

# A novel approach in delivering Immunobiologicals : A Glimpse

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## Abstract

To find an appropriate, needle free alternative to the traditional needle for the delivery of vaccines remains an important goal. One of the major challenges is that there are no such things in a global immunobiologicals industry. What is required for the developing countries is not necessarily the same as that for industrialized nation or for emergency. In developing countries, pricing and access to new technologies appear to be the most important features: Especially those technologies that (a) Improve stability thus removing the need for cold chain storage and (b) Improve the ease of use with minimum cross contamination. Industrialized nations cost of the producing the immunobiologicals and therefore the selling prices are significantly higher than in the developing countries. Only in these industrialized nations patient afford the preavilage of pain free alternative to needles with no adverse effects or cross contamination, but these often come at an increased price. Many immunobiologicals has to be delivered several times to attain full immunity. Patients having needle phobia regrets to take booster doses which lean them to fully unprotected state. A range of alternative delivery system is being developed for vaccines. This review article deals with diverse techniques obtainable for pain free delivery of immunobiologicals.

**Key words :** Needle free, Delivery, Safety, Immunobiologicals

## Introduction

With few exceptions, immunobiologicals are delivered by injection at the intramuscular, subcutaneous, or intra dermal space. This practice has led patients, parents, and practitioners to refer to vaccine administration as getting one's shots. Although vaccination delivered by injection has led to tremendous advances in the control of many infectious diseases, this technique is not without risks or discomfort, leading to the search for alternate means of vaccine delivery. Increasing concerns over bioterrorism has also led to new needle-free vaccine delivery research [Levine, M.M., Sztein, M.B., 2004]. This article deals with diverse techniques obtainable for pain free delivery of immunobiologicals. Needle free vaccine delivery is desirable for many reasons. In fact, most descriptions of an ideal vaccine include a needle-free method of administration [Galen, J.E., *et al.*, 2004]. Needle free vaccine administration has the potential to lead to the following significant advances in immunization delivery improved safety for the

vaccinator, vaccine and community; better compliance with immunization schedules; decreased injection site pain, easier and faster immuno biologicals delivery; and reduced cost. For these reasons, needle-free vaccine delivery is supported by many prominent public health organizations involved in the delivery of vaccines, including the World Health Organization, the Global Alliance for Vaccines and Immunization, and the Centers for Disease Control and Prevention.

## Safety

The traditional administration of vaccines via needle and syringe posses' safety risks for patients healthcare providers, and the community. A primary safety concern is the risk of transmission of infectious diseases or between patients and healthcare providers [Simonsen, L., *et al.*, 1999]. In both the developed and developing world, the administration of vaccines posses an occupational risk, through needle stick injuries. Among the estimated 12 billion injections per year worldwide, approximately 1 billion are childhood vaccinations, and thus a significant proportion of needle stick injuries may be attributed to vaccinations [Simonsen, L., *et al.*, 1999]. Even in regions with relatively low occupational risk for needle-stick injuries under normal circumstances, the risk may substantially increase in the event that mass vaccinations were necessary, such as with a bioterrorism emergency or a natural pandemic [Levine, M.M., Campbell, J.D., 2004]. Improper injection practices used commonly in the developing world include reuse of contaminated needles and syringes without sterilization between patients, changing needles but not syringes between patients, and improper disposal of used needles and syringes practices are common, which increase the risk of blood-borne pathogen transmission during vaccinations [Levine, M.M., Campbell, J.D., 2004].(putting the community at risk of needle sticks).For the estimated 1 billion injections that are given yearly in the course of childhood vaccination programs, according to a WHO study, at least 50% of injections were unsafe in 14 countries located in 5 different developing world regions [Simonsen, L., *et al.*, 1999]. Many studies report a link between unsafe injections and the transmission of infectious agents including HBV, HCV, and HIV. Studies have shown that a striking 2080% of new hepatitis B infections are due to unsafe injections [Simonsen, L., *et al.*, 1999]. It is clear that the burden of unsafe injections are high.

## Compliance

Poor compliance with schedules is often due to parental concern regarding the number of vaccine injections administered to children and

to “needle phobia”, which is common in both adults and children. Several recent studies have addressed fear of injections and methods to minimize pain associated with vaccines. These studies indicate that approximately 20% of children suffered “serious distress” from vaccinations and 8.2% of young adults had an unreasonably intense fear of injections [ Nir, Y., *et al.*, 2003]. This pain and distress is one reason that for some patients (and parents of patients) in industrialized nations are hesitant to be immunized. A major impetus for the development of combination vaccines for infants is the concern among parents and healthcare providers about the number of injections required for the recommended childhood vaccinations schedule. Unfortunately, although combination vaccines decrease the number of injections necessary, some candidate combinations have shown diminished immune response to antigens administered together. Combination vaccines administered mucosally have generally been better at allowing delivery of multiple vaccines in combination without losing immunogenicity.

### Discomfort

In addition to increasing compliance with vaccine-delivery, needle free immunobiological delivery is expected to decrease pain and suffering, including actual injection site pain, anticipatory and perceived pain, and reactivity from injections. Mucosal and transcutaneous vaccines are not expected to cause pain or discomfort. In addition to immediate pain, later reactivity must also be examined. Reactogenicity of vaccines delivered by needle and syringe varies depending upon vaccine and age of the recipient. Adults report pain or tenderness at the injection site following 50% to 80% of tetanus toxoid boosters whereas approximately 20% of children have soreness the injection site following administration of trivalent inactivated influenza vaccine [Moylett, E.H., *et al.*, 2004].

### Cost

Introduction of new vaccine delivery method cannot add significant costs and remain viable, especially considering the limited resources for vaccination in the developing world. Currently, the material costs for delivery of vaccines by needle and syringe are small (approximately 2.82 rupees per injection). However, in considering differences in cost of various methods of vaccine delivery, it is important to evaluate not only differences in cost of the devices but also differences in cost from medical care necessary due to iatrogenic blood-borne pathogen transmission. For instance, according to a recent theoretical cost model analysis, when the cost of medical care and lost productivity due to iatrogenic blood-borne pathogen transmission is included in the cost of re-sterilizable needles and syringes, in developing countries, the societal cost per injection may be as high as 1177.88 rupees per injection. Even when only costs of medical care due to iatrogenic infections relevant to the health care payer's perspective are taken into account, disposable and re-sterilizable needles and syringes cost approximately 29.48 rupees per injection. By this calculation, the use of alternate methods for vaccine delivery may result in cost-reduction or at least no net increase in cost of vaccine services.

## Types of needle free delivery system

### Liquid jet injector

Liquid jet injections employ a high-speed jet to puncture the skin and deliver drugs without the use of a needle. Research on jet injectors began in the early 1930s with Arnold Sutermeister, an engineer who noticed accidental injections of diesel oil into the hands of workers when small leaks occurred in high-pressure lines (Bremseth, D.L., and Pass, F., 2001). Since then, two main classes of liquid jet injectors have been developed. These are single-dose jet injectors, known as Disposable Cartridge Jet Injectors (DCJIs) and Multi-Use-Nozzle Jet Injectors (MUNJI's) (Mitragotri, S., 2006). Some DCJIs are only partly disposable while others are fully disposable. MUNJIs did not have any disposable parts and were introduced for rapid mass immunization. Their use, however, was discontinued in the wake of reports of spread of hepatitis B in the 1980's due to their use.

### Mechanism

The basic design of commercial liquid jet injector consists of a power source (compressed gas or spring), piston, drug-loaded compartment and a nozzle with orifice size typically ranging between 150 and 300µm (Mitragotri, S., 2006). Upon triggering the actuation mechanism, the power source pushes the piston which impacts the drug-loaded compartment, thereby leading to a quick increase in pressure. This forces the drug solution through the nozzle orifice as a liquid jet with velocity ranging between 100 and 200m/s. A schematic of injection

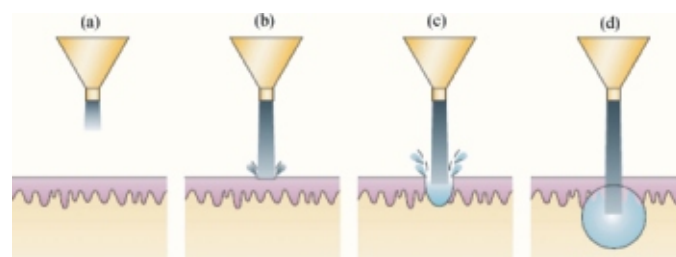


Fig.1. Schematic of drug delivery using liquid jet injector (Mitragotri, S., 2006):  
 (a) Formation of liquid jet,  
 (b) Initiation of hole formation due to impact of jet on skin surface  
 (c) Development of hole inside skin with progress of injection,  
 (d) Deposition of drug at the end of hole in a near spherical or hemi spherical pattern ( spherical pattern shown).

process is shown in Fig.1. The jet is turbulent in nature and the diameter of the jet is comparable to that of the orifice but increases with distance traveled. Upon implunging on skin, the jet punctures through the skin and initiates hole formation. The formation of a hole is believed to be due to a combination of skin erosion and fracture and is completed during the first few hundred micro seconds (Baxter, J., and Mitragotri, S., 2005). As the jet progresses deeper in the skin, velocity decreases until it does not have sufficient energy to continue hole formation. This completes the first phase of injection i.e. unidirectional skin puncture and is followed by these second phase, multidirectional jet dispersion from the end point of penetration. Further, the dispersion of liquid from this point appears to be approximately hemi spherical, whose shape is governed by jet power.

### Design parameters

The depth of penetration and shape of liquid dispersion is governed by the orifice diameter and jet exit velocity. Nozzle diameters between 31 and 559 $\mu\text{m}$  and exit velocities between 115 and 200m/s have been used in experimental studies (Baxter, J., and Mitragotri, S., 2005,2006). An increase in penetration depth is reported both with increasing nozzle diameter at constant exit velocity and increasing jet exit velocity at constant diameter, when injection volumes were kept constant. Increasing diameter also increased size of dispersion. More recently, jet power ( $P_o$ ) has been suggested as a combined parameter for describing dependence of jet penetration depth and dispersion on velocity and nozzle diameter. Jet power is calculated as:  $P_o = 1/8 \rho D_o^2 u_o^3$

Where  $D_o$  is nozzle diameter,  $u_o$  is exit velocity and  $\rho$  is liquid density. Penetration depth increased from 0.2mm at a power of 1W to 2.8mm at a power of 62.4W. With increasing power, the shape of liquid dispersion at the end of hole also changed from resembling a lower hemisphere with end of eroded hole as center to an upper hemisphere with end of hole lying at the top of hemisphere. With variation in jet parameters, it is possible to span the full thickness of skin and control the depth where the bulk of drug solution is being delivered. The percent completeness of injection, defined as the percent of drug solution delivered across the skin, also increased linearly from near zero at a power of 1W to >90% at a power of ~30W, beyond which the delivery remained constant at or above which 90%. Other factors may also affect penetration depth but need further investigation include mechanical properties of skin, injection volume and stand-off distance. The stand-off distance is defined as the distance which the liquid jet travels after leaving the injector's orifice until it makes contact with the skin.

### Powder injectors

Powder jet injectors deliver vaccines or drugs in dry powdered form into superficial layers of skin. The terms biolistic injectors and gene guns have also been commonly used for these injectors, with the latter term used exclusively for DNA delivery (Kendall, M., 2006). The early work on injecting solid micro-particles in biological samples was reported by Klein, T.M., *et al* (1987), who demonstrated transfection of plant cells with DNA and RNA using nucleic acid-coated tungsten particles. The potential of this technique for applications in protein delivery, gene therapy as well as traditional and DNA vaccination was analyzed (Burkoth, T.L., *et al.* 1999; Chen, D.X., *et al.*, 2002).

### Mechanism

Basic design of solid jet injectors include compressed gas as the power source, a drug compartment containing particulate drug formulation, and a nozzle to direct the flow of particles (Mulholl, W.J., *et al.*, 2004). The drug compartment is closed with diaphragms on either sides, which are typically few microns thick. Upon triggering the actuation mechanism, compressed gas from a storage canister expands and pushes against the diaphragms, sequentially rupturing them. The flow of gas carries the drug particles with it. The particles then exit through a nozzle and impinge on skin Fig.2 & Upon impacting on the skin, particles puncture micron- sized holes into stratum corneum by virtue of their momentum. Another design used for studying powder injection mechanisms is light gas gun, which uses an accelerating piston for

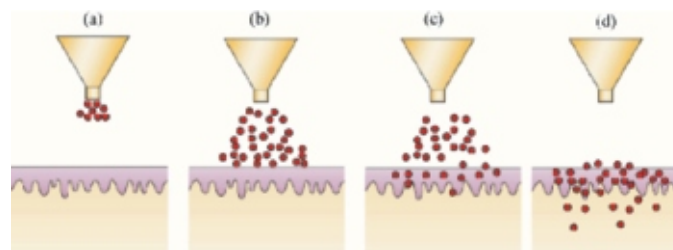


Fig.2. Schematic of drug delivery using powder injector (modified from Mitragotri, S., (2006))

- Ejection of particles from nozzle
- Impact of particles on skin surface of jet on skin surface, development of hole inside skin with progress of injection,
- Deposition of drug at the end of hole in an earspherical or hemispherical pattern (spherical pattern shown). Penetration of particles a cross stratum corneum,
- Completion of delivery. Particles which penetrate into the skin are mostly distributed in stratum corneum and viable epidermis.

imparting desired particle velocity (Crozier, W.D., and Hume, W., 1957). Upon triggering the actuation mechanism; the piston accelerates and carries the particles with it. A deceleration mechanism forces the piston to slow down and makes the particles leave the surface of piston. The particles are ejected and they impact on target tissue surface.

### Design parameters

Key parameters in determining particle delivery across the stratum corneum are impact velocity, particle radius and particle density. The particles constitute of powdered preparation of drugs or immunobiologicals and range between 10 and 20m. For DNA vaccination, coated metal particles between 0.5 and 3m have been used. A much broader range of particle sizes (0.552.6m) and densities (1.0818.2g/cm<sup>3</sup>) have been studied for injector development (Kendall, M., *et al.*, 2004a). For studying correlations between particle properties and skin penetration, a combined parameter, namely particle impact parameter, has been defined as  $\rho v r$ , where,  $\rho$ ,  $v$  and  $r$  are particle density, impact velocity and radius, respectively. Particle impact parameter represents momentum per unit cross sectional area of the particle. Depth of penetration and fraction of particles penetrating stratum corneum were found to be directly proportional to this parameter. At a fixed value of particle impact parameter, an increase in particle radius corresponds to a decrease in particle velocity at constant density and resulted in a decrease in penetration depth. For a given set of particle properties, velocity of particles can be controlled by varying gas pressure (200-900psi). Since keeping particle impact parameter uniform is necessary for targeting specific skin layers, various internal contour designs have been studied for achieving narrow velocity profiles. This has led to optimization of internal sections of the injector, namely driver tube and shock tube through which the carrier gas flows before reaching the nozzle (Kendall, M., *et al.*, 2004a). A recent study has revealed a correlation between epidermal cell death and particles delivered per unit area of target tissue, making particle pay load another important parameter (Raju, P.A., *et al.*, 2006).

### Microneedles

Microneedles may be sufficient for transport across the 1020m-thick stratum corneum was first proposed in the 1970s (Gerstel, M.S., and Place, V.A., 1976) but progress was delayed largely due to lack of

techniques to fabricate such small structures. The first work on use of microneedles for transdermal drug delivery was reported in the late 1990s (Henry, S., *et al.*, 1998). Established techniques of the microelectronics industry are now being adapted and expanded upon for microneedle fabrication. Earlier designs of microneedles had silicon as the fabrication material due to easy adaptability to microelectronic fabrication processes. Current design emphasize metal and polymeric microneedles and four different types of microneedle designs have been developed, which include solid microneedles that pierce the skin to make it more permeable, solid microneedles coated with dry powder drugs or immunobiologicals for dissolution in the skin, microneedles prepared from polymer with encapsulated vaccine for rapid or controlled release in the skin, and hollow microneedles for injections (Cormier, M., *et al.*, 2004 Prausnitz, M.R., 2004 Birchall, J., *et al.*, 2006, Sivamani, R.K., *et al.*, 2007). Metals used in solid microneedles include stainless steel, titanium and nickel-iron. Polymeric needles use engineering plastics, biodegradable polymers and water soluble polymers such as polycarbonate, polylactic co glycolic acid, and carboxy methyl cellulose, respectively.

### Mechanism

The mechanism of action depends on the microneedle design and is summarized in Fig.3 & Fig 3a All types of microneedles are typically fabricated as an array of up to hundreds of microneedles over a base substrate. Solid microneedles can either be pressed on to the skin or scraped on the skin for creating microscopic holes, thereby increasing



Fig 3a. Mono dose Needle Free Injector (courtesy Aventis Pasteur in France and Am-O- Jet in USA)

skin permeability by up to four orders of magnitude (McAllister, D.V., *et al.*, 2003). This is followed by application of drugs or immunobiologicals from a patch. Residual holes after micro needle removal measure microns in size and have a life time of more than a day when kept under occlusion, but less than 2 hours, when left uncovered. The second strategy is to have immunobiologicals in a dry coating onto solid microneedles (Gill, H.S., and Prausnitz, M.R., 2007). This coating can dissolve within 1 minute after insertion into skin, after which the microneedles can be withdrawn and discarded. As an alternative for using insoluble metal or polymer microneedles, complete microneedles have been fabricated out of biodegradable or water-soluble polymers. Model drugs have been encapsulated within PLGA microneedles for controlled release over hours to months (Park *et al.*, 2006) and, more recently, water-soluble carboxymethyl-cellulose, polyvinyl-pyrrolidone and maltose for rapid release within minutes (Lee, J.W., *et al.*, 2008; Sullivan, S.P., *et al.*, 2008) The final approach consists of using hollow microneedles to puncture the skin followed by infusion of liquid formulation through the needle bores in a manner similar to hypodermic injection (Wang, P.M., *et al.*, 2006).

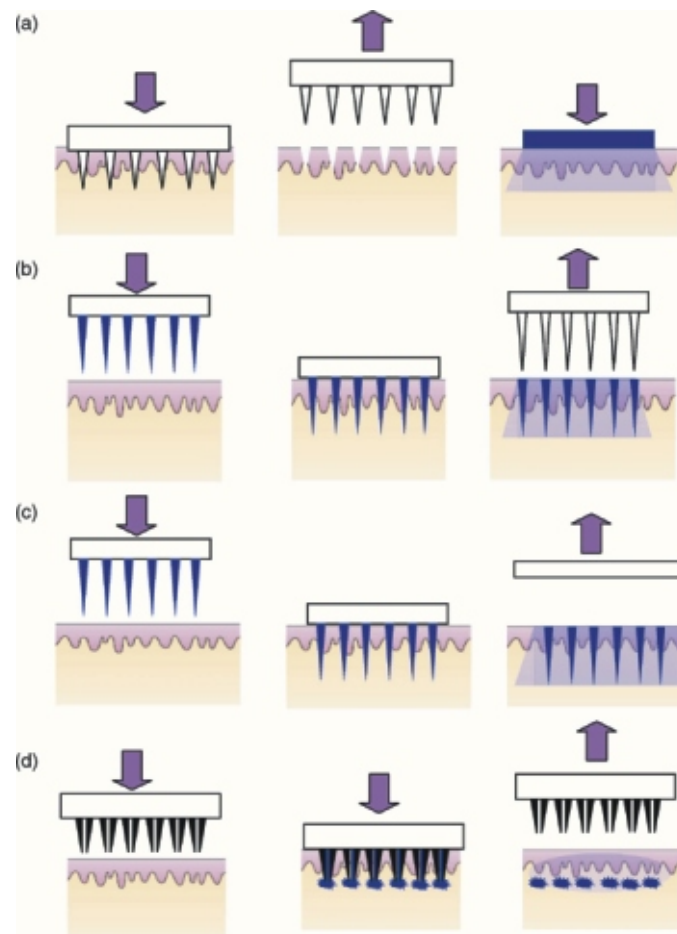


Fig.3. Schematic of drug delivery using different designs of micro needles (a) Solid micro needles for permeabilizing skin via formation of micron-sized holes across stratum corneum. The needle patch is withdrawn followed by application of drug-containing patch (b) Solid micro needles coated with dry drugs or vaccine for rapid dissolution in the skin (c) Polymeric micro needles with encapsulated drug or vaccine for rapid or controlled release in the skin, (d) Hollow micro needles for injection of drug solution.

### Design parameters

Microneedle design is constrained by a number of parameters. First, microneedles must be capable of inserting into skin without breaking. While metals are typically strong enough polymers must be selected to have sufficient mechanical strength. Microneedle geometry is also important, where sharpness of tip strongly affects the force required for micro needle insertion into skin. Other parameters, including micro needle length, width and shape all influence force required for microneedle fracture (Park, J.H., *et al.*, 2005). Typical micro needle geometries vary from 150 to 1500 micrometers length, 50 to 250 micrometers base width and 1 to 25 micrometers tip diameter. Microneedles can also be designed to minimize pain. Initial studies showed that specific microneedles of a couple hundred micrometers length were reported painless (Kaushik, S., *et al.*, 2001). More recently, a detailed study has shown that micro needle length strongly affects pain, whereas 3-fold increase in needle length (i.e. 500 - 1500 micrometers) increased pain 7-fold (i.e., from 5% to 35% of the pain caused by a hypodermic needle) (Gill, H.S., and Prausnitz, M.R., 2008). Increasing the number of microneedles

(620m long) 10-fold from 5 to 50 increased pain by a factor of three. Fabrication methods for microneedles need to be designed appropriately. As single-use, disposable devices, manufacturing costs should be kept low. Lithographic etching and micro-molding methods are typically used and are expected to have mass production costs well under 44 rupees and possibly as low as 4.4 rupees per device. Fabrication methods also need to avoid denaturing of vaccines and drugs and have therefore emphasized room temperature processing with aqueous solvents (Prausnitz, M.R., *et al.*, 2004).

### Thermal ablation

Use of thermal energy for surgical removal of selected tissue has been reported by medical practitioners as early as Hippocrates (460-370 BC), who used hot iron rods for cauterization of wounds. In modern medicine, thermal ablation generally refers to tissue removal due to high temperature induced by various energy resources. Percutaneous thermal ablation for tumor targeting is well established but does not use devices with micron-sized operating dimensions (DeSanctis, J.T., *et al.*, 1998). More recently, devices with micro-scale ablation elements have been developed for controlled removal of stratum corneum and thus thermally microporate the skin for enhanced transdermal drug delivery.

### Mechanism

Thermal ablation of skin that selectively removes stratum corneum without damaging deeper tissues is achieved through careful control of skin surface temperature over short duration of time. By heating the skin surface briefly (e.g.  $t \ll 1s$ ), heat penetration is largely limited to stratum corneum, with local temperatures up to hundreds of degrees Celsius, while deeper viable tissue remains much cooler and structurally intact. Formation of micropores of the 30 $\mu$ m diameter and 70 $\mu$ m depth and absence of necrosis in surrounding tissue has been reported using selective ablation techniques. In another study, micropores exhibiting an elliptical geometry of 80 $\mu$ m width and 300 $\mu$ m length and 40-50 $\mu$ m depth were formed corresponding to the geometry of ablation elements. One mechanistic hypothesis is that bound water in the stratum corneum must be heated beyond its boiling point, upon which the water vaporizes. This sudden increase in volume of water blasts locally in stratum corneum. In this way, thermal treatment of the stratum corneum triggers a mechanical event that actually causes tissue ablation. Other experiments suggest that temperatures much higher than boiling point of water which are needed for extensive tissue ablation and that stratum corneum combustion is mechanistically responsible (Park, J.H., *et al.*, 2008).

### Design parameters

The temperature, duration, and localization of thermal energy applied to the skin are all critical design parameters. Skin should be heated well above 100°C and possibly up to many hundreds of degrees Celsius. Because skin heating is done for a very short time and extreme temperature gradients exist within skin (e.g. > 10,000°C per mm), it has been difficult to make precise measurements of skin temperature. To localize heating within the stratum corneum, thermal pulses are applied typically on the millisecond time scale or shorter. Longer pulses lead to heating of deeper skin tissue, which can cause undesirable damage to living tissues. Heating should also be localized to specific areas on the

skin surface. By employing an array of these micro-heaters, large area of skin can be treated for drug delivery, but only small spots of stratum corneum area within the treated area. One approach achieving controlled heating in this way involves a two dimensional grid of wires having micron-scale resistors between each of the nodes. Using such a device, a brief surge electric current through the network causes the resistors to suddenly heat up due to ohmic resistance. The electrodes cool down as soon as the current is turned off. This transiently heats the skin surface and ablates stratum corneum. PassPort™ system fabricated by Altea Therapeutics Corp. (Atlanta, GA, USA) (Banga, A.K., 2006) is based on this concept. Another approach involves an array of electrodes that are activated one by one through a feedback mechanism to briefly pass radiofrequency (RF) current into the skin. The resulting heat generated within the stratum corneum selectively heats this tissue for localized ablation. One such handheld device based on RF energy is ViaDerm™ which has been developed by Trans Pharma Ltd. (Israel) (Levin, G., *et al.*, 2005). The device employs a disposable array of stainless steel micro-electrodes (100 $\mu$ m length and 40 $\mu$ m diameter: 200 electrodes per/cm<sup>2</sup>) mounted on a polycarbonate body. The activation of device is governed by pressure as the device is pressed on the skin at the site of application. Repeated applications of up to 250 and 380 V for in vivo and in vitro respectively were used at a frequency of 100 kHz for duration of 1 millisecond each.

### Discussion

Auto-disable technology, which prevents re-use and is now the standard for needle and syringe vaccine delivery in much of the world, will alternate methods of vaccine delivery discussed in this article offer the distinct advantage of significantly decreased or minuscule risk of blood-borne pathogen transmission. Cost analyses comparing vaccine delivery via needle and syringe with vaccine delivery via jet injectors and aerosol vaccines are encouraging (Ekwuene, D.U., *et al.*, 2002). For the anticipated future immunobiologicals manufacturers in developing

Table.1 Diverging Immunization Realities

Immunobiologicals	Industrial countries	Developing countries
Infant combination vaccines	Acellular pertussis-based	Whole-cell pertussis based
Measles vaccines	Trivalent MMR	Monovalent measles
Polio vaccine	Inactivated parenteral	Live oral
BCG	Uncommon	Routine
<i>Varicella, pneumococcal conjugate</i>	Increasingly common	Not introduced yet
Immunization schedule extends to year 2 life source of vaccine	Routine	Uncommon
Source of vaccine	Industrialized country manufacturers	Mostly developing country manufacturers
Use of multi dose vials	Minority of vaccine used	Majority of vaccine used
Public perception	Concerns over vaccine safety	Fear of disease

countries will continue to produce multi dose vials containing thiomersol, because a switch to single dose presentation will require large investments to amend to production lines and would increase cost per dose and volume to be handled by the cold chain. In contrast to the situations summarized here has recently has the mid 1980's there was little difference in the array of vaccines given to infants in the developed and developing world. (Table 1)

### Why needle free immunization is desirable

A concern about the number of injections that must be given to infants and toddler is driving the development of parenteral combination vaccines. In developed countries, immunization without needles or syringes would increase acceptability, and would enhance occupational safety for vaccinators and other health providers. This could be particularly critical in the future. Needle-free immunization is even more critical for developing countries, where expanded immunization coverage and the addition of new vaccines could prevent millions of childhood deaths. Since the mid-1970s, the World Health Organization's Expanded Programme on Immunization (EPI) has recommended six basic vaccines for infants in developing countries: diphtheria and tetanus toxoids, whole-cell pertussis, bacillus Calmette-Guerin (BCG), and attenuated polio and measles; hepatitis B and Haemophilus influenza type b (Hib) conjugate were recommended subsequently. Developing countries are also increasingly using mass immunization campaigns to drive measles from communities and to crucial meningococcal epidemics. The Global Alliance for Vaccines and Immunization (GAVI) and its associated Vaccine Fund are addressing the long delay in the introduction of lifesaving vaccines between industrialized versus developing countries. In developing countries, delivery of immunization would be more efficient and economical if all immuno biologicals were temperature stable, requiring less than three doses to immunize, and could be administered without needles. However, except for the oral polio vaccine, all EPI vaccines are now given using needle and syringe. This is problematic because in developing countries injection safety is a not-orious problem. Improper practices involving non sterile needles and syringes cause abscesses and transmit blood-borne pathogens (such as hepatitis B and C and HIV) 2. Single-use 'auto-disable syringes' provide a partial solution by preventing reuse, but generate infectious waste that must be properly handled less it endanger by standers. Although parenteral vaccination accounts for only a fraction of the needles used by health workers. Immunization is held to a higher standard than other uses of needles because it involves healthy individuals.

### Safety

Thermal ablation devices have shown acceptable safety profiles. In a recent human clinical trial for evaluating safety, administration sites were examined and results quantified using Draize irritation index for irritation on a scale of 0-8 and Visual Analogies Scale (VAS) for pain on a scale of 0-100. Draize index was 0.75 while VAS score was 5, conforming low degree of erythema and pain. Similar results have been reported in clinical trial for grainsetron delivery, where no irritation was detected after 24hours patch application. Slight erythema has been reported for the use of prototype of PassPort™ system (Badkar *et al.*, 2007). Although data has not yet been published from ongoing human

clinical trials, their progression to phases II and III suggests an acceptable safety profile other data from animal and human studies have been published and generally report no significant adverse reactions to micro needles. More specifically, no infections were caused by microneedles which have been reported (Matriano, J.A., *et al.*, 2002; Cormier, M., *et al.*, 2004; Widera, G., *et al.*, 2006). In addition, skin irritation has been reported to be mild and transient when it exists at all (Lin, W.Q., *et al.*, 2001; Matriano, J.A., *et al.*, 2002; Gardeniers, *et al.*, 2003; McAllister, D.V., *et al.*, 2003; Martanto, W., *et al.*, 2004; Wang, P.M., *et al.*, 2006), and bleeding is generally not associated with use of microneedles (McAllister, D.V., *et al.*, 2003; Dean, C.H., *et al.*, 2005; Alarcon, J.B., *et al.*, 2007). As discussed above, a variety of microneedle designs have been reported to be painless in human subjects. Additional studies is needed for fully assess safety. Human clinical trials have reported painless delivery at the time of injection with DNA vaccines being well tolerated (Roy, M.J., *et al.*, 2000; Drape, R.J., *et al.*, 2006). Post injection symptoms have been reported to develop quickly after the injection and include mild erythema, hyper-pigmentation, a flaking and discoloration at the injection site. In some cases, transient sensations of mild tingling, tightening has also been reported. Most symptoms disappeared within the first month except mild discoloration, which has been reported to persist for up to 6 months. The acceptance of conventional jet injectors has been mixed due to variable reactions at the administration site. Some reports state no difference in level of pain compared to that experienced by hypodermic needles (Sarno, M.J., *et al.*, 2000), but others have reported higher levels of pain (Jackson, L.A., *et al.*, 2001). Variable reports in local reactions further augmented this fact, with some researchers reporting absence of local reactions (Resman, Z *et al.*, 1985) while others have reported significantly more reactions including pain, bleeding and haematomas (Houtzagers, C.M.G.J., *et al.*, 1988). It has been shown that the depth of penetration and percent delivery decrease with increasing Young's modulus (i.e. mechanical strength) of skin (Baxter, J., and Mitragotri, S., 2005). Commercial injectors comes with very limited choice of settings and owing to the person-to-person variability in skin's mechanical properties. Variability in patient response may be due to the failure of this "one size fits all" approach of current devices. Future devices such as pulsed micro jets are being designed to address these problems by offering superior control over injection profile.

### Applications

A number of *in vitro* and *in vivo* studies has been carried out in ViaDerm™ for the delivery of testosterone, grainsetron, dichloro fenac sodium and plasmid DNA (Levin *et al* 2005, Birchall *et al* 2006). Following the invitro studies in vivo studies were carried out in human with the help of Via Derm™ for the delivery of grainsetron and a steady observation was maintained up to 12 hours and the experiment proved to have constant level till patching for 24 hours. Another Phase I human study in the delivery of hPTH(1-34) was carried for 7 days and it resulted in absence of drug accumulation or dehydration of hPTH and drug bio availability f 40% is also been reported Phase II and phase III clinical trial in ViaDerm™ is currently on its way along with human clinical study in the delivery of insulin Trans Pharma 2008, thermal ablation by PassPort™ has been tested for the delivery of adenovirus,

interferon  $\alpha$ , influenza antigens, tetanus antigen, erythropoietin and fentanyl citrate in pre clinical studies. Human clinical trials are currently underway in insulin, hydromorphone HCl, fentanyl citrate, apomorphine HCl Altea Therapeutics.

Microneedles have been studied *in vitro*, in animals and in humans for a variety of applications. Microneedles piercing has been shown to increase skin permeability by orders of magnitude to a variety of compounds ranging from low molecular weight tracers to proteins, DNA and even nano particles (McAllister, D.V., *et al.*, 2003). A recent study reported on delivery of naltrexone, which is used to treat alcohol and opioid addiction, at therapeutic levels in normal human subjects using this approach (Wermeling, D.P., *et al.*, 2008). Solid micro needles have also been coated with a number of different compounds, including low molecular weight drugs, proteins, DNA, virus particles and microparticles (Gill, H.S., and Prausnitz, M.R., 2007), human clinical trials by Zosano Pharmaceuticals (Freemont, CA, USA) had completed Phase II trial for delivery of parathyroid hormone from coated microneedles. Dissolving polymer microneedles have similarly encapsulated various compounds, including erythropoietin and enzymes that were shown to retain activity after encapsulation and even after at least two months of storage at room temperature. (Lee, J.W., *et al.*, 2008; Sullivan, S.P., *et al.*, 2008). Hollow microneedles have been shown to deliver insulin to rodent models and modulate blood glucose levels (McAllister, D.V., *et al.*, 2003). Recent work in human subjects have demonstrated insulin delivery to control blood glucose levels in diabetic human subjects and lidocaine delivery to induce local anesthesia in normal human subjects. Vaccine delivery via microneedles has attracted considerable attention, for example, administration of influenza vaccine via microneedles elicited immune responses comparable to or better than intra muscular injections in mouse model (Alarcon, J.B., *et al.*, 2007). Human clinical trials on influenza vaccination using microneedles have completed phase III and have been submitted as the basis for registration in Europe through collaboration between Becton Dickinson (Franklin Lakes, N.J, USA) and Sanofi Pasteur (Lyon, France). Other vaccines studies include administration of ChimeriVaxTM- JE for yellow fever, plasmid DNA encoding hepatitis B surface antigen, and recombinant protective antigen of bacillus anthrax. In all the studies, microneedles generated immune responses at least as strong as those generated by subcutaneous or intra-muscular injections. Studies also demonstrated dose sparing ability of micro needles, where lower antigen dosage via microneedles elicited immune responses comparable together with antigen doses via alternate route, i.e., subcutaneous and intramuscular injections. Recently, a device which uses an electrically active microneedle array causes electroporation in the skin that effectively enhanced DNA vaccination. Solid jet injectors have been studied for the delivery of DNA encoding for viral and bacterial antigens using coated gold micro-particles (Matthews, K., *et al.*, 2007a). Induction of humoral and cell mediated immune response against influenza, Hepatitis B and rabies has been shown in mice (Lodmell, D.L., *et al.*, 2000; Chen, D.X., *et al.*, 2002). Protection against tumors has also been demonstrated by injecting DNA coated gold microparticles and DNA encapsulated in polymeric particles (Han, R.C., *et al.*, 1999; McKeever, U., *et al.*, 2002; Frelin, L., *et al.*, 2003). An extensive review of preclinical DNA vaccination studies using solid jet injector systems in large animal

models has been published by (Fuller, D.H., *et al.*, 2006). Clinical efficacy in humans has been demonstrated by induction of cell mediated and humoral immune response against hepatitis B using DNA coated gold micro particles (Roy, M.J., *et al.*, 2000). Phase I clinical studies for delivery of DNA vaccine against influenza showed humoral response (Drape, R.J., *et al.*, 2006). Another human clinical study used cross immunization regime with primary immunization using powder injector followed by intra dermal injection as booster, and showed cell mediated response against malaria. MUNJIs have been used for mass immunization programs for diseases including measles, smallpox, cholera, hepatitis B, influenza and polio (Weniger, B.G., 2003). DCJIs have been used for delivery of several proteins. Most work has been done on delivery of insulin (Lind Mayer, I., *et al.*, 1986) and growth hormones (Verhagen, A., *et al.*, 1995; Bareille, P., *et al.*, 1997; Ageroso, H., *et al.*, 2002), while erythropoietin (Suzuki, T., *et al.*, 1995) and interferon (Brodell, R.T., *et al.*, 1995) have also been delivered. Insulin administration by jet injectors led to a faster delivery into systemic circulation, possibly due to better dispersion at the injection site. However, the acceptance of jet injectors has been low due to variable reactions at the site of administration. To counter the challenges faced by traditional jet injectors, a novel pulsed micro-jet has been developed (Arora, A., *et al.*, 2007). This new approach focuses on minimizing pain and bruising by minimizing injection volumes and depth of penetration. The actuation mechanism is based on a piezoelectric transducer and offers strict control over delivery volumes and injection velocity. The high velocity (>100m/s) of micro jets allowed their entry into skin, whereas the small jet diameters (50-100 $\mu$ m) and extremely small volumes (215 $\mu$ l) limited the penetration depth (~200 $\mu$ m). The efficacy of this design was confirmed by delivering therapeutic doses of insulin in a rat model.

Orally active vaccines are sort because they avert the need for injection equipment with its associated cost and the risk of unsafe injection. The pre-requirement description use of the disposable syringes and needle are the present continuing impediments to the delivery of immunobiologicals. Of great concern is the high risk of unsafe injection caused by re use, poor sterilization and mishandling was arisen to those unskilled health officials. Oral activity also is important because it permits vaccines to be delivered in a wider range of service provides a regulation of production process that might less rigorous than those governing inject products which are more relevant pioneering works are in progress in the field of stable system for the production of plant derivatives proteins. (Jagannathan, S., *et al.*, 2008). The needle free immunobiological delivery has become a very distinctive discovery in this modern world, where science and technology goes in hand in hand. They are a quite number of advantages first being the delivery of the vaccine in a hygienic way and next major advantage is during immunization there is no need of formal health care trainees but during the immunization camps in other vaccination in health care centre's the trainees are given training and are monitored by the WHO team leads. They are very helpful especially when mass vaccinations are required especially during national immunization days (campaigns), natural pandemics and bioterrorism emergencies. A lot of time could be also saved in mass vaccination campaigns with the help of needle free immunobiologicals.

**Table. 2 impact of technology change on immunization services**

	Safer multi dose vaccine delivery	Mono dose pre-filled injection devices
Equity of access to new vaccines	Safe injection devices and disposal technology assured for mass immunization. Lowest cost per delivered multivalent dose of new vaccine	Ease of administration permitting community to immunize. A single dose available to a single child always
Safety of vaccine administration	No reuse of syringes possible. Reduced needle stick risks. Sterilization assured by monitoring- or eliminated	No reuse of injection devices possible. Vaccine dose integrity and sterility guaranteed to the point of use. No possibility of manual manipulation of vaccine
Simplicity and efficiency of vaccine delivery	Progressive elimination of complex and risky sterilization procedures. Progressive improvement in waste management systems. Higher cost for improved safety	Elimination of administrative vaccine wastage, resulting in lower costs. Reduced reliance on refrigeration and ice making at the peripheral level where 75% of distribution costs are concentrated. Less equipment maintenance. Easier stock control.

There are a number of benefits in the development of immunobiologicals during the delivery of immunobiologicals through the needle free system. In the multi dose vaccine delivery it is safer, hygienic and less cost effective, whereas in the mono dose pre filtered injection single dose is available, hygienic and prevents wastage of the vaccines too and maintenance free (Table 2). A hypodermic needle constructed from a biodegradable material possibly (WHO-Geneva 2000) even a sugar, which would achieve the safety advantages of needle free injection with a simpler, more conventional technology. The realization of this concept is not yet on the perspective but should be pursued so that the safety of needle based immunobiologicals systems can be maximized. This having some time frames with that time frame each technology passes through the phases of research and development, product, launch, market development and post market monitoring.

## References

1. Agero, H., Moller Pedersen, J., *et al.*, 2002. Pharmacokinetics and Pharmacodynamics of new formulation of recombinant human growth hormone administered by Zoma jet 2 vision. A new needle free device, compared to subcutaneous administration using a conventional syringe. *J. Clin. Pharmacol.*, **42**: 1262-1268.
2. Alarcon, J.B., Hartley, A.W., *et al.*, 2007. Preclinical evaluation of microneedle technology for intra dermal delivery of influenza vaccines. *Clin. Vacc. Immunol.*, **14**:375381.
3. Arora, A., Hakim, I., *et al.*, 2007. Needle-free delivery of macromolecules across the skin by nano liter-volume pulsed microjets. *Proc. Natl. Acad. Sci. U.S.A.*, **104**: 42554260.
4. Badkar, A.V., Smith, A.M., *et al.*, 2007. Transdermal delivery of interferon alpha 2B using micro poration and ion topophoresis in hairless rats. *Pharm. Res.*, **24**:1389-1395.
5. Banga, A.K., 2006. New technologies to allow transdermal delivery of therapeutic proteins and small water-soluble drugs. *Am.J. Drug. Deliv.*, **4**:221-230.
6. Bareille, P., MacSwiney, M., *et al.*, 1997. Growth hormone treatment without a needle using the Preci-Jet 50 transjector. *Arch. Dis. Child.*, **76**:6567.
7. Baxter, J., Mitragotri, S., 2005. Jet-induced skin puncture and its impact on needle free jet injections: experimental studies and a predictive model. *J. Control. Release.* **106**:361-373.
8. Baxter, J., Mitragotri, S., 2006. Needle-free liquid jet injections: mechanisms and applications. The concepts which form the basis of transdermal micro-devices. *Expert Rev. Med. Devices.*, **3**:565574.
9. Birchall, J., Coulman, S., *et al.*, 2006. Cutaneous gene expression of plasmid DNA in excised human skin following delivery via micro channels created by radio frequency ablation. *Int.J. Pharm.*, **312**:15-23.
10. Bremseth, D.L., Pass, F., 2001. Delivery of insulin by jet injection: Recent observations. *Thabetus technol. Ther.*, **3**: 225-232.
11. Brodell, R.T., Bredle, D.L., 1995. The treatment of palmar and plantar warts using natural alpha-interferon and a needle less injector. *Dermatol. Surg.*, **21**:213218.
12. Burkoth, T.L., Bellhouse, B.J., *et al.*, 1999. Transdermal and transmucosal powdered drug delivery. *Crit. Rev. Ther. Drug Carrier Syst.*, **16**:331384.
13. Chen, D.X., Endres, R.L., *et al.*, 2002. Epidermal powder immunization using non-toxic bacterial enterotoxin adjuvants with influenza vaccine augments protective immunity. *Vaccine.*, **20**:2671-2679.
14. Cormier, M., Johnson, B., *et al.*, 2004. Transdermal delivery of desmopressin in using a coated micro needle array patch system. *J. Control. Release.*, **97**:503-511
15. Crozier, W.D., Hume, W., 1957. High velocity, light gas gun. *J. Appl. Phys.*, **28**:892894.
16. Dean, C.H., Alarcon, J.B., *et al.*, 2005. Cutaneous delivery of a live, attenuated chimeric flavivirus vaccine against Japanese encephalitis (chimeriVaxJE) in non human primates. *Hum. Vacc.*, **1**:106-111.
17. DeSanctis, J.T., Coldberg, S.N., *et al.*, 1998. Percutaneous treatment of hepatic neoplasms: A review of current techniques. *Cardio Vasc. Interv. Radiol.*, **21**:273-296
18. Drape, R.J., Macklin, M.D., *et al.*, 2006. Epidermal DNA vaccine for influenza is immunogenic in humans. *Vaccine.*, **24**:44754481.
19. Ekwueme, D.U., Weniger, B.G., Chen, R.T., 2002. Model-based estimates of risks of disease transmission and economic costs of seven injection devices in sub-Saharan Africa, *Bull. World Health Organ, Suppl.*, **80**:859870.

20. Frelin, L., Alheim, M., et al., 2003. Low dose and gene gun immunization with hepatitis C virus non structural 1 (NS) 3 DNA based vaccine containing NS4 A inhibit N S3 /4 A expressing tumors in vivo. *Gene Ther.*, 10:686699.
21. Fuller, D. H., Loudon, P., et al., 2006. Preclinical and clinical progress of particle mediated DNA vaccines for infectious diseases. *Methods.*, 40:8689.
22. Galen, J.E., Zhao, L., Chinchilla, M., Wang, J.Y., Pasetti, M.F., Green, J., Levine, M.M., 2004. Adaptation of the endogenous *Salmonella enterica* serovar Typhi clyA-encoded hemolysin for antigen export enhances the immunogenicity of anthrax protective antigen domain 4 expressed by the attenuated live-vector vaccine strain CVD 908-htrA. *Infect. Immun.*, 72:70967106.
23. Gerstel, M.S., Place, V.A., 1976. Drug Delivery Device., 3:482.
24. Gill, H.S. Prausnitz, M.R., 2007. Coated microneedles for transdermal delivery *J. Control. Release.*, 117:227-237.
25. Gill, H.S., Prausnitz, M.R., 2008. Effect of microneedle design on pain in human subjects. *Clin. J. Pain.*, 24:585-594.
26. Gardeniers, H.J.G.E., Luttge, R., et al., 2003. Silicon micro machined hollow micro needles for transdermal liquid transport. *J. Microelectromech. Syst.*, 12:855-862.
27. Han, R.C., Cladel, N.M., et al., 1999. Protection of rabbits from viral challenge by gene gun-based intracutaneous vaccination with a combination of cotton tail rabbit papillomavirus E1, E2, E6, and E7 genes. *J. Virol.*, 73:7039-7043.
28. Henry, S., McAllister, D.V., et al., 1998. Microfabricated microneedles: a novel approach to transdermal drug delivery. *J. Pharm. Sci.*, 87:922925.
29. Houtzagers, C.M.G.J., Visser, A.P., et al., 1988. The Medi-Jector II-efficacy and acceptability in insulin-dependent diabetic patients with and without needle phobia. *Diabetic Med.*, 5:135-138.
30. Ito, Y., Yoshimatsu, J.J., et al., 2006b. self dissolving microneedles for the percutaneous absorption of EPO in mice. *J. drug target.*, 14: 255-261.
31. Jackson, L.A., Austin, G., et al., 2001. Safety and immunogenicity of varying dosage of trivalent inactivated influenza vaccine administered by needle free jet injectors. *Vaccine.*, 19: 4703-4709.
32. Jacobson, R.M., Swan, A., Adegbenro, A., Ludington, S.L., Wollan, P.C., Poland, G.A., 2001. Making vaccines more acceptable-methods to prevent and minimize pain and other common adverse events associated with vaccines. *Vaccine.*, 19: 24182427.
33. Jagannathan, S., Shivanandappa, K.C., Chandraraj, Venkataraj, K.N., Sundaran, B., 2008. Plant Mediated Pharmaceuticals (PMP): A review for drug discovery and bioprocess-Part I. *Lifesciences industry news.*, vol. 1; Issue 2116-31.
34. Kaushik, S., Hord, A.H., et al., 2001. Lack of pain associated with micro fabricated microneedles. *Anesth. Analg.*, 92: 502-504.
35. Kendall, M., Mitchell, T., et al., 2004a. Intra-dermal ballistic delivery of micro particles into excised human skin for pharmaceutical applications. *J. Biomech.*, 37: 17331741.
36. Kendall, M., 2006. Engineering of needle-free physical methods to target epidermal cells for DNA vaccination. *Vaccine.*, 24:4651-4656.
37. Klein, T.M., Wolf, E.D., et al., 1987. High velocity micro projectiles for delivering nucleic acids into living cells. *Nature.*, 327:70-73.
38. Levine, M.M., Szein, M.B., 2004. Vaccine development strategies for improving immunization: the role of modern immunology. *Nat. Immunol.*, 5:460-464.
39. Lee, J. W., Park, J.H., et al., 2008. Dissolving microneedles for transdermal drug delivery. *Biomaterials.*, 29: 2113-2124.
40. Levin, G., Gershonowitz, A., et al., 2005. transdermal delivery of human growth hormone through RF-Microchannels. *Pharm. Res.*, 22:550-555.
41. Levine, M.M., Campbell, J.D., and Kotloff, K.L., 2002. Overview of vaccines and immunizations. *Br. Med. Bull.*, 62:1-13.
42. \*\*\*Levine, M.M., Campbell, J.D., Mucosal immunization and needle-free injection devices, in: Levine, M.M., Kaper, J.B., Rappuoli, R., Liu, M.A., Good, M.F., (Eds.) 2004. *New Generation Vaccines.*, Marcel Dekker, Inc., New York, N.Y.: 393399.
43. Lin, W.Q., Cormier, M., et al., 2001. Transdermal delivery of antisense oligonucleotides with microprojection patch (Macroflux(R)) technology. *Pharm. Res.*, 18:1789-1793.
44. Lindmayer, I., Menassa, K., et al., 1986. Development of new jet injector for insulin therapy. *Diabetes Care.*, 9:294297.
45. Lodmell, D.L., Ray, N.B., et al., 2000. DNA vaccination of mice against rabies virus: effects of the route of vaccination and the adjuvant mono phosphoryl lipid A (MPL (R)). *Vaccine.*, 18:1059-1066.
46. Martanto, W., Davis, S.P., et al., 2004. Transdermal delivery of insulin using micro needles in vivo. *Pharm. Res.*, 21:947-952.
47. Matriano, J.A., Cornier, M., et al., 2002. Macro flux Micro projection array patch technology: new and efficient approach for intracutaneous immunization. *Pharm. Res.*, 19:63-70.
48. Matthews, K., Rhind, S. M., et al., 2007a. The effects of gene gun delivered p IL-3 adjuvant on skin pathology and cytokine expression. *Vet. Immunol Immunopathol.*, 119:233242.
49. Matthews, K., Rhind, S.M., et al., 2007b. The effect of gene gun-delivered pGM-CSF on the immunopathology of the vaccinated skin. *Scand. J. Immunol.*, 65: 298307.
50. McAllister, D.V., Wang, P.M., et al., 2003. Microfabricated needles for transdermal delivery of macromolecules and nanoparticles: Fabrication methods and transport studies. *Proc. Natl. acad. sci. U.S.A.*, 100: 13755-13760.
51. McKeever, U., Barman, S., et al., 2002. Protective immune responses elicited in mice by immunization with formulations of poly (lactide co-glycolide) microparticles. *Vaccine.*, 20: 15241531.
52. Mikszta, J. A., Alarcon, J.B., et al., 2002. Improved genetic immunization via micromechanical disruption of skin barrier function and targeted epidermal delivery. *Nat. Med.*, 8:415-419.

53. Mitragotri, S., 2006. Innovation- current status and future prospects of needle free jet injectors. *Nat. Rev. Drug Discov.*,5: 543-548.
54. Morel, P.A., Falkner, D., et al., 2004. DNA immunisation: altering the cellular localisation of expressed protein and the immunisation route allows manipulation of the immune response. *Vaccine.*,22: 447456.
55. Moylett, E.H., Hanson, I.C., 2004. Mechanistic actions of the risks and adverse events associated with vaccine administration, *J. Allergy Clin. Immunol.*, 114: 1010-1020.
56. Mulholl W.J., Kendall, M.A.F., et al., 2004. Characterization of powdered epidermal vaccine delivery with multi photon microscopy. *Phys. Med. Biol.*, 49: 5043-5058.
57. Nir, Y., Paz, A., Sabo, E., Potasman, I., 2003. Fear of injections in young adults: prevalence and associations, *Am. J. Trop. Med. Hyg.*, 68: 341-344.
58. Park, J. H., Allen, M.G., et al., 2005. Biodegradable polymer microneedles: fabrication, mechanics and transdermal drug delivery. *J. Control. Release.*, 104: 51-56.
59. Park, J.H., Lee, J.W., et al., 2008. The effect of heat on skin permeability. *Int. J. Pharmaceuticals.*, 359: 94-103.
60. Peachman, K.K., Rao, M., et al., 2003. Immunization with DNA through the skin. *Methods.*, 31: 232-242
61. Prausnitz, M.R., 2004. Microneedles for transdermal delivery. *Adv drug deliv. Rev.*, 56: 581-587.
62. Raju, P.A., McSloy, N., et al., 2006. Assessment of epidermal cell viability by near infrared multiphoton microscopy following ballistic delivery of gold micro particles *Vaccine.*, 24: 46444647.
63. Resman, Z., Metelko, Z., et al., 1985. The application of insulin using the jet injector *Dg-77. Acta Diabetol. Latina.*, 22: 119-125.
64. Roy, M. J., Wu, M.S., et al., 2000. Induction of antigen specific CD8+ T cells, The helper cells, and protective levels of antibody in humans by particle mediated administration of a hepatitis B virus DNA vaccine. *Vaccine.*, 19: 764-778.
65. Sarno, M. J., Blase, E., et al., 2000. Clinical immunogenicity of measles, mumps and rubella vaccine delivered by the Injex jet injector: comparison with standard syringe injection. *Pediatr. Infect. Dis. J.*, 19: 839-842.
66. Schramm-Baxter, J., Katrencik, J., et al., 2004. Jet injection into polyacrylamide gels: investigation of jet injection mechanics. *J. Biomech.*, 37: 1181-1188.
67. Sintov, A.C., Krymberk, I., et al., 2003. Radio frequency- driven skin micro channeling as a new way for electrically assisted transdermal delivery of hydrophilic drugs. *J. Control. Release.*, 89: 311-320.
67. Simonsen, L., Kane, A., Lloyd, J., Zaffran, M., Kane, M., 1999. Unsafe injections in the developing world and transmission of blood borne pathogens: a review, *Bull. World Health Organ, Suppl.*, 77: 789-800.
68. Sivamani, R. K., Liepmann, D., et al., 2007. Microneedles and transdermal applications. *Expert Opin. Drug delivery.*, 4: 19-25.
69. Sullivan, S.P., Murthy, N., et al., 2008. Minimally invasive protein delivery with rapidly dissolving polymer microneedles. *Adv. mater. J.*, 20: 933-938.
70. Suzuki, T., Takahashi, I., et al., 1995. Daily subcutaneous erythropoietin by jet injection in pediatric dialysis patients. *Nephron.*, 69: 347.
71. Verhagen, A., Ebels, J.T., et al., 1995. Pharmacokinetics and Pharmacodynamics of a single dose of recombinant human growth hormone after subcutaneous administration by jet injection comparison with conventional needle injection. *Eur. J. Clin. Pharmacol.*, 49: 6972.
72. Wang, P.M., Cornwell, M., et al., 2006. Precise microinjection into the skin using hollow microneedles. *J. invest. dermatol.*, 126: 1080-1087.
73. Weller, C., Linder, M., 1966. Jet injection of insulin vs syringe and needle *Am. Med. Assoc.*, 195, 844847.
74. Weniger, B.G., 2003. Jet Injection of Vaccines: Overview and Challenges for Mass Vaccination with Jet Injections (JIs). *Innovative Administration Systems for Vaccines*, Rockville, Maryland.
75. Wermeling, D.P., Banks, S.L., et al., 2008. Microneedles permit transdermal drug delivery of a skin-impermeant medication to humans. *Proc. Natl. Acad. Sci. USA.*, 105: 2058-2063.
76. Widera, G., Johnson, J., et al., 2006. Effect of delivery parameters on immunization to ovalbumin following intracutaneous administration by a coated micro needle array patch system. *Vaccine.*, 24: 1653-1664. 90.
77. WHO Geneva 2000, Department of vaccine and biologicals. In collaboration with USAID, PATH and UNICEF.

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