AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Microneedle based drug delivery system and advantages in ocular drug delivery.

GuravAtul S[1*], DaptardarTanvi A.[1], KhakalNilima N.[2], GavhaneYogesh N.[1], SayyadFahim J.[1]

1.Government College of pharmacy,Karad, Maharashtra, India- 415 124.

2.Swami Vivekanand Nursing Home Trust's College of B. Pharmacy, Shivaji Nagar, Rahuri, Tal- Rahuri, Dist- Ahamednagar, Maharashtra, India-413 705.

ABSTRACT

The stratum corneum of the skin or the sclera/cornea of the eye, the outermost layer of the skin and others has the principal barrier to topically-applied medications. Thus, many therapeutic agents are limited by their inability to reach the systemic circulation. Transdermal delivery of hydrophilic drugs and macromolecular agents of interest is problematic. Therefore, facilitation of drug penetration through this barrier may involve by-pass or reversible disruption of molecular architecture. Microneedles, when used to puncture skin, will by-pass the barrier and create transient aqueous transport pathways of micron dimensions and enhance the transdermal permeability. These micropores are orders of magnitude larger than molecular dimensions, and, therefore, should readily permit the transport of hydrophilic macromolecules. This microneedle based drug delivery system will useful for the eye treatments by avoiding drawbacks of topical administration of drug, multiple drug treatment and other dosage form intended in the eye. This review gives the details of history, various types of microneedles, drug delivery, fabrication methods, manufacturing, evaluation and, importantly, investigations of clinical safety of microneedles.

Keywords: Microneedle, Ocular drug delivery system, Fabrication of microneedles.

*Corresponding Author Email:atulgurav_25@rediffmail.com

Received 30 June 2014, Accepted 08 July 2014

Please cite this article in press as: Atul G *et al.*,Microneedle based drug delivery system and advantages in ocular drug delivery.American Journal of PharmTech Research 2014.

INTRODUCTION

The eye is carefully organized to allow light transmission in order to provide sight. The tissue and fluids that make the eyes are centered on this function as well as the need to keep the eye free from diseases[1]. The eye is a spherical structure with a wall consisting of three layers; the outer sclera, the middle choroid layer, Ciliary body and iris and the inner nervous tissue layer retina. The sclera is tough fibrous coating that protects the inner layers. It is white except for the transparent area at the front, the cornea which allow light to enter the eye. The choroid layer, situated inside the sclera, contains many blood vessels and is modified at the front of the eye as pigmented iris. The iris is the colored part of the eye in shades of blue, green, brown, hazel, or grey[2]. This shows in following figure. 1.

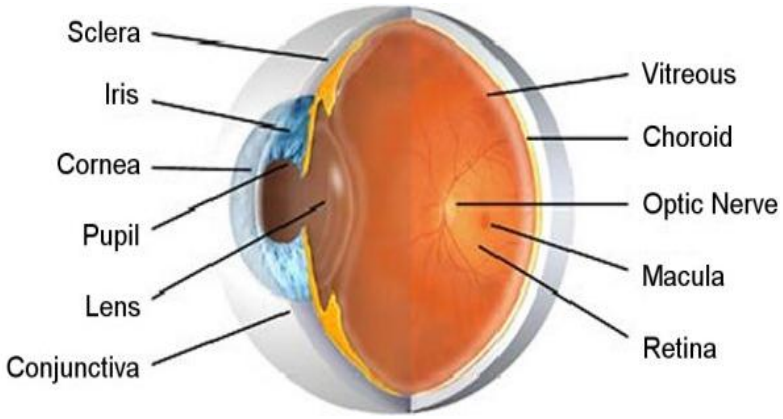

Figure. 1: Human eye

Advantages:

- Possibility of targeting internal ocular tissue through non-corneal routes
- Reduction of systemic side effects and thus reduced adverse effects.
- Administration of an accurate dose in the eye, which is fully retained at the administration site, thus a better therapy.
- Increase ocular residence, hence, improving bioavailability.
- Reduction of the number of administration and thus better patient compliance.
- Possibility of providing a prolonged drug release and thus a better efficacy.
- Lower incidence of visual and systemic side effects.
- Increased shelf life with respect to aqueous solutions.
- Exclusion of preservatives, thus reducing the risk of sensitivity reactions

Novel drug delivery system:

Novel drug delivery system is a method of delivering drugs to a patient by applying new ideas used in controlling pharmacokinetics, pharmacodynamics, non-specific toxicity, immunogenicity,

and other factors. Different methods of novel drug delivery systems are liposomes, colon delivery, cubosomes, colloidosomes, ethosomes etc. Advantages of novel drug delivery system are as follows:

- Provides selective passive targeting to tissues.
- It may reduces first pass metabolism
- Increases efficacy and therapeutic index of drugs.
- Delivery of large molecules is possible and it has higher patient compliance[3].

VARIOUS EYE DISEASES ARE AS FOLLOWS:

Combined Anterior and Posterior Segment Diseases

Endothalmititis, uveititis, and glaucoma are combined anterior and posterior segment diseases.

Degenerative Diseases

The two other classes of visually significant retinal diseases are degenerative and tumorigenic diseases: retinitis pigmentosa, Leber's congenital amaurosis, and the chorioretinal tumors, which include retinoblastoma, choroidal melanoma, and intraocular lymphoma[4].

Drugs or dosage form of drugs needed for administration in eye / systematically:

Some of drug and dosage form are explained as follows. It shows the necessity of microneedle based drug delivery system.

Topical administration

It means the administration of the drug at the site of action directly. It includes the following parts

- Eye drops

Principally absorbed through the cornea but absorption through conjunctival mucosa also occurs, giving rise to systemic effects. Drops may be in solution form (clear - eg, anaesthetic drops) or in suspension (cloudy - eg, steroids).

- Eye ointments

Ointments allow a prolonged contact time; therefore, less frequent applications are required (good for night use).Ointments allow a prolonged contact time; therefore, less frequent applications are required (good for night use).

- Eye lotions

These are solutions used for irrigation of the conjunctival sac (to flush out particles and chemical irritants). Sterile normal saline is the norm but clean water will do in an emergency.

Multiple drug treatment

Topical and systemic treatment: check for duplication (eg, beta-blockers) and drug interactions (eg, carbonic anhydrase inhibitors and loop diuretics).

Avoiding microbial contamination

Use single application packs in the surgery clinic/emergency department (eg, fluorescein drops).

- Local injections
- An anaesthetic drop will be administered.

MICRONEEDLES:

Introduction and definition.

The term microneedle is used to describe a class of micrometer scale needles that can be used in a variety of ways to deliver drugs locally to the body. "Microneedles can be defined as solid or hollow cannula with an approximate length of 50-900 microns and an external diameter of not more than 300microns". [5]

History:

Microneedles were first proposed in 1976, the technology needed to make needles of micron dimensions was not available until 2000s. Since then using the low-cost mass-production tools of the microelectronics industry, needles have been fabricated out of silicon, metals and other materials. Microneedles are supposed to be more efficient in transporting drug across the skin as compared to other devices. Active research in the field of microneedles began in mid 1990's, largely through three isolated efforts operated in parallel at BecktonDikinson (BD), Alza corporation and Georgia institute of technology[6].

Advantages of micro needles[7]:

1. Large molecules can be administered.
2. Painless administration of the active pharmaceutical ingredient.
3. Faster healing at injection site than with a hypodermic needle.
4. Decreased microbial penetration as compared with a hypodermic needle, the microneedle punctures only the epidermis.
5. Enhanced drug efficacy may result in dose reduction.
6. Rapid drug delivery can be achieved by coupling the microneedles with an electrically controlled micro pump

Disadvantages of micro needles[8]:

1. 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 differing degrees.
2. The thickness of the stratum corneum and other skin layers varies between individuals and so penetration depth of particles could vary too.

3. The external environment, like hydration of the skin, could affect delivery.
4. The tip of the microneedle may break off and remain within the skin on removal of the patch.
5. Compressed tissues can block hollow micro needles.

VARIOUS TYPES OF MICRONEEDLES[9]:

Depending on materials used in their fabrication, types of micro needles are as follows:

Solid microneedles:

Solid microneedles are penetrating into the skin or scrape the skin by making holes. Through this holes drug can be transported easily. The fabrication of microneedles has given focus on providing sufficient mechanical strength reducing the strength required to insert microneedles by increasing their sharpness and formulating them with various materials of choice.

Silicon microneedles:

Short silicon microneedles have been prepared using a silicon dry-etching process based on reactive ion etching with a chromium mask, as well as isotropic etching in an inductively coupled plasma etcher.

Metal microneedles:

Metal microneedles have been prepared by three-dimensional laser ablation, laser cutting, wet etching and electroplating methods. Two dimensional microneedles have also been prepared by electroplating or electro-less plating.

Dissolving microneedles:

Polymer microneedles have been developed to completely dissolve in the skin and there by leave behind no biohazardous sharps waste after use. These microneedles are typically made solely of safe, inert, water soluble materials, such as polymers and sugars that will dissolve into the skin after insertion.

Hollow microneedles:

Hollow microneedles provide a defined conduit for drug delivery into the skin or other tissue. Similar to hypodermic needles, hollow microneedles enable pressure-driven flow of a liquid formulation.

Ceramic microneedles:

Ceramic microneedles have been fabricated using ceramic micro molding and sintering.

Drug Delivery through Microneedles:

Poke and apply:

One of the strategies is to simply insert solid microneedles in vivo a tissue and remove the microneedles leaving behind channels or pores that make the tissue more permeable. It is a two step procedure of delivering drug:

- Creation of the channels and
- Application of the formulation to be delivered.

Coat and poke method:

This approach requires the drug formulation to be coated on the surface of a microneedle. Upon insertion of the coated microneedle into a wet tissue, the coating formulation dissolves off of the microneedle and the microneedle can be removed, leaving the coating formulation inside the tissue within the channels created by the microneedles.

Poke and release:

This approach involves loading a drug formulation within a microneedle and leaving the microneedle in the tissue as it releases the loaded drug formulation into the tissue[10].

Poke and flow:

A hollow microneedle is inserted within a tissue and a liquid formulation can be injected through the hollow cannula microneedle for delivering within a tissue at depths of less than a millimeter.

Dip and scrape:

Microneedles are first dipped into a drug solution and then scrapped across the skin surface to leave behind the drug within micro abrasions created by the needles[11].

Fabrication of Microneedles:

Individual small needles have been hand crafted for research purposes for decades and already in the 1970's low cost micro needle arrays were thought of for drug delivery. In 1990's the microelectronics industry provided the micro fabrication tools needed to make microneedles suitable for pharmaceutical application. Each of differed microneedle design enabled drug delivery by different mechanism[12]. As-

- Solid microneedles can be used as a skin pre-treatment.
- Pores created by penetration of solid microneedles shows slow diffusion of drug in skin.
- Microneedles may be made of water-soluble or biodegradable polymer that encapsulates the drug and these microneedles completely dissolve or degrade in the skin thereby releasing the encapsulated drug[13, 14].

Fabrication of solid microneedles:

Fabrication of solid microneedles has focused on providing sufficient mechanical strength through

choice of microneedle material and geometry and reducing the force needed to insert microneedles into tissue by increasing tip sharpness. Solid microneedles have been fabricated out of various materials like silicon, non-degradable polymers, bio-degradable polymers [polyglycolic acid], stainless steel, titanium, nickel and ceramics[15, 16].

Fabrication of silicon microneedles:

Micro-electromechanical systems (MEMS) technology utilizes a number of tools and methodologies to create small three-dimensional (3D) structures, with dimensions ranging from sub-centimeter to sub-micrometer[17].

Lithography

Both in microelectronics and micromachining, fabrication starts with lithography which is the technique used to transfer the master pattern onto the surface of a substrate (e.g. silicon wafer), previously coated with a photosensitive material, by selective exposure to a radiation source (e.g. UV light). The most widely used type of lithography is photolithography[18].

Thin-film deposition on substrate

One of the basic steps in the micro-electromechanical systems (MEMS) process is the deposition of a thin film on the substrate surface (e.g. silicon wafer). These films can then be patterned using photolithographic techniques and suitable etching techniques. Common materials include silicon dioxide (oxide) and silicon nitride (nitride) . Thin-film deposition on the substrate can be also be achieved by using a wide range of materials, which include noble metals such as gold[19].

Etching

It is necessary to etch the thin-films previously deposited and/or the substrate itself. In general, there are two classes of etching processes: wet etching and dry etching.

Wet etching is theprocess in which the material is removed by immersing the wafer in a liquid bath containing a chemical etchant. The two main wet etching techniques are isotropic and anisotropic etching.

Dry etching is the form of etching is carried out at low pressure in the presence of inert or reactive gases. Dry etching is categorized into two main types: reactive ion etching (RIE), which involves chemical processes, and ion-beam milling, which involves purely physical processes[20].

Manufacturing methods of metal and other types of microneedles:

A number of approaches have been investigated for fabricating metal microneedles, such as electroplating, photochemical etching, and laser cutting. The simplest form of solid metal microneedles were made by assembling stainless steel wires of 200 μm diameter and 300 μm height, where the tips were cut tangentially to obtain sharp tips. Some utilized a novel

microneedledevice called a Derma Roller, with different stainless steel needle lengths (150, 500, and 1500 μm) coming out from a cylindrical assembly containing 24 circular arrays of eight needles each (192 needles in total). Martanto fabricated arrays of MN shafts from stainless steel sheets (75 μm thick) using an infrared laser, where the shape and orientation of the arrays were drafted by AutoCAD software[21, 22].

Manufacturing methods for polymeric microneedles

Polymeric microneedle fabrication and reproducibility is considerably more cost-effective when compared to that of typical micro-electromechanical systems (MEMS) processes. For achieving accuracy in production of micro-scale dimensions of polymer microneedles, a variety of mould-based techniques, such as casting, hot embossing, injection molding, and investment molding have been investigated. Polymeric materials which have been efficiently fabricated into microneedles include; poly methylmetha-acrylate, poly-L-lactic acid, poly-glycolic acid , and poly-lactic-co-glycolic acid , cyclic-olefin copolymer, poly vinyl pyrrolidone, and sodium carboxymethyl cellulose. Sugars have also been used to fabricate the microneedles, such as galactose, maltose, and dextrin[23].

EVALUATION OF MICRONEEDLES[24]

Scanning electron microscopy can be used to determine the base radius, tip radius and wall thickness of the microneedles. Interfacial area can be calculated in two ways:

(1) The annular surface area, A_a; at the needle tip

- $A_a = \pi(r_t t - t^2/4)$……equation (1)

And (2) the full cross-sectional area, A_f; at the needle tip

- $A_f = \pi r_t^2$…… Equation (2)

Needle wall angle, α, is calculated as

- $\alpha = \tan^{-1}(r_b - r_t/h)$…….. equation (3)

Where r_t is the outer radius of the microneedle tip, r_b is the outer radius at the needle base, t is the wall thickness and h is the height.

Functional capacity test

The test setup consisted of a syringe pump system with a dye-filled syringe, a polymer tube and microneedle array. This syringe pump system is used to examine the formation of the microneedle lumens by allowing dye to flow from the syringe to the microneedle orifice.

Measurement of insertion force into human skin

A displacement–force test station was used to measure the force applied to a needle, needle position and skin resistance during the sequence of the needle's translation, deflection of tissue around the needle and insertion into the skin of human subjects. A drop in electrical resistance of the skin is used to identify needle penetration since visual observation of needle insertion is extremely difficult.

Margin of safety

The margin of safety is the ratio between the force required for piercing the stratum corneum and the force at which microneedles broke. For compressive failure force measurement, Enduratec station is used in which microneedles are placed between punch and load cell[25].

Measurement of fracture force

The force required for mechanical fracture of a microneedle was tested by employing an axial load test station that drove the microneedle against a flat block of aluminum at a rate of 0.01 mm/s until a preset displacement of 500 µm is reached.

Penetration/diffusion test

In-vitro/ex-vivo tests are performed on isolated animal or human dermatomed skin to study penetration or diffusion of drug from a dosage form to its site of application. These tests can also be used to compare the depth of penetration of the molecule[26].

Transepidermal water loss (TEWL)[27]

TEWL can be determined by employing a diffusion cell and intact animal skin

Biological safety test[28, 29]

Extraction of chemicals from microneedles was done by immersing microneedles in physiological saline at 37°C for 72 hrs. The extract was then directly applied on shaved intact human skin for checking dermal irritation. If the test result are negative, it confirms the biological safety of microneedles.

APPLICATIONS OF MICRONEEDLES:

The microneedle drug delivering system can be used for treatment of various diseases in relation to genetics, malignancies and other infectious diseases. Use of microneedles is a better technique as compared to microinjections as many cells can be treated at once. Various applications of microneedle drug delivery system are as follows:

Immunobiologicals[30]:

Microneedles form transient conduits and enhance passage of the vaccine molecule across the skin barrier. Using microneedles, vaccines are able to cross the stratum corneum and stimulate a clinical response.

Bioactive macromoleculs[31]:

Microneedle arrays enhanced the transport across dermatomed human skin for both low and high molecular weight compounds. Insulin, heparin and other growth hormones can be administered through this technique.

Drugs[32]:

The bioavailability of drugs can be increased, with reduced side effects and complications associated with intraocular injection and systemic administration, by the use of coated microneedles.

Phlebotomy[33]:

Phlebotomy is the withdrawal of blood for diagnostic purpose. Microneedles can be used to obtain precise body fluids as well as blood samples from the capillaries, which are situated at a distance of 500–2000 µm in the dermis layer beneath the skin.

SAFETY ISSUES OF MICRONEEDLES[34, 35]:

Microneedles have been used for the safe and efficient delivery of drugs and vaccines by creating reversible micro channels in the skin. Disruption of the stratum corneum by using conventional needles for the delivery of drugs and vaccines may cause pain, bleeding, skin irritation, skin redness and infection.

Applications were found to be tolerable. Efficacious vaccination could be achieved using microneedles as compared with conventional intramuscular delivery of vaccines like influenza vaccine and rabies vaccine. Increase in length and number of microneedles causes slight increase in the pain experienced.

Micro channels created by needles may cause chances of infection at the injection site because of permeation of pathogenic microbes or any toxic substances.

Challenges in development of microneedle[36]:

1] Safety issues are to be taken into considerations before developing microneedles for delivering of small and large molecules.

2] Metallic microneedles used in delivering drug may retain traces of metal which may cause irritation, erythma, swelling etc.

3] Variations in thickness of skin cause variations in bioavailability of drugs and this factor should be considered in manufacturing microneedles.

CASE STUDY[37, 38]

Various Experiments Are Being Performed To Judge The Efficiency Of Suprachoroidal Drug Delivery System Using Microneedles Over Other Methods Of Drug Delivery System. Following Is

A Brief Extract Demonstrating The Efficiency Of Microneedles Over Other Methods Of Drug Delivery from the work:

Method:

Suprachoroidal injection, a new approach for drug delivery to the posterior segment of the eye was validated using a 34 G needle and Indian ink injections in Sprague Dawley rats, followed by histology. Delivery of NaF (Sodium fluoride) was compared in Sprague Dawley rats after suprachoroidal, posterior subconjunctival, or intravitreal injections. NaF levels were monitored noninvasively up to 6 hours using Fluorotron Master™, an ocular fluorophotometer Pharmacokinetic parameters were estimated using WinNonlin.

Results:

Histological analysis indicated localization of Indian ink to the suprachoroidal space below sclera, following injection. NaF delivery to choroid-retina was in the order: suprachoroidal>intravitreal>posterior subconjunctival injection. Peak NaF concentration (C_{max}) in choroid-retina was 36-fold ($p = 0.001$) and 25-fold ($p = 0.001$) higher after suprachoroidal (2744±1111 ng/ml) injection when compared to posterior subconjunctival (76±6 ng/ml) and intravitreal (108±39 ng/ml) injections, respectively. NaF exposure ($AUC_{0–360min}$) to choroid-retina after suprachoroidal injection was 6-fold ($p = 0.001$) and 2-fold ($p = 0.03$) higher than posterior subconjunctival and intravitreal injections, respectively. Choroid-retina T_{max} was observed immediately after dosing with suprachoroidal injections and at 10 and 27.5 minutes, respectively, with subconjunctival and intravitreal injections.

Thussuprachoroidal injections are possible in a rat model using microneedles. Suprachoroidal injections resulted in the highest bioavailability, that is, the extent and rate of delivery of NaF to choroid-retina, when compared to intravitreal and posterior subconjunctival injections.

FUTURE TRENDS:

Microneedles composed of biodegradable and biocompatible materials have been explored. Microneedles approach of drug delivery is currently being evaluated for a number of drugs, but extensive studies would be required to enhance the application of these delivery modes in the clinical setup. Results from several researches suggest that microneedles are a promising, possible powerful technology for the administration of therapeutics into the skin. Fewer issues like microneedle penetration, various ways of limiting the visco-elasticity of the skin, coating the needles and further improving needle array design should be studied in depth.

CONCLUSIONS

Drug delivery to eyes using microneedles is a novel approach of delivering drugs through ocular route. It is convenient, painless, and less invasive alternative to injection and it can be used as a common method for administering large proteins and peptides, antibiotics, vaccines in low manufacturing cost. Microneedles overcome the limitations of other routes of drug administration to eyes. There is no molecular size limit, no molecular electrical charge requirement and no specific formulation pH restraint. Over conventional drug delivery system, this is more potent and can be used for sustained release.

REFERENCES:

1. Alm A, Nilson SF. Uveosceral Outflow-a Review. Experimental eye research 2009; 760-768.
2. Chein YW. Occular Drug delivery and delivery systems. Special edition, 269-296.
3. Kalagar AA, Nitave SA. Approach on Novel Drug Delivery System. World Journal of Pharmacy and Pharmaceutical Sciences 2014; 2: 3449-3461.
4. Edelhauser HF, Rowe-Rendleman CL, Robinson MR et al. Opthalmic Drug Delivery Systems for the Treatment of Retinal Diseases. Basic Research to Clinical Application-Invest Opthamol 2010.
5. Donnelly RF, Majithiya R, Singh TR, Morrow DI, Garland MJ, Demir YK, et.al.. Design, Optimisation and Characterization of Polymeric microneedle arrays prepared by a novel laser-based micromoulding technique. Pharm Res 2011; 41-57.
6. Ai Ling Teo, Christopher Shearwood, KianChye Ng, Jia Lu, ShabbirMoochhala,. Transdermal microneedles for drug delivery applications. Mater sciEng 2006; 151-154.
7. Windera.G , Johnson J, Kim L, Libiran L, Nyam K, Daddona PE, et al.. Effect of delivery parameters on immunization to ovalbumin following intra cutaneous administration by a coated microneedle array patch system. Vaccine 2006; 1653-1664.
8. Gerstel MS, Place VA. Drug Delivery Device. United States Patent1976; 964, 482.
9. Yeu-Chun Kim, Jung-Hwan Park, Mark R.Prausnitz. Microneedles for drug and vaccine delivery. Advanced drug delivery reviews 2012; 1547-1568.
10. V.PShah. Transdermal Drug Delivery. Marcel Dekker. Inc., 2nd edition. New York, 200; 3365.
11. Barry B, Williams A. Penetration enhancers. Advanced Drug Delivery ReviewS, 2004; 603-618.

12. Bariya SH, Gonel MC, Mehta TA, Sharma OP. Microneedles: an emerging transdermal drug delivery system. Pharm Pharcol 2012; 11-29.
13. Vandervoot J, Ludwig A. Microneedles for transdermal drug delivery:aminireview. Front Bioscience 2008; 13: 1711-1715.
14. Madou MJ. Fundamentals of microfabrication and nanotechnology, CRC press, Boca Raton 2011.
15. Henry S, McAllister DV, Allen MG, Prausnitz MR. MicrofabricatedMicroneedle: a novel approach to transdermal drug delivery. Pharm Sci 1998; 87: 922-925.
16. Moon SJ, Lee SS, Lee HS, Known TH. Fabrication of microneedle array using LIGA and hot embossing process. MicrosystTechnol 2005; 11: 311-318.
17. Li GH, Badkar A, Nema S, Kolli CS, Banga AK. In vitro transdermal drug delivery of therapeutic antibiotics using maltose microneedles. International journal of pharmacy 2009; 368: 109-115.
18. Chandrashekaran.S, Brazzle JD, Frazier AB. Surface micromachined metallic microneedles. MEMS 2003; 281-288.
19. Clark LD, Edell DJ, Hyannis MA. KOH: HO etching of (110) Si, (111) SiO2, and Ta: an experimental study; Proceedings of the IEEE. Micro-Robots and teleoprators workshop 1987.
20. Daddona PM. Transdermal technology development for the delivery of therapeutic peptides and proteins. Drug delivery Tech 2002.
21. Gill HS, Prausnitz MR. Coated microneedles for transdermal delivery. Control Releases 2007; 227-237.
22. Han M, Hyun DH, Park HH, Lee SS, Kim CH, Kim CG. A novel fabrication process for out-of-plane microneedle sheets of biocompatible polymer. MicromechMicroengg 2007; 1184-1191.
23. Jeong WL, Park JH, Prausnitz MR. Dissolving microneedles for transdermal drug delivery. Biomaterials 2008; 113-124.
24. Davis SP, Landis BJ, Adams ZH, Allen MG, Prausnitz MR. Isertion of microneedles into skin: measurement and prediction of insertion force and needle fracture force. Biomech 2004; 1155-1163.

25. Forvi, Soncini, Bedoni, Rizzo, Casella, O'Mahony, et al.. A method to determine the margin safety for microneedles arrays. Proceedings of the world congress on Engineering 2010.
26. Wu Y, Qiu Y, Zhang S, Qin G, Gao Y. Microneedle-based drug delivery: studies on delivery parameters and biocompatibility. Biomed Microdevices 2008; 601-610.
27. MikolajMilewski, Nicole K. Brogden, and Audra L. Stinchcomb., Current Aspects of Formulation Efforts and Pore Lifetime Related to Microneedle Treatment of Skin. Expert Opin Drug Deliv.2010; 2010 May;7(5):617-29.
28. Häfeli UO, Mokhtari A, Liepmann D, Stoeber B.. In vivo evaluation of a microneedle-based miniature syringe for intradermal drug delivery. BioMedMicrodevices 2009; 943-950.
29. Martanto W, Davis SP, Holiday NR, Wang J, Gill HS, Prausnitz MR. Transdermal delivery of insulin using microneedles in vivo. Pharm Res 2004; 47-952.
30. Verbaan FJ, Bal SM, van den Berg DJ, Groenink WH, Verpoorten H, Lüttge R, et al. Assembled microneedlearrys enhance the transport of compounds varying over a large range of molecular weight across human dermatomed skin. Control Release2007; 238-245.
31. So JW, Park HH, Lee SS, Kim DC, Shin SC, Cho CW. Effect of microneedle on the pharmacokinetics of ketoprofen from its transdermal formulations. Drug Delivery2009; 52-56.
32. AggarwalP, Johnston CR. Geometrical effects in mechanical characterizing of microneedle for biomedical application. Sens Actuators B 2004; 226-234.
33. Suzzane NM, Julia C, Stan P, Joke AB. In vivo assessment of safety of microneedle arrays in human skin. European Journal of Pharmaceutical Sciences 2008; 193-202.
34. Gupta J, Gill HS, Andrews SN, Prausnitz MR. Kinetics of skin resealing after insertion of microneedles in human subjects. Control Release 2011; 148-155.
35. Van DP, Oosterhuis-KF, Van der Wielen M, Almagor Y, Sharon O, Levin Y. Safety and efficacy ofa novel microneedle device for a dose sparing intradermal influenza vaccination in healthy adults. Vaccine 2009; 454-459.
36. Godin B, Touitou E. Transdermal skin delivery: Predictions for humans from In vivo, ex vivo and animal models. Advance Drug Delivery Reviews 2007; 1152-1161.

37. Puneet T, Rajendra.SK,Kompella UB. Comparison of Suprachoroidal Drug Delivery with Subconjuctival and Intravitreal Routes Using Noninvasive Fluorophotometry. PMC 2012.

38. Jagger RD, Mieler WF, Miller JW. Age-related macular degeneration. N Engl J Med 2008; 2606-2612.