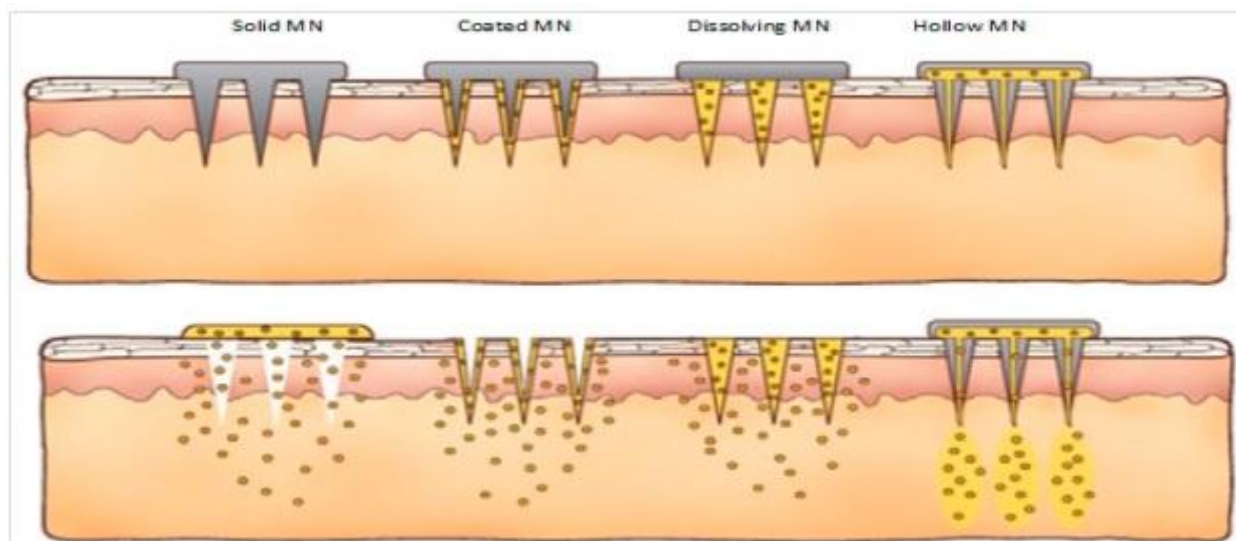


Figure 2: Different types of microneedles: solid, coated, dissolving and hollow.

4.5 Hydrogel-forming microneedles

Hydrogel-forming microneedles are the latest development in microneedle technology. These are arrays of microneedles made up of a swelling material with a drug reservoir attached to the baseplate of the array³¹⁻³⁴. After insertion into the skin, the array absorbs interstitial fluid and swells to form continuous conduits between the dermal microcirculation and an attached patch-type drug reservoir leading to the diffusion of the drug into the skin^{32, 33}. Such microneedles act initially as a tool to penetrate the stratum corneum barrier. Upon swelling, they become a rate controlling membrane. These microneedle arrays are produced mainly using synthetic polymers such as an aqueous polymer gel that can be easily cross-linked by chemical or physical methods³⁵. These materials, once swollen, should maintain structural integrity and be reasonably robust during handling³³. Although hydrogel-forming microneedles are made from polymers, there are distinct differences between these microneedles and the regular dissolving polymer microneedles. Advantages of hydrogel-forming microneedles are that they can be fabricated in a wide range of patch sizes and geometries, can be easily sterilized, resist hole closure while in place and are removed completely intact from the skin³². However more effort should be directed at widening the range of materials that can be used to fabricate these useful transdermal drug delivery systems. Hydrogel-forming MN arrays have been employed to deliver a wide range of different molecules from small molecules^{32-33, 36-37} to high molecular weight compounds^{32-33, 37}. The use of hydrogel-forming microneedle arrays for minimally-invasive extraction and quantification of drug substances and glucose from skin *in vitro* and *in vivo* strongly illustrates the potential of hydrogel-forming microneedles in minimally-invasive patient monitoring and diagnosis³⁸.

5. METHODOLOGY FOR DRUG DELIVERY

A number of delivery strategies have been employed to use the microneedles for transdermal drug delivery.

These include

- Poke and patch
- Coat and poke
- Poke and release
- Poke and flow

5.1 Poke and patch

It involves piercing an array of solid microneedles into the skin followed by application of the drug patch at the treated site. Transport of drug across skin can occur by diffusion or possibly by iontophoresis if an electric field is applied³⁹. This technique was also tried to extract the interstitial fluid to measure the glucose level by non-invasive method⁴⁰.

5.2 Coat and poke

In this approach needles are first coated with the drug and then inserted into the skin for drug release by dissolution. The entire drug to be delivered is coated on the needle itself⁵. Dip and scrape approach is a variation of this approach, where microneedles are first dipped into a drug solution and then scraped across the skin surface to leave behind the drug within the micro abrasions created by the needles³⁹. A limited amount of drug could be coated over the microneedles (only about 1 mg) and extensive optimization was required for uniform coating in this 'coat and poke' approach.

5.3 Poke and release

It involves release of encapsulated drug into the skin from the microneedles made from polymers and polysaccharides that either slowly dissolves or degrades after administration. Advantage of the 'poke and release' approach was that the drug release could

be modulated as per the requirement using a variety of available polymers and polysaccharides²⁸.

5.4 Poke and flow

In this approach first the skin is pierced (external pressure) and then the drug is allowed to flow through hollow microneedles from the reservoir in the patch⁴¹.

A large amount of drug can be administered by poke and flow method. Additionally, pressure-driven delivery adds the possibility to precisely steer the flow rate and to obtain a more controlled delivery.

All of the above-mentioned approaches can be employed to deliver drugs either systemically or at a restricted site (local action).

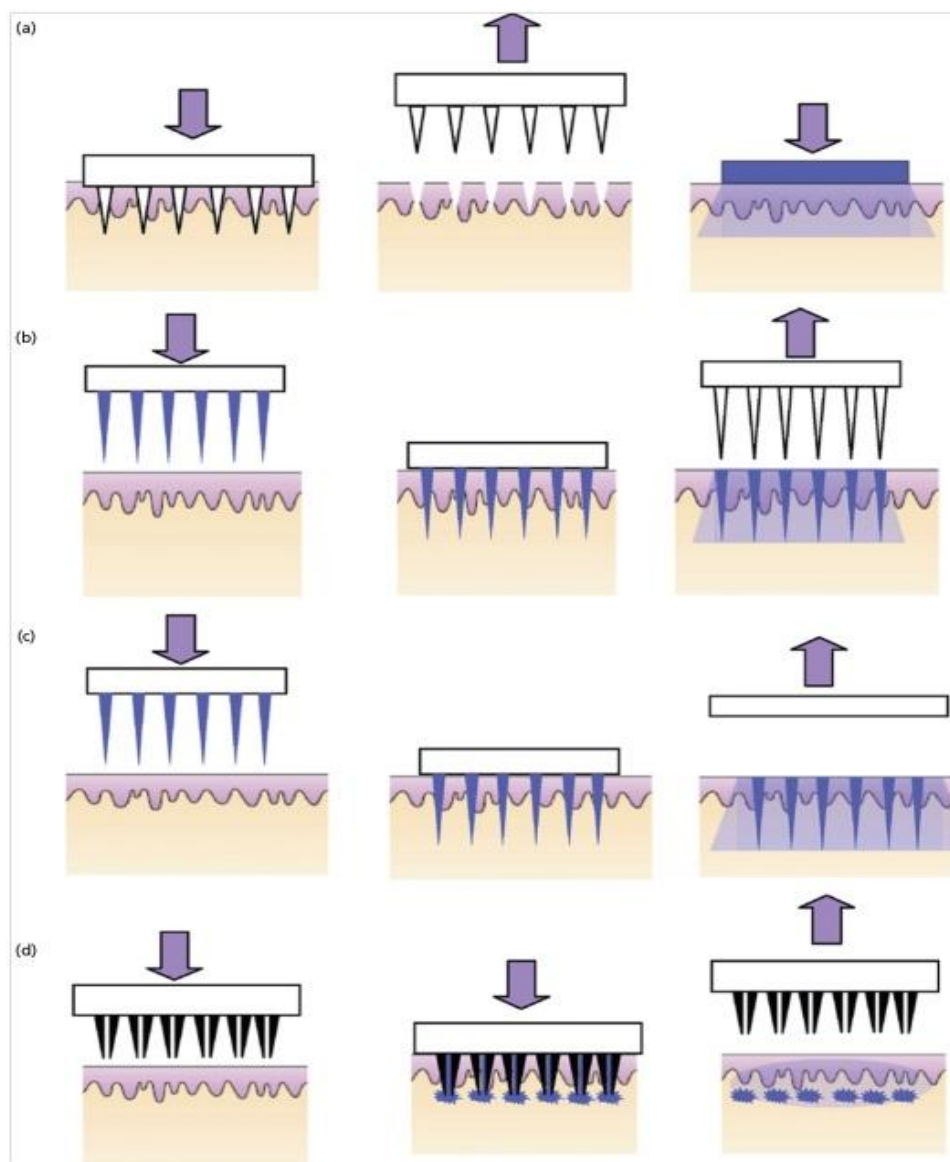


Figure 3 : Methodology for drug delivery by microneedles: (a) ‘poke and patch’ using solid microneedles, (b) ‘coat and poke’ using coated solid microneedles, (c) ‘poke and release’ using polymeric microneedles, (d) ‘poke and flow’ using hollow microneedles.

6. APPLICATIONS OF MICRONEEDLES

Microneedles have been explored for different applications and are extended to many fields. Owing to benefit of piercing in a minimally invasive manner, apart from being an alternative to conventional hypodermic therapy, they have also been employed for ocular, systemic and intracellular delivery. Microneedles can be used to deliver high molecular weight compounds like proteins and peptides, immunobiologicals like vaccines, antibodies etc.⁴²,

bioactive agents or bio macromolecules like insulin, heparin, albumin, growth hormones⁴³. Microneedles have also gained prominent attention in the field of cosmetics and various cosmeceuticals have been used for the treatment of acne, pigmentation, scars and wrinkles as well as for skin toning⁴².

6.1 Immunobiologicals

Attributing to the drawbacks of conventional vaccination procedure of needle phobia and the pain

associated with insertion of needle into the skin, recent studies have focused on development of needle-free vaccination like liquid jet injectors, powder injectors, thermal ablation and microneedles for the administration of immunobiologicals via the subcutaneous, intramuscular or intradermal route for prevention of infectious diseases. Microneedles have an edge over the other methods due to lack of pain, self-administration and quick delivery of vaccine⁴²⁻⁴³. Making use of the microneedles allows vaccines to cross the stratum corneum and stimulate a clinical response. In case of dissolving microneedles, controlled and complete penetration is an important parameter to be considered. These are capable of delivering a small dose, less than several milligrams, of peptides, proteins, vaccines, hormones and organic compounds. Numerous studies have been performed and reported on vaccine delivery using microneedles including human IgG²¹, tetanus toxoid²³, DNA vaccine³⁹, influenza vaccine⁴⁴, hepatitis B vaccine⁴⁵, human papilloma virus vaccine⁴⁶, west Nile virus vaccine⁴⁷, chikungunya virus vaccine⁴⁷, herpes simplex virus vaccine⁴⁸⁻⁴⁹, Bacillus Calmette Guerin vaccine⁵⁰, ovalbumin⁵¹, live attenuated chimeric JE vaccine (ChimeriVax)-JE/Flavivirus vaccine⁵², diphtheria⁵³, malaria⁵³, combination and recombinant protein vaccines for anthrax, botulism, plague and staphylococcal toxic shock⁵⁴.

6.2 Bioactive macromolecules (Biopharmaceuticals)

Owing to the proteolytic degradation and hindered absorption, bioactive macromolecules such as insulin, heparin, and growth hormones are not administered orally. The majority of commercially available biopharmaceuticals are administered via the parenteral route and hence a suitable non-invasive route is desirable. Microneedle arrays have been found to enhance the transport across dermatomed human skin for both low and high molecular weight compounds and also that the length of the microneedles and the depth up to which the microneedles penetrated in the skin had no effect on the transport of either low or high molecular weight compounds⁵⁵. For rapid release as well as controlled release of molecules, an approach of preparing dissolving microneedles constituting water-soluble polysaccharides has been done^{20, 22}. Studies performed and reported on administration of biopharmaceuticals using microneedles include delivery of low molecular weight heparin²², insulin⁵⁶, L-Carnitine⁵⁷, calcein and bovine serum albumin⁵⁸, desmopressin⁵⁹, recombinant human growth hormone and desmopressin⁶⁰, albumin⁶¹, calcein⁶², erythropoietin⁶³, oligonucleotides⁶⁴, porphyrin precursor 5-aminolevulinic acid (ALA)⁶⁵, salmon calcitonin⁶⁶, daniplestim⁶⁷, leuprolide acetate⁶⁸, Parathyroid hormone⁶⁹, human growth hormone⁷⁰ etc.

6.3 Drugs

It is essential for a drug molecule to possess the necessary physicochemical properties to cross the skin barrier. Transport of a drug molecule through the skin and also the rate of transportation are governed by these physico-chemical properties like hydrophilic-

lipophilic balance, solubility, molecular weight, etc. The challenges posed in transdermal drug delivery can be overcome by use of microneedles. Use of microneedles enhances the bioavailability of drugs and can exclude the need for penetration enhancers, which may induce irritation. Microneedles may also reduce the side effects and complications associated with systemic administration. Drugs administered via microneedles include L-Ascorbic acid¹⁹, riboflavin²⁷, galanthamine⁷¹, aspirin⁷², docetaxel⁷³, pilocarpine⁷⁴, methotrexate⁷⁵, prochlorperazine edisylate⁷⁶, lidocaine hydrochloride⁷⁷, ropinirole hydrochloride⁷⁸, ketoprofen⁷⁹, naltrexone with diclofenac sodium⁸⁰, phenylephrine⁸¹, naltrexone⁸², mannitol⁸³, glycerol⁸⁴ etc.

6.4 Phlebotomy

Phlebotomy refers to the withdrawal of blood samples for analysis of specific blood constituents for diagnosis of a disease. Blood samples are generally collected from capillaries by pricking the skin or from veins using evacuated collection tube, depending on the volume of blood required for analysis. These methods are associated with disadvantages such as excessive bleeding, infection, scarring, fainting or feeling light-headed. People may hesitate to give blood due to fear of needles and the moderate pain associated with the procedure. In such case, painless blood sampling using microneedles can be a very good alternative to hypodermic needles⁸⁵⁻⁸⁶. Microneedles situated at a distance of 500–2000 μm in the dermis layer beneath the skin can be used to obtain precise body fluids as well as blood samples from the capillaries. Apart from making the procedure painless, microneedles also reduce the blood sample requirements (up to 200 nanolitres). However the most essential requirement is that the microneedle must penetrate to sufficient depth; hence care should be taken in the design, material selection and dimensions of the microneedle, to ensure penetration at low pressure without breakage. Painless hollow microneedle-based micro sampling can be used instead of traditional methods for glucose estimation in case of disease diabetes which requires frequent monitoring of blood for estimation of glucose concentration or disease severity⁸⁷. Microneedles can also be used for monitoring of therapeutic drug levels⁸⁸. A jagged-shaped, hollow in-plane silicon microneedle resembling the proboscis of a mosquito has been prepared for collection of blood for testing⁸⁹. Arrays of 350 μm long, hollow, out-of-plane microneedles demonstrated in-situ analysis biological fluid extraction through capillary action⁹⁰.

6.5 Diagnosis

The use of microneedles can also be employed in the field of diagnosis. Hollow microneedles, along with quantum dots, help in medical diagnosis. Quantum dots are Nano scale crystals with a light-emitting property. The multiphoton microscopy method could rapidly diagnose cancers or other medical problems⁹¹. Recent research focuses on the development of magnetic nanoparticles along with magnetic microneedle tips those safely collect biomarkers that indicate early-stage

osteoarthritis in knees, hips and other joints⁹². Work has been carried out on development of two sensing device based on hybrid microneedles array for diagnostic and therapeutic applications. Hybrid microneedles having a porous structure were prepared, which can include a variety of biological molecules, as bioprobes or drugs. The first device is an electrochemical sensor where microneedles contain enzymes in their matrix that interact with glucose. The redox reaction with glucose, mediated by ferrocene, creates a charge transfer resulting in a current proportional to the glucose concentration. The second device is a therapeutic tool with optically controlled release of drug. In this case the device includes a porous silicon membrane with a Bragg's mirror, whose reflection wavelength is related to the drug's concentration in the microneedle⁹³. Microneedle sensors fabricated at the end of an endoscope using poly caprolactone (PCL) microneedles coated with poly (3, 4-ethylenedioxythiophene) (PEDOT), functionalized with hemin molecules on the surface were employed for both endomicroscopic imaging and biosensing of colon cancer by real-time electrical detection of nitric oxide⁹⁴. Microneedles have also been developed as sensors for hydrogen peroxide, lactate, dissolved oxygen and glutamate⁹⁵⁻⁹⁷. Microneedles have been employed as bioelectrical interfaces, especially for neural recording and stimulation⁹⁸⁻¹⁰⁶, as well as for electrocardiography (ECG)¹⁰⁷ and electroencephalography (EEG) measurements¹⁰⁸⁻¹⁰⁹.

6.6 Cosmeceuticals

Cosmeceutical industry has shown great interest in microneedle technology, the majority of cosmetic products are lending themselves to microneedle technology for non-surgical and non-ablative treatment of skin conditions such as ageing (wrinkles, lax skin), scarring (acne, surgical), photo damage, hyperpigmentation (age/brown spots) and hair loss (alopecia). The process facilitates and stimulates skin's natural repair without causing permanent epidermal damage. Microneedles can be used for cosmetic applications, mainly for treatment of skin blemishes and the delivery of active cosmetic ingredients¹¹⁰⁻¹¹¹. Marketed microneedles like Derma rollers® and stamps are available for treatment of skin problems as well as to improve looks. MTS Dermaroller® marketed by Clinical Resolution Laboratory is a cosmetic aid possessing needles that penetrate the skin up to a depth of 0.2–0.3 mm. It contains 200 very fine stainless-steel needles that pierce the epidermis, creating a micro-channel effect. Clinical studies from various countries have proven that therapeutic serum absorption is increased by as much as 1000 times when applied using the MTS Dermaroller®¹¹². A hyaluronic acid based dissolving microneedle patch developed for the intradermal delivery of ascorbic acid and retinyl retinoate displayed improved skin appearance in terms of roughness and wrinkle appearance when tested in human volunteers¹¹³. An enhancement in the local delivery of eflornithine (used to reduce facial hirsutism) was observed both in vitro and in vivo when

the skin was exposed to microneedle pretreatment followed by local administration of an eflornithine cream¹¹⁴. Microneedle technology can be used to treat different types of scars by dermabrasion¹¹⁵⁻¹¹⁹. Dermabrasion involves piercing the skin multiple times with microneedles to induce collagen growth. A noticeable improvement was observed in the treatment of patients with dermal scars (striae distensae) using a disc microneedle therapy¹¹⁶. However, similar to the treatment of acne, this treatment too is associated with limitations of skin bleeding and painful treatment. An aesthetic improvement in patients with acne scars was reported with a reduction in scar severity in all subjects when treated with Dermaroller®¹¹⁷. Similar results were obtained for treatment of atrophic facial scars using Dermarollers®¹²⁰. An alternative approach to the use of Dermarollers® for the treatment of acne scars reported is the fractional radio frequency microneedle system. This system was introduced recently as a new device for facial rejuvenation. It involves creating radiofrequency-induced thermal zones. This causes damage to the reticular dermis followed by the dermal thickening. This system was clinically evaluated and the results suggested that this novel technique is efficacious for the treatment of acne scars, as it reduced the severity of the scars in more than 80% of volunteers¹²¹.

7. MICRONEEDLES IN COMBINATION WITH OTHER ENHANCING TECHNIQUES FOR IMPROVING TRANSDERMAL DRUG DELIVERY

In order to improve the skin permeability, different techniques have been developed and employed such as using permeation enhancers, either chemical or physical. These enhancers may exhibit different mechanisms of action however their sole aim is to modify the structure of stratum corneum which is considered as the main barrier to the permeation of substances. Even though the use of permeation enhancers increases the penetration of drugs through the skin, in many cases it proves to be insufficient in achieving the therapeutic levels. To overcome this, several experiments in which two or even more enhancing techniques are combined have been attempted to obtain a synergistic effect, thus achieving the required effect of enhanced drug delivery and better control of drug delivery across the skin. Following section gives an overview of the enhancing techniques.

7.1 Microneedles in combination with Iontophoresis

The technique of transdermal iontophoresis involves the application of a physiologically acceptable low electric current (<0.5 mA/cm²) to the skin and works on the principle of "like repels like", in order to promote the transfer of charged and polar substances across the stratum corneum of the skin¹²². The barrier disruption thus caused by employing iontophoresis in combination with microneedles increases the possibility of variety of drugs that can be administered transdermally. It also allows achieving controlled delivery of drug by controlling the current¹²³.

Iontophoretic transdermal transport mainly involves mechanisms of electromigration and electroosmosis. Electromigration is the movement of ionic species in response to the application of the electric field, and is the most important mechanism for the delivery of ionic drugs. Electroosmosis is the aqueous flow that occurs during the movement of charged species, carrying all the dissolved drugs across the skin. The technique of iontophoresis might be still limited by the molecular size of the drug candidate even though it offers an attractive alternative for the delivery of macromolecules¹²⁴.

7.2 Microneedles in combination with Sonophoresis

Sonophoresis involves application of ultrasound (frequency, 20 kHz to 10 MHz; intensity, up to 3 W/cm²) causing perturbation of lipid bilayer inducing change in the lipid arrangement of the stratum corneum, forming cavitation thus enhancing transportation of drugs across the skin. Drug permeation can be controlled by controlling the frequency of the ultrasound. An increase in the sound frequency from 20 kHz to 1 MHz causes 1000 fold increase in skin perturbation¹²⁵. A synergistic effect on the permeation of molecules through the skin can be achieved by combining sonophoresis with microneedles. Very few studies have been performed on employing this strategy and thus needs to be explored further.

7.3 Microneedles in combination with Electroporation

Electroporation involves application of high voltage short-duration (milliseconds) current (generally 50–1000 V/cm) causing a transitory, localized perturbation of lipid bilayer inducing structural rearrangements of the cell membrane due to the electric fields that are produced during current application. The aqueous pores formed as a result act as the aqueous pathways and provide a local driving force that facilitates the transport of molecules across the stratum corneum. A trans-membrane potential up to 1 kV for 10 ms to 500 ms was used for in-vitro electroporation of stratum corneum¹²⁵. Although this process is useful for delivering large hydrophilic drug species like small molecules, proteins, peptides and oligonucleotides, including biopharmaceuticals with molecular weights greater than 7 kilo Daltons, electroporation was also used for permeation enhancement of larger molecules having molecular weight up to several kilo Daltons¹²⁶. Combining electroporation with microneedles resulted in microneedles that behaved as microelectrodes for electroporation, which eradicated the need for electrodes. This combination provides a synergistic effect on the permeation of molecules through the skin.

7.4 Microneedles in combination with Vibratory actuation

Precise control of insertion force is required for the penetration of a microneedle into the skin and it should not exceed the fracture force of the microneedle. Structure rigidity and miniaturization of microneedles should be well considered and a satisfactory balance

between these two should be maintained. Vibration actuation causes tissue damage via fluid cavitation and thermal damage due to frictional interaction. When this mechanism of vibration actuation is combined with microneedles, a reduction in the microneedle insertion force is achieved as a result. A study employing this combination was performed wherein the combination of vibration actuation and microneedles helped in the preparation of microneedles using metals and polymers with low value of Young's Modulus also the effect of vibratory actuation on microneedle insertion force was evaluated and greater than 70% reduction in the insertion force was observed¹²⁷.

7.5 Microneedles in combination with Vesicles

Several studies have been performed and reported on employing lipid vesicles for the transdermal drug delivery. However these systems are surrounded with controversy related to their delivery mechanism which is reported to be associated with the accumulation of the vesicle and the encapsulated drug in the stratum corneum because of their inability to penetrate the skin deep enough rendering them with little value as carriers for transdermal drug delivery. This paved the way for employing other vesicular systems which are able to penetrate into deeper skin layers, such as niosomes (vesicles composed of nonionic surfactants), ethosomes (vesicles with high alcohol content), transferosomes (vesicles composed of phospholipids as their main ingredient, with 10-25% surfactant and 3-10% ethanol), invasomes (vesicles formed by phospholipids, ethanol and terpenes as penetration enhancers) etc. Combining microneedles with these vesicular systems holds potential for enhanced transdermal drug delivery. Depending on the diameter and height of microneedles, liposomes can be located in different parts of the skin. Results may vary according to the needle geometries. Microneedles of short height render vesicles on the surface of the stratum corneum, while longer microneedles lead vesicles into deeper skin layers.

7.6 Microneedles in combination with Microparticles and Nanoparticles

The versatility of microparticles and nanoparticles for the effective and targeted release of different therapeutic agents to different anatomical regions makes them a good candidate as drug carriers or vehicles for topical and transdermal drug delivery for enhanced drug permeation¹²⁸. Microparticles and nanoparticles are distinct with respect to their size (micrometric and nanometric, respectively), biopharmaceutical properties and their therapeutic applications. These carriers can be constituted of biodegradable polymers or lipid materials. Penetration of nanoparticles through human skin depends on the nanoparticle size and shape, material properties etc. Nanospheres or nanocapsules of different sizes can be obtained by varying the materials employed and the method of preparation. A dramatic increase in cellular uptake can be achieved by reducing the size of nanoparticles. Few nanoparticle systems have a characteristic capability of diffusing into the cells, which makes them candidates to reach the drug target

in a specific cellular compartment. Combining microneedles with these nanoparticulate systems may significantly enhance the transdermal drug delivery wherein microchannels created by the microneedles may prove to be large enough to deliver drug-loaded nanostructures into the skin. Delivery of microparticles into the skin sets a challenge. In such case combining microneedles with these solid particles could facilitate the delivery of the later into the skin. By making use of appropriate microneedle design and insertion methods, even relatively large microparticles can be delivered into the skin. Perhaps the greatest shortcoming of controlled-release microneedles is that limited dose can be administered. Since the drug is encapsulated within microneedles and microneedles are, of course, very small, the maximum total dose that can be administered is likely to be less than 1 mg⁵⁸.

7.7 Microneedles in combination with Micro pumps

Conjunction of microneedles with micro pumps provide precise delivery of drug as the pumps control the flow rate and pressure for delivery of concentrated

drug solution as required. This combination was employed in preparing an integrated system, with micro valves and micro-pumps, which was capable of controlling fluid withdrawal for medical analysis and delivering the drug in response to metabolites levels¹²⁹.

7.8 Pocketed and Grooved Microneedles

Modification of the microneedle surface can be employed for targeted drug delivery to a specific depth in the skin and for loading greater amount of drug onto the microneedle. This can be achieved by applying the protective coat or second drug coat on the same microneedles after filling the first part in the pockets. Such microneedles are known as pocketed microneedles. These can also be obtained by fabricating microneedles with one or more holes cut through the center¹³⁰. Also grooved microneedles that capable of loading greater amount of drug onto the microneedle can be employed for enhanced drug delivery¹³¹.

Table 2: Summary of few results of the delivery of drugs to the skin via microneedles in combination with carriers and/or enhancers

S.N.	Type of microneedle	Enhancer	Carrier	Drug	Model	Result
1	Coated microneedles ²	-----	1- μ m diameter barium sulfate particles and 10- μ m diameter latex particles	-----	Porcine cadaver skin, in vitro	Relatively large microparticles can be delivered into the skin for controlled drug release using appropriate microneedle design and insertion methods
2	Solid stainless microneedle arrays ¹²	Iontophoresis (0.2 mA/cm ²)	Nanovesicles	Insulin	Pig skin, in vitro Rats, in vivo	Combination approach of microneedles and iontophoresis showed significant increase in the permeation of charged nanovesicles. Comparable levels to subcutaneous injection of insulin was achieved
3	Beveled-tip and tapered-cone biodegradable polymeric microneedles ⁵⁸	-----	carboxymethyl cellulose or poly-L: - lactide microparticles	Calcein and bovine serum albumin	Human cadaver skin and test in saline, in vitro	Microneedles capable of encapsulating drug and providing controlled-release delivery in skin for hours to months.
4	Macroflux® titanium or stainless Steel microprojection Array ⁶⁴	Iontophoresis (100 μ A/cm ²)	-----	Oligodeoxynucleotide ISIS 2302 (ODNs)	Hairless guinea pigs, in vivo	Delivering ODNs into and through the skin in therapeutically relevant amounts
5	Maltose microneedles ⁶⁶	Iontophoresis (0.2 mA/cm ² for 1 hr)	-----	Salmon Calcitonin (SCT)	Hairless rat skin, in vivo	Transdermal delivery of SCT was achieved with highest delivery

						flux.
6	Maltose Microneedles ⁶⁷	Iontophoresis (0.5 mA/cm ²)	-----	Daniplestim	Hairless rat skin, in vivo	The combination approach yielded much higher flux values of daniplestim compared to iontophoresis alone
7	Maltose microneedles ⁶⁸	Iontophoresis (0.1 mA/cm ² for 4 hrs)	-----	Leuprolide acetate	Hairless rats, in vivo	The combination approach yielded much faster and higher flux values of leuprolide acetate compared to iontophoresis and microneedles alone respectively
8	Microneedle Array ⁷³	-----	Elastic liposomes	Docetaxel (DTX)	Rat skin, in vitro Porcine skin, in vitro	Microneedle treatment led to increased steady-state flux of DTX from all formulations and shorter lag time when applying elastic liposomes through microneedle
9	Maltose Microneedles ⁷⁵	Iontophoresis (0.4 mA/cm ² for 1 hr)	-----	Methotrexate	Hairless rat skin, in vitro and in vivo	Enhanced transdermal delivery of methotrexate both in vitro and in vivo.
10	DermaRoller® ⁷⁶	Iontophoresis (0.4 mA/cm ²)	-----	prochlorperazine edisylate	dermatomed human skin, in vitro	Significantly enhanced the transdermal delivery of prochlorperazine edisylate
11	AdminPatch® ⁷⁸	Iontophoresis	-----	Ropinirole hydrochloride	Porcine ear skin, in vitro	Continuous iontophoresis in combination with microneedles showed enhanced reflux of Ropinirole hydrochloride
12	DermaRollers® ⁸³	-----	Invasomes	Mannitol	Human abdominal skin, in vitro	Enhanced penetration and permeation of hydrophilic model drugs due to skin perforation by DermaRollers® particularly when combined with invasome formulation.
13	Microneedle roller ⁸⁵	Sonophoresis	-----	Glycerol	Porcine skin, in vitro	The combination method effectively enhanced transdermal delivery of glycerol by accelerating the diffusion rate through the skin barrier.
14	Silicon microneedle electrode array ¹²⁶	Electroporation	-----	Fluorescein isothiocyanate (FITC)-dextran	Male hairless rats, in vivo	Enhanced permeation of FITC-dextran observed as a synergistic effect of the

						combination approach
15	Hollow microneedles ¹²⁷	Vibratory actuation	-----	-----	Excised animal skin, in vitro	Greater than 70% reduction in microneedle insertion force by using vibratory actuation
16	Pocketed solid stainless steel microneedle ¹³⁰	-----	-----	Sulforhodamine, sodium fluorescein, luciferase plasmid DNA, vit B2	Abdominal porcine cadaver skin, in vitro	Rapid release of the model drugs at a targeted depth in the skin.
17	Grooves-embedded microneedle arrays of poly-L-lactic acid ¹³¹	-----	-----	Ovalbumin	Mice, in vivo	Microneedles coated with ovalbumin render higher antibody response as the number and depth of grooves increase
18	Maltose Microneedles ¹³²	Iontophoresis (0.5 mA/cm ²)	-----	Low mol. wt heparin (LMWH)	Hairless rat skin, in vitro	Increased skin permeation of LMWH
19	Microneedle Array ¹³³	Iontophoresis (0.3 mA/cm ²)	-----	FITC-dextrans	Hairless rat skin, in vitro	Enhanced transdermal delivery of dextrans
20	Silicon and glass hollow microneedle array ¹³⁴	Sonophoresis	-----	Calcein and bovine serum albumin (BSA)	Pig skin, in vitro	In vitro results showed a significant improvement in the delivery rate even when large molecular weight compounds were used.
21	Stainless steel & silicon solid microneedles ¹³⁵	Sonophoresis	-----	FITC-dextrans	excised porcine skin, in vitro	Significantly increased skin permeation of FITC-dextrans.
22	Stainless steel solid microneedles ¹³⁶	Sonophoresis	Cross-linked hydrogel of NaCMC and gel emulsion	Lidocaine	Porcine skin, in vitro	Slight increase in the permeability of lidocaine due to the combination approach.
23	Dissolving microneedles ¹³⁷	Electroporation	-----	p2CMV mL-12	Mice, in vivo	in-situ cutaneous transfer of p2CMV mL-12 to successfully treat B16F10 subcutaneous tumors
24	Silicon microneedle electrode arrays ¹³⁸	Electroporation	-----	-----	-----	A uniform drug release through hollow microneedle electrodes into tissue is expected to be achieved during electroporation.
25	Coated microneedle array ¹³⁹	Electroporation	-----	Plasmid DNA	Mice, in vivo	Robust antibody responses.
26	Solid microneedles ¹⁴⁰	-----	L595 vesicles (composed of sucrose-laurate ester & octaoxyethylene-laurate ester) and sPC	hepatitis B surface antigen (HBsAg)	Mice, in vivo	Successful transcutaneous immunization (TCI) of hepatitis B surface antigen (HBsAg)

			vesicles (composed of soybean- phosphatidylchol ne and Span- 80)			
27	Solid microneedles ¹⁴¹	-----	anionic surfactant-based vesicles	diphtheria toxoid	BALB/c mice, in vivo	Enhancement in the immunogenicity of topically applied diphtheria toxin due to microneedle pretreatment
28	Dermaroller® ¹⁴²	-----	liposomes	-----	Human skin, iv vitro	Evaluation of potential of Dermaroller®, with different needle geometries, to deliver a lipophilic fluorescent compound encapsulated in liposomes across the human skin
29	Nanopore turbo roller ¹⁴³	-----	nanoliposomes	sepia melanin	Human skin, iv vitro	Accelerates the delivery of melanin into hair structures allowing an even absorption, larger pigment deposits, and deeper penetration of the formulation into the hair.
30	Silicon microneedle arrays ¹⁴⁴	-----	Polystyrene nanospheres	-----	Human skin, iv vitro	Potential for significant enhancement the intra/transdermal delivery of nanoparticle formulations.
31	Solid silicon microneedle arrays ¹⁴⁵	-----	poly(d,l-lactic- co-glycolic acid) (PLGA) nanoparticles	-----	Human skin, iv vitro	Permeation of nanoparticles into the skin was enhanced by microneedles
32	Microneedle arrays ¹⁴⁶	-----	Microparticles	Gene	-----	Microneedles increase the penetration depths of the particles.
33	Hollow microneedle ¹⁴⁷	-----	nanoparticle and microparticle suspensions	-----	whole rabbit, pig, and human eyes ex vivo	Microneedles were shown for the first time to deliver nanoparticle and microparticle suspensions into the suprachoroidal space of rabbit, pig and human eyes.
34	Solid silicon microneedle Arrays ¹⁴⁸	-----	Polystyrene latex nanospheres	-----	Human cadaver epidermis, in vitro	A significant number of latex nanoparticles cross the skin.
35	Silicon microneedle arrays ¹⁴⁹	-----	Fluorescent latex nanospheres	-----	Human skin, in vitro	Topical application of nanospheres followed by the microneedle treatment resulted in the migration of the

						charged fluorescent particles into the microchannels, & to the cells of the viable epidermis
36	Silicon-based Microneedles ¹⁵⁰	-----	Fluorescent polystyrene nanospheres	Lipid: polycation : pDNA (LPD) nonviral gene therapy vectors.	Heat-separated human epidermal sheets, in vitro	Microneedle membrane treatment resulted in enhanced diffusion of fluorescent polystyrene nanospheres
37	Silicon Microneedles ¹⁵¹	-----	Hydrogel / Fluorescent Nanoparticle Loaded	-----	Human skin, in vitro	Microneedle treatment resulted in successful targeting of nanoparticles within the viable epidermis
38	Hollow Microneedles ¹⁵²	-----	Nanospheres of PLA with Nile red and latex fluorescein labeled microspheres	-----	Human corpse sclera, in vitro	Successful delivery of soluble molecules and nanoparticle Suspensions into the sclera.
39	Pocketed stainless steel microneedles ¹⁵³	-----	-----	Botulinum toxin A (BT)	Excised human skin, in vitro	Microneedles provide less invasive alternative that is suited to delivery of large proteins such as BT. Microneedles were not only able to accommodate therapeutic doses of a model protein but they were also able to deliver an inactivated form of the toxin into human skin.
40	Coated microneedles ¹⁵⁴	-----	Poly(lactic-co-glycolic) acid (PLGA) nanoparticles	Doxorubicin	porcine cadaver buccal tissues, in vitro	Coated microneedles provide uniform and effective delivery of drugs to localized oral cancers.
41	Silicon microneedles ¹⁵⁵	-----	Hypoxia-sensitive hyaluronic acid-based vesicles	Insulin	Mice, in vivo	Glucose responsive vesicles loaded microneedles provided convenient, painless, and continuous administration of insulin. They were found to be responsive to glucose challenge and also efficiently minimized the risk of hypoglycemia in the in vivo glucose tolerance test.

42	Silicon microneedles ¹⁵⁶	-----	Lipid microparticles	Quercetin	Pig skin, in vitro	Compared to intact skin, a marked increase in quercetin levels permeated into the stratum corneum and viable epidermis was achieved when skin was treated with the flavonoid-loaded LMs in combination with microneedle arrays
43	Solid silicon microneedles ¹⁵⁷	-----	Poly-D, L-lactic acid (PDLLA) nanoparticles	Ketoprofen	Porcine skin, in vitro	An enhanced flux of ketoprofen was observed in the skin treated with silicon microneedles over a prolonged period of time.

CONCLUSION

Various research studies carried out on microneedles are evident enough to state that microneedles are the efficient and potential transdermal delivery systems. They have been gaining a lot of importance due to their key advantages over the conventional delivery systems of being safe, convenient and painless. Microneedles have proved to be the solution for overcoming the barrier imposed by the stratum corneum, thus broadening the number of drugs and other molecules to be administered by this route. Extensive work has been carried out to create advances and to explore the wide applications of microneedles. Apart from playing the vital role in transportation of numerous drug molecules including poorly permeable ones, macromolecules and biopharmaceuticals, microneedles have also been applied in phlebotomy, diagnosis and cosmeceuticals. Numerous studies have been performed and reported on the synergistic effect of combining microneedles with other techniques for enhanced drug delivery such as sonophoresis,

electroporation, iontophoresis, vibratory actuation etc. and have turn out to be beneficial in efficient transportation of drug and other macromolecules across the skin. Conjugating microneedles with carriers such as vesicles, nanoparticles, microparticles etc. allows achieving a controlled delivery of drug molecules. Precise drug delivery has been possible by employing microneedles in combination of micro pumps while greater amount of drug can be delivered by loading into the pocketed and grooved microneedles. Thus it was concluded that microneedles are more efficient as compared to the hypodermic needles and the other conventional delivery systems.

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*** Corresponding Author**

Smita Nayak, Department of Quality Assurance, Gahlot Institute of Pharmacy

E-mail: smitanayak125@yahoo.com, Tel: 022-27550816

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