- Medical microneedle to deliver DNA plasmids for vaccination to novel corona virus

Rapid dermal inducing microneedle system for DNA vaccination

Vaccine

Vaccines contain dead or inactivated organisms or purified products derived from them.
There are several types of vaccines in use.[32] These represent different strategies used to try to reduce the risk of illness while retaining the ability to induce a beneficial immune response.

Inactivated

Attenuated

Toxoid

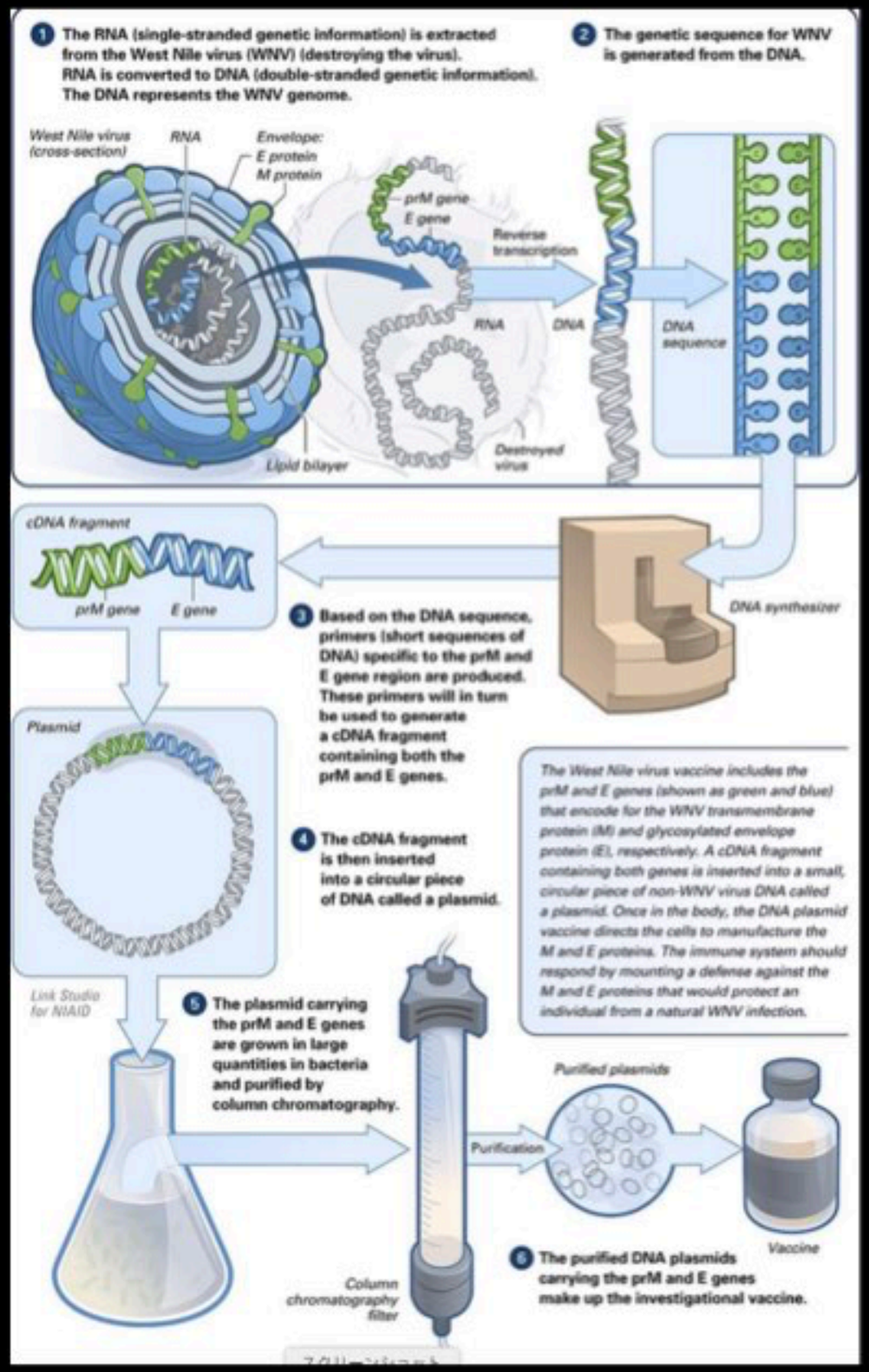
Subunit

Conjugate

Experimental

Dendritic cell vaccines combine dendritic cells with antigens
Recombinant vector
DNA vaccination
T-cell receptor peptide vaccines
Targeting of identified bacterial proteins

DNA vaccination



DNA vaccination is a technique for protecting against disease by injection with genetically engineered plasmid containing the DNA sequence encoding the antigen(s) against which an immune response is sought so cells directly produce an antibody, producing a protective immunological response[1]. DNA vaccines have potential advantages over conventional vaccines, including the ability to induce a wider range of immune response types.
Several DNA vaccines are available for veterinary use. Currently no DNA vaccines have been approved for human use. Research is investigating the approach for viral, bacterial and parasitic diseases in humans, as well as for several cancers.

Wikipedia

DNA vaccines are third generation vaccines. They contain DNA that codes for specific proteins (antigens) from a pathogen. The DNA is injected into the body and taken up by cells, whose normal metabolic processes synthesize proteins based on the genetic code in the plasmid that they have taken up. Because these proteins contain regions of amino acid sequences that are characteristic of bacteria or viruses, they are recognized as foreign and when they are processed by the host cells and displayed on their surface, the immune system is alerted, which then triggers immune responses.[2][3]
Alternatively, the DNA may be encapsulated in protein to facilitate cell entry. If this capsid protein is included in the DNA, the resulting vaccine can combine the potency of a live vaccine without reversion risks. In 1983, Enzo Paoletti and Dennis Panicali at the New York Department of Health devised a strategy to produce recombinant DNA vaccines by using genetic engineering to transform ordinary smallpox vaccine into vaccines that may be able to prevent other diseases.[4] They altered the DNA of cowpox virus by inserting a gene from other viruses (namely Herpes simplex virus, hepatitis B and influenza).[5][6]
In 2016 a DNA vaccine for the Zika virus began testing at the National Institutes of Health. The study was planned to involve up to 120 subjects between 18 and 35. Separately, Inovio Pharmaceuticals and GeneOne Life Science began tests of a different DNA vaccine against Zika in Miami. The NIH vaccine is injected into the upper arm under high pressure. Manufacturing the vaccines in volume remains unsolved.[7]
Clinical trials for DNA vaccines to prevent HIV are underway.[8]

DNA vaccine for novel corona virus is developing,
so problem is how to apply to mass patients efficiently.

Application procedures for DNA plasmid vaccine

1, Secure system

2, Easy to apply, i.e. Mass administration

3, Induce directly to immune cells, Langelhans cell, in epidermis directly

4, Effectve with small amount

5, Storage, room temperature

Drug delivery

1, Intra venous administration (IV)
2, Intra muscular administration (IM)
3, Sub dermal administration
4, Intra dermal administration
5, Sub epidemal administration
6, Intra epidermal administration
7, External applicatiom (ointment, cream, lotion etc.)

Trans dermal drug delivery system (TDDDS) ie; 4、5、6、 are difficult with conventional needle with syringe.

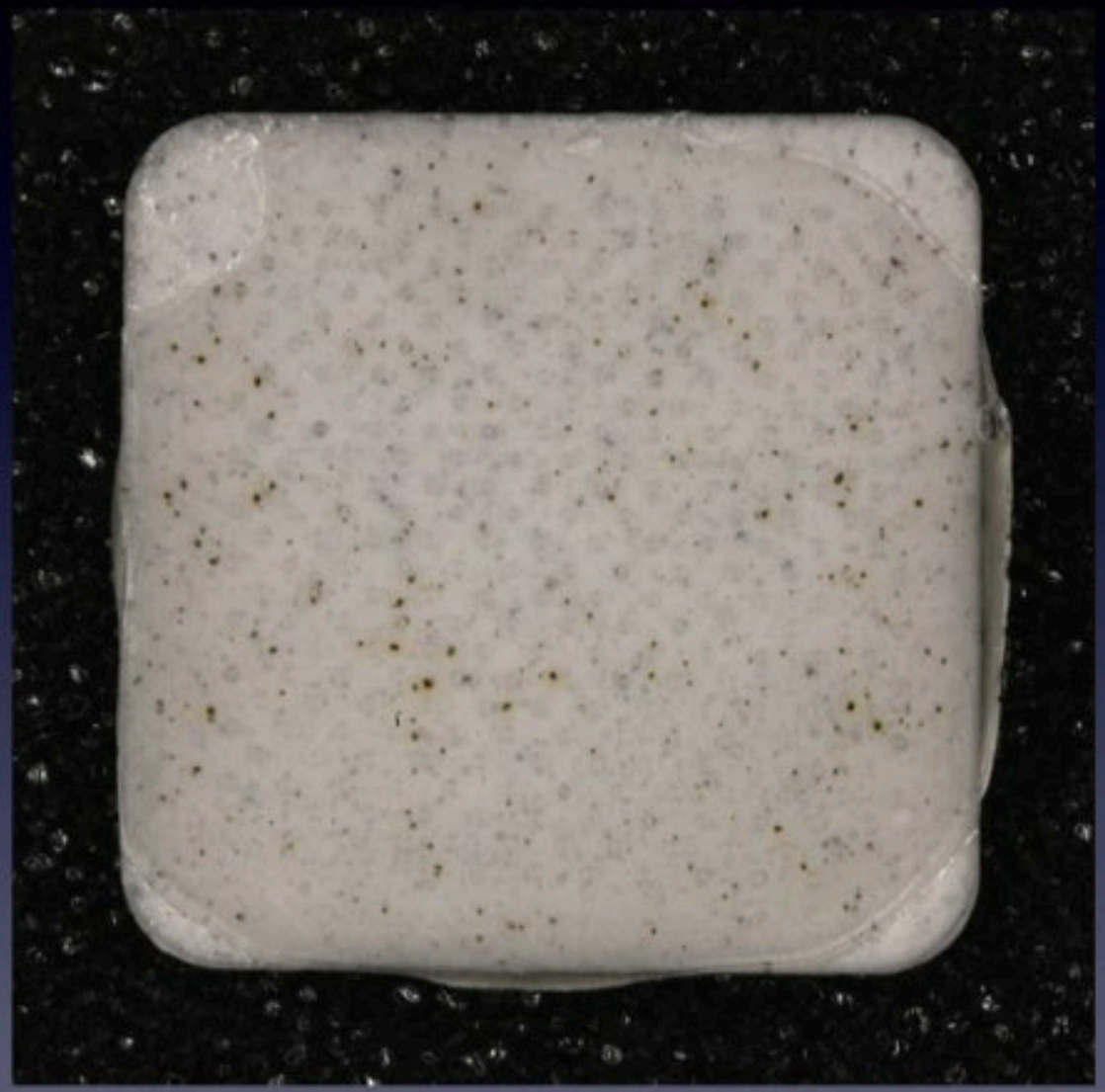
High molecular weight drugs such as growth factors can not be absorbed from skin.
To enhance the skin permeability of drugs, absorption enhancers, iontophoresis, electroporation and ultrasound have been studying. Eventhough, trans dermal drug delivery is severely limited by the poor permeability of drugs through the human skin, ie most drugs do not permeate through the skin at therapeutically relevant rates.

Rapid dermal inducing microneedle system is
an ideal sytem for gene vaccination

Effectiveness of this system is already confirmed and appliable to
DNA vaccination,
after the completion DNA plasmid for novel corona virus

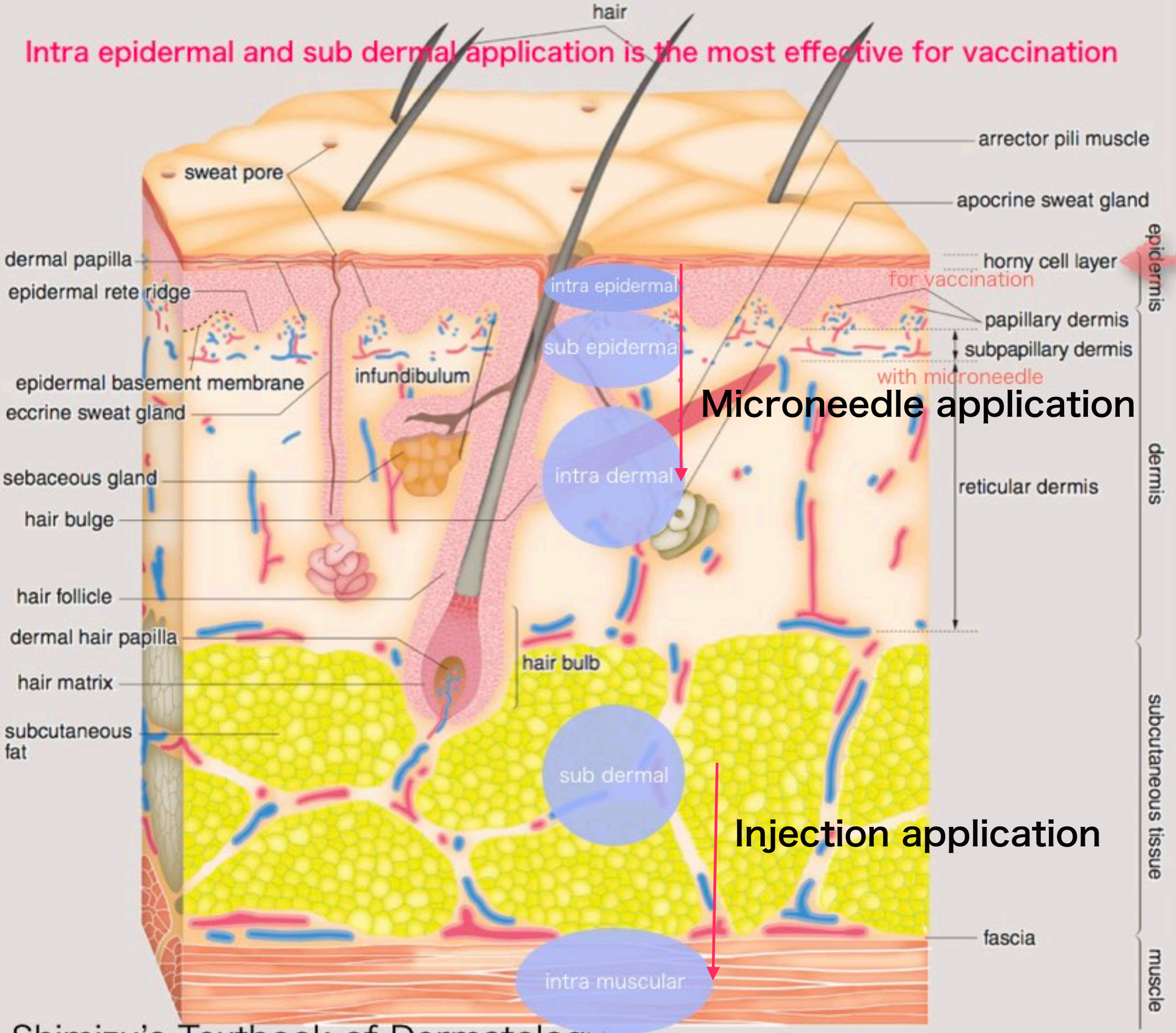
Shapes of microneedle

round shape



square shape

Intra epidermal and sub dermal application is the most effective for vaccination



Shimizu's Textbook of Dermatology

Srong barrier

High molecular substance dose not penetrate through horny layer.

Delivery systems

The development of new delivery systems raises the hope of vaccines that are safer and more efficient to deliver and administer. Lines of research include liposomes and *ISCOM* (immune stimulating complex).[92]

Notable developments in vaccine delivery technologies have included oral vaccines. Early attempts to apply oral vaccines showed varying degrees of promise, beginning early in the 20th century, at a time when the very possibility of an effective oral antibacterial vaccine was controversial.[93] By the 1930s there was increasing interest in the prophylactic value of an oral typhoid fever vaccine for example.[94]

An oral polio vaccine turned out to be effective when vaccinations were administered by volunteer staff without formal training; the results also demonstrated increased ease and efficiency of administering the vaccines. Effective oral vaccines have many advantages; for example, there is no risk of blood contamination. Vaccines intended for oral administration need not be liquid, and as solids, they commonly are more stable and less prone to damage or to spoilage by freezing in transport and storage.[95] Such stability reduces the need for a "cold chain": the resources required to keep vaccines within a restricted temperature range from the manufacturing stage to the point of administration, which, in turn, may decrease costs of vaccines.

Woman receiving rubella vaccination, Brazil, 2008.

A microneedle approach, which is still in stages of development, uses 'pointed projections fabricated into arrays that can create vaccine delivery pathways through the skin'.[96]

An experimental needle-free[97] vaccine delivery system is undergoing animal testing.[98][99] A stamp-size patch similar to an adhesive bandage contains about 20,000 microscopic projections per square cm.[100] This dermal administration potentially increases the effectiveness of vaccination, while requiring less vaccine than injection.[101]

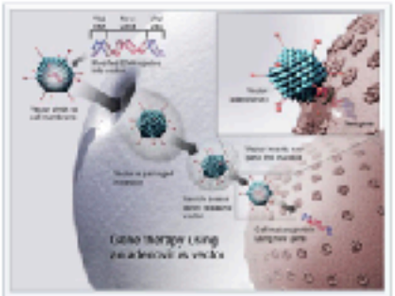
Plasmids

The use of plasmids has been validated in preclinical studies as a protective vaccine strategy for cancer and infectious diseases. However, in human studies, this approach has failed to provide clinically relevant benefit. The overall efficacy of plasmid DNA immunization depends on increasing the plasmid's immunogenicity while also correcting for factors involved in the specific activation of immune effector cells.[102]

DNA vaccine and RNA plasmid vaccines are under developing.
Self-dessolving MN is ideal drug delivery system for plasmid to induce immunological response for Novelcorona virus through Langelhans cells existing in epidermis and upper part of dermis.

Delivery [edit]

DNA vaccines have been introduced into animal tissues by multiple methods.



DNA vaccine and Gene therapy techniques are similar.

Saline injection [edit]

The two most popular approaches are injection of DNA in saline, using a standard hypodermic needle and gene gun delivery.[25] Injection in saline is normally conducted intramuscularly (IM) in skeletal muscle, or intradermally (ID), delivering DNA to extracellular spaces. This can be assisted by electroporation,[25] by temporarily damaging muscle fibres with myotoxins such as bupivacaine; or by using hypertonic solutions of saline or sucrose.[2] Immune responses to this method can be affected by factors including needle type,[11] needle alignment, speed of injection, volume of injection, muscle type, and age, sex and physiological condition of the recipient.[2]

Gene gun [edit]

Gene gun delivery ballistically accelerates plasmid DNA (pDNA) that has been absorbed onto gold or tungsten microparticles into the target cells, using compressed helium as an accelerant.[2][15]

Dosage [edit]

The delivery method determines the dose required to raise an effective immune response. Saline injections require variable amounts of DNA, from 10 µg-1 mg, whereas gene gun deliveries require 100 to 1000 times.[27] Generally, 0.2 µg – 20 µg are required, although quantities as low as 16 ng have been reported.[2] These quantities vary by species. Mice for example, require approximately 10 times less DNA than primates.[3] Saline injections require more DNA because the DNA is delivered to the extracellular spaces of the target tissue (normally muscle), where it has to overcome physical barriers (such as the basal lamina and large amounts of connective tissue, to mention a few) before it is taken up by the cells, while gene gun deliveries bombard DNA directly into the cells, resulting in less "wastage".[2][3]

Alternatives [edit]

Alternatives included aerosol instillation of naked DNA on mucosal surfaces, such as the nasal and lung mucosa,[15] and topical administration of pDNA to the eye[28] and vaginal mucosa.[15] Mucosal surface delivery has also been achieved using cationic liposome-DNA preparations,[9] biodegradable microspheres,[28][15] attenuated *Salmonella*,[30] *Shigella* or *Listeria* vectors for oral administration to the intestinal mucosa[31] and recombinant adenovirus vectors.[15] Another alternative vector is a hybrid vehicle composed of bacteria cell and synthetic polymers. An *E. coli* inner core and poly(beta-amino ester) outer coat function synergistically to increase efficiency by addressing barriers associated with antigen-presenting cell gene delivery which include cellular uptake and internalization, phagosomal escape and intracellular cargo concentration. Tested in mice, the hybrid vector was found to induce immune response.[32][33]

Another approach to DNA vaccination is expression library immunization (ELI). Using this technique, potentially all the genes from a pathogen can be delivered at one time, which may be useful for pathogens that are difficult to attenuate or culture.[2] ELI can be used to identify which genes induce a protective response. This has been tested with *Mycoplasma pulmonis*, a murine lung pathogen with a relatively small genome. Even partial expression libraries can induce protection from subsequent challenge.[34]

e. Langerhans cell

The Langerhans cell is a bone marrow-derived dendritic cell specific to stratified squamous epithelia such as the skin. Langerhans cells are frequently seen isolated in the middle and upper suprabasal cell layers (**Fig. 1.20**). The cells are distributed at a density of 400/mm^2 to 1,000/mm^2. They lack tonofilaments and cell attachment structures, such as desmosomes, and they migrate. By electron microscopy, a few fibrillary components and Birbeck granules, whose cross-section is a characteristic tennis racquet shape, are observed in the cell cytoplasm (**Fig. 1.21a**). Birbeck granules are known to be Golgi-apparatus-derived or membrane-derived, and carry antigens in the cells.

Langerhans cells present antigens to T cells (see Chapter 3 for immune reactions in the epidermis). Since the Langerhans cell is ATPase positive, CD1a positive and S-100 protein stain positive, it is easily distinguished from other kinds of cells.

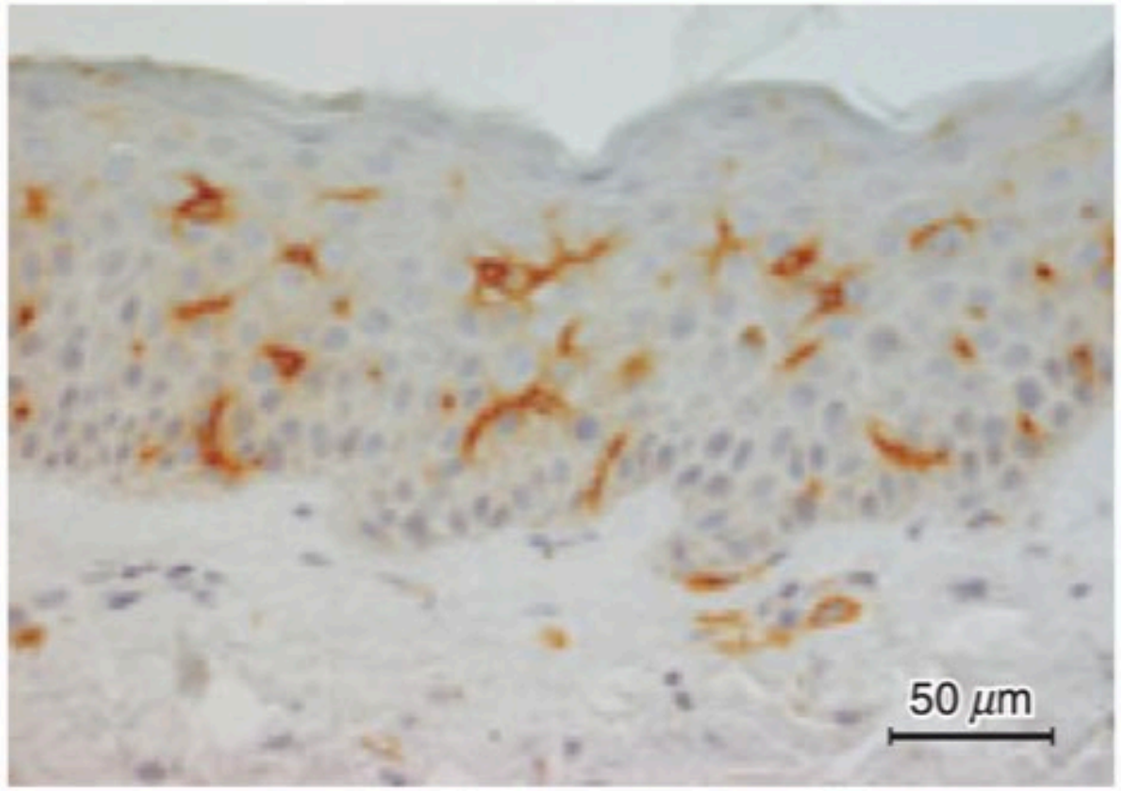


Fig. 1.20 Langerhans cell (immunostaining against CD1a).

Langerhans cells are bone marrow-derived cells and appear as dendritic cells. They contain the characteristic racquet-shaped Birbeck granules in the cellular cytoplasm (**Figs. 3.7** and **3.8**). Langerhans cells are antigen-presenting cells that are specific to the skin. Langerhans cells adhere to the epidermal keratinocytes by E-cadherins, functioning as sentinels against foreign antigens. When presenting an antigen to T cells, Langerhans cells are known to detach from the epidermis to reach the regional lymph nodes through the lymphatic vessels (**Fig. 3.9**). On the surface of the human Langerhans cells are MHC class II, CD1a, and S-100 proteins; this is useful for identifying them. With stimulation by antigens, they express CD80 and CD86 by the functions of GM-CSF and TNF-α secreted from keratinocytes to strongly activated T cells.

Needle-free vaccine delivery ☆

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Show more

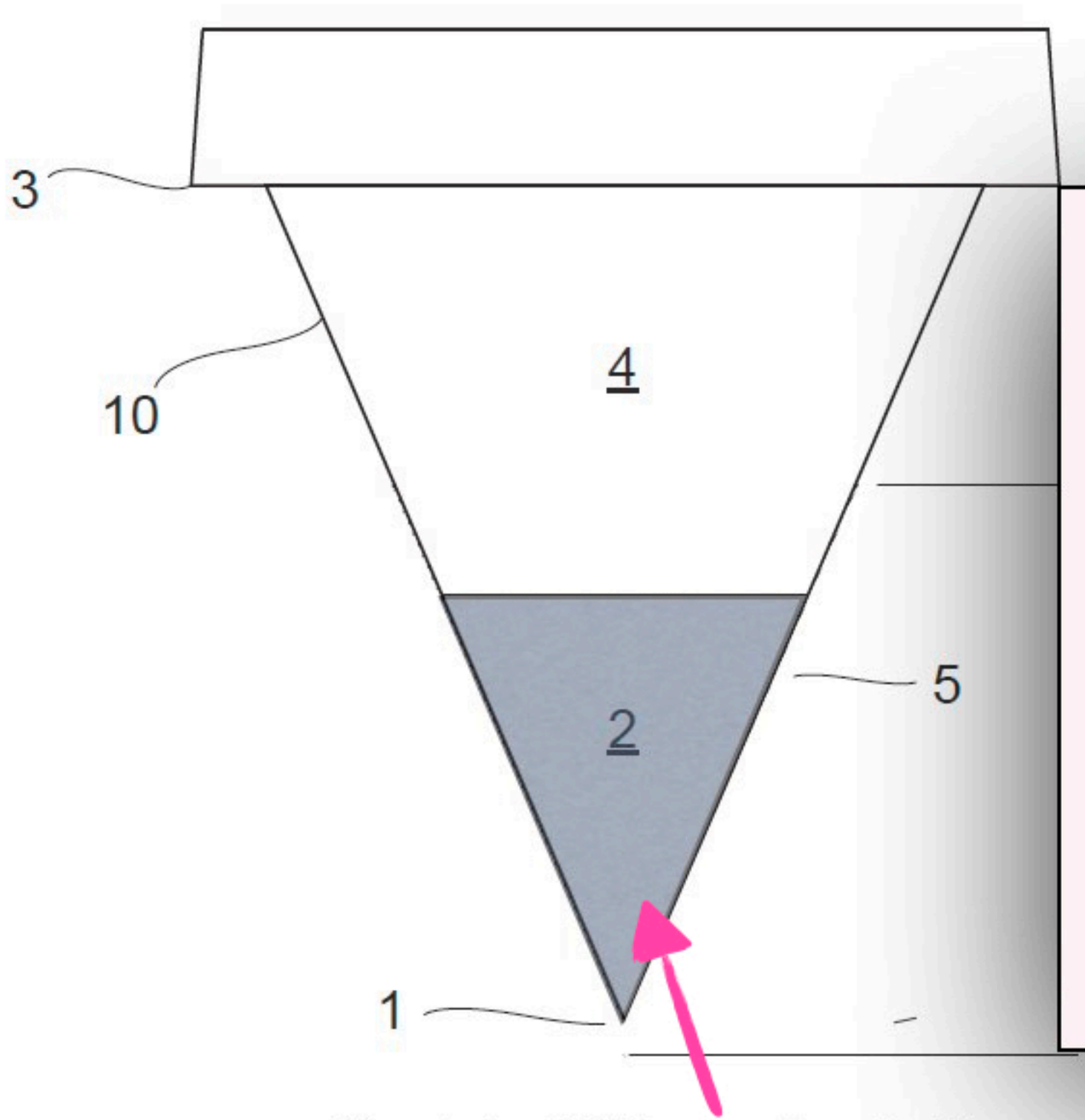
https://doi.org/10.1016/j.addr.2005.12.003

Abstract

The search for methods of vaccine delivery not requiring a needle and syringe has been accelerated by recent concerns regarding pandemic disease, bioterrorism, and disease eradication campaigns. Needle-free vaccine delivery could aid in these mass vaccinations by increasing ease and speed of delivery, and by offering improved safety and compliance, decreasing costs, and reducing pain associated with vaccinations. In this article, we summarize the rationale for delivery of needle-free vaccines and discuss several methods currently in use and under development, focusing on needle-free injection devices, transcutaneous immunization, and mucosal immunization. Jet injectors are needle-free devices that deliver liquid vaccine through a nozzle orifice and penetrate the skin with a high-speed narrow stream. They generate improved or equivalent immune responses compared with needle and syringe. Powder injection, a form of jet injection using vaccines in powder form, may obviate the need for the "cold chain." Transcutaneous immunization involves applying vaccine antigen and adjuvant to the skin, using a patch or "microneedles," and can induce both systemic and mucosal immunity. Mucosal immunization has thus far been focused on oral, nasal, and aerosol vaccines. Promising newer technologies in oral vaccination include using attenuated bacteria as vectors and transgenic plant "edible" vaccines. Improved knowledge regarding the immune system and its responses to vaccination continues to inform vaccine technologies for needle-free vaccine delivery.

Microneedle system combined with impact applicator is a perfect solution for DNA vaccinanion

Double layered medical microneedle



1 0 , Double layered microneedle

1 , Needle tip

2 , First portion contain drug

3 , Supporting plate

4 , Second basal portion

Part 2 : drug (+)

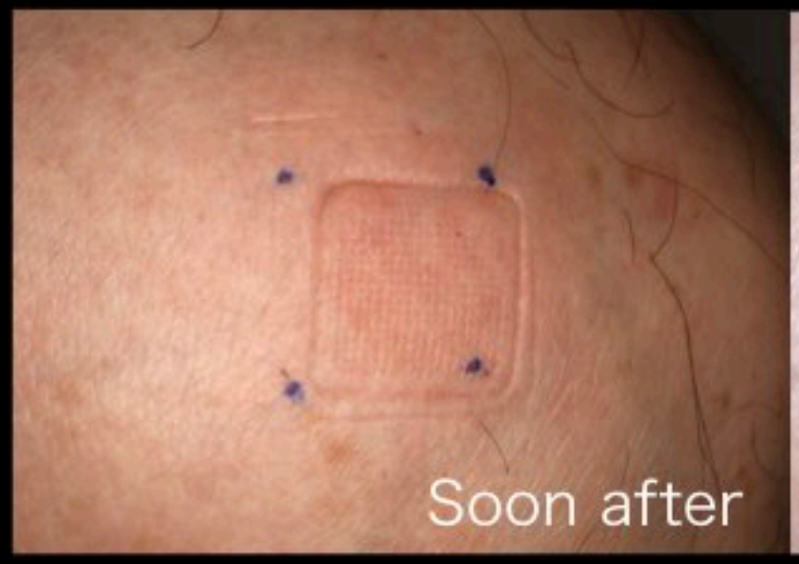
Part 4 : drug (-)

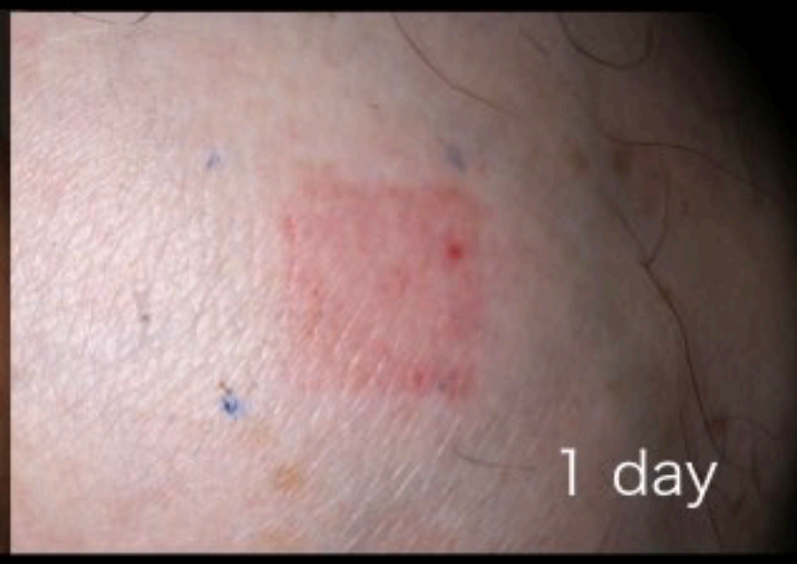
For medical purpose targeting dermis, more than 500μm is essential.

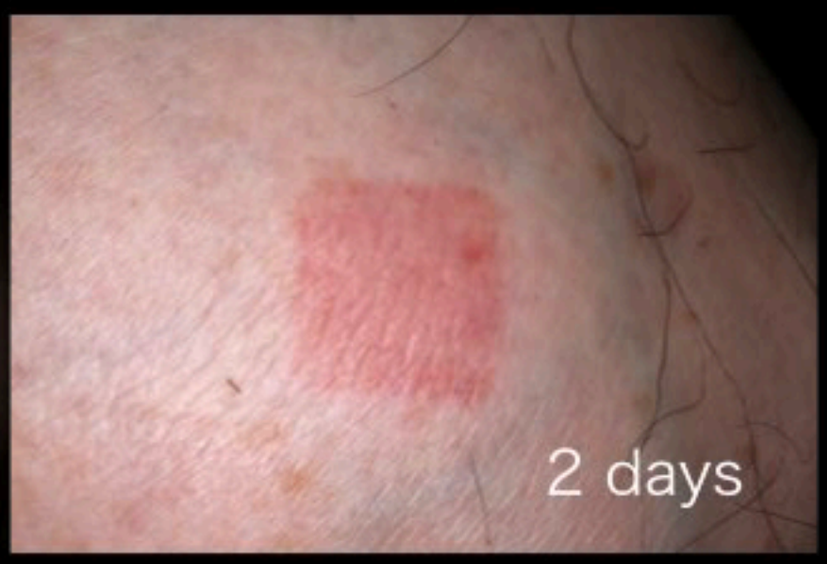
Contain DNA vaccine in the part 2

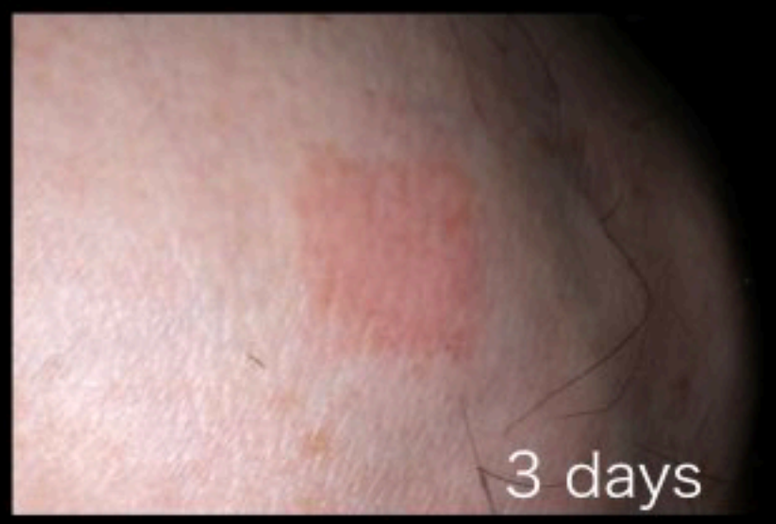
Applicator of microneedle

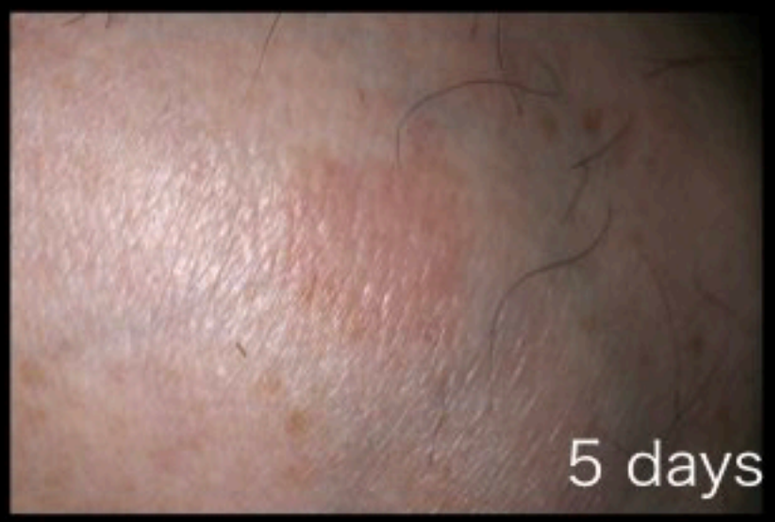
Due to the inherent elasticity and irregular surface topography of the skin, it remains a major challenge to the reproducibility of MN penetration. Therefore, in order to achieve uniform and reproducible MN penetration into skin, an external source of assistance could be very useful. Recent Pat Drug Deliv Formul. 2011 Jan;5(1):11-2

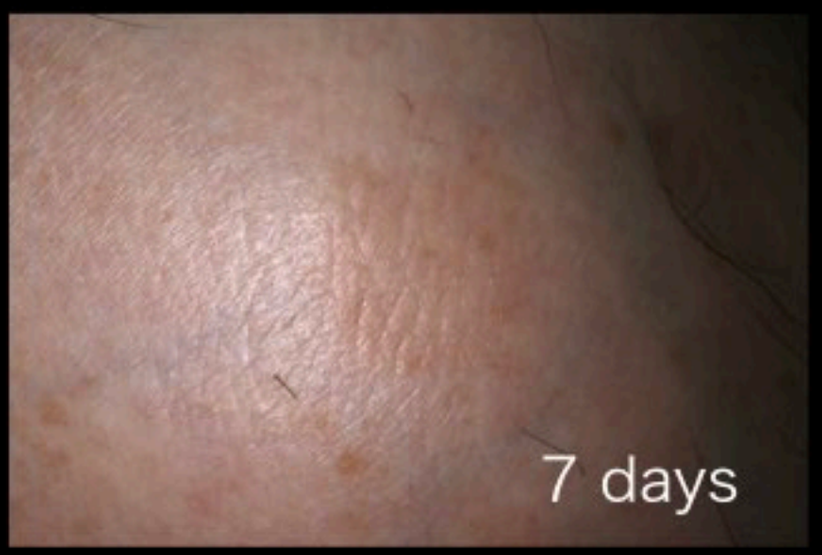


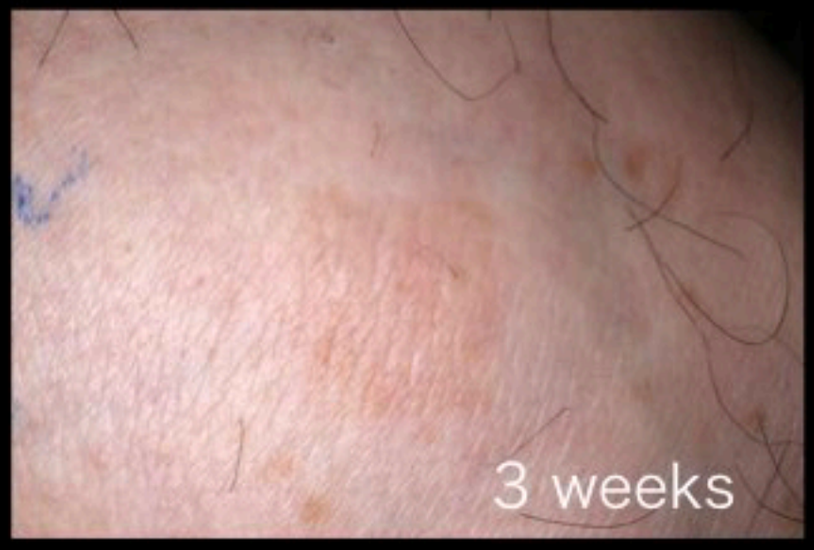


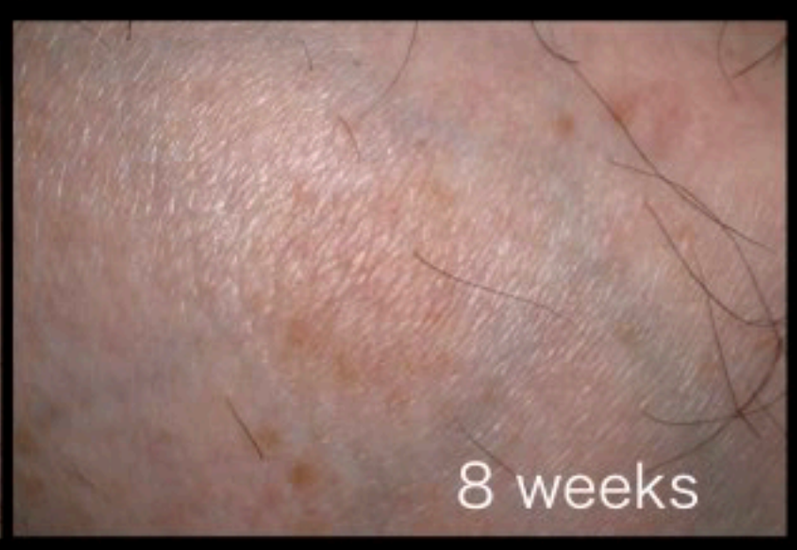


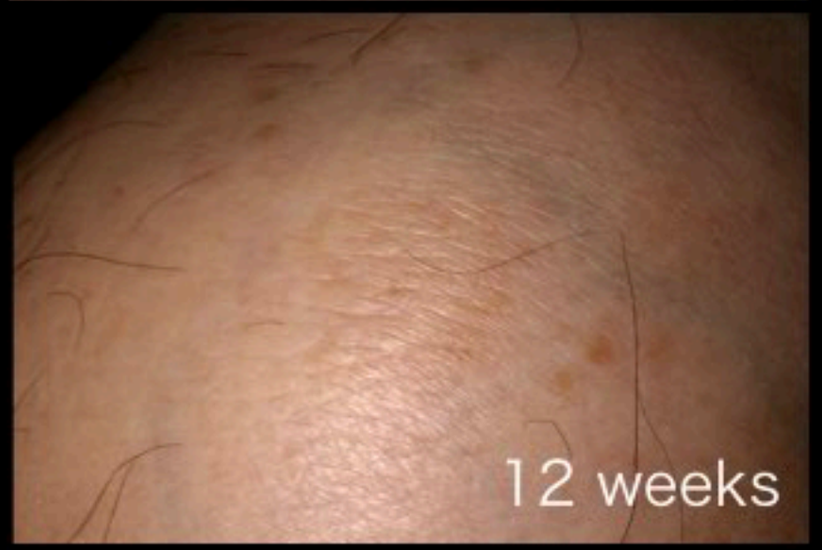




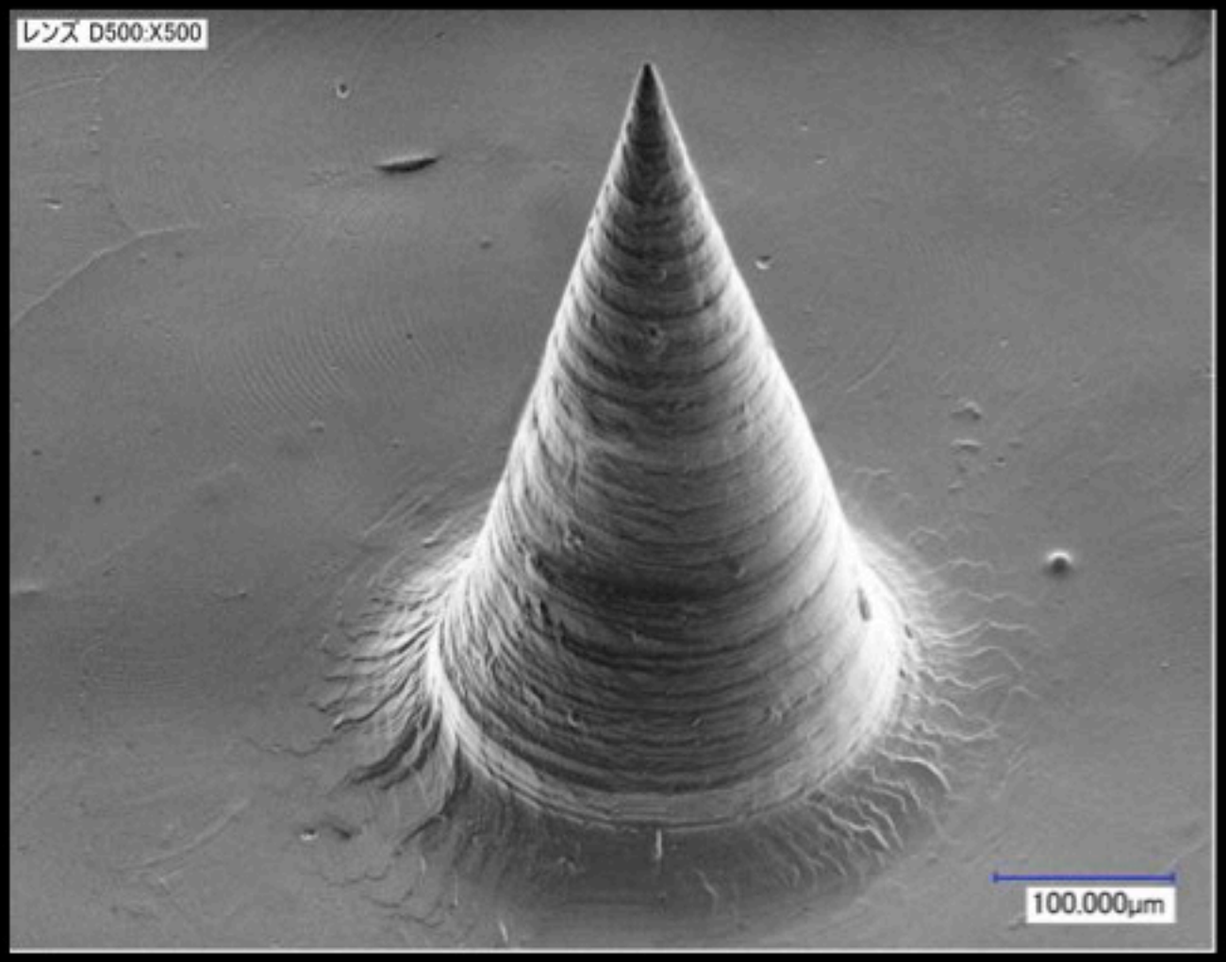




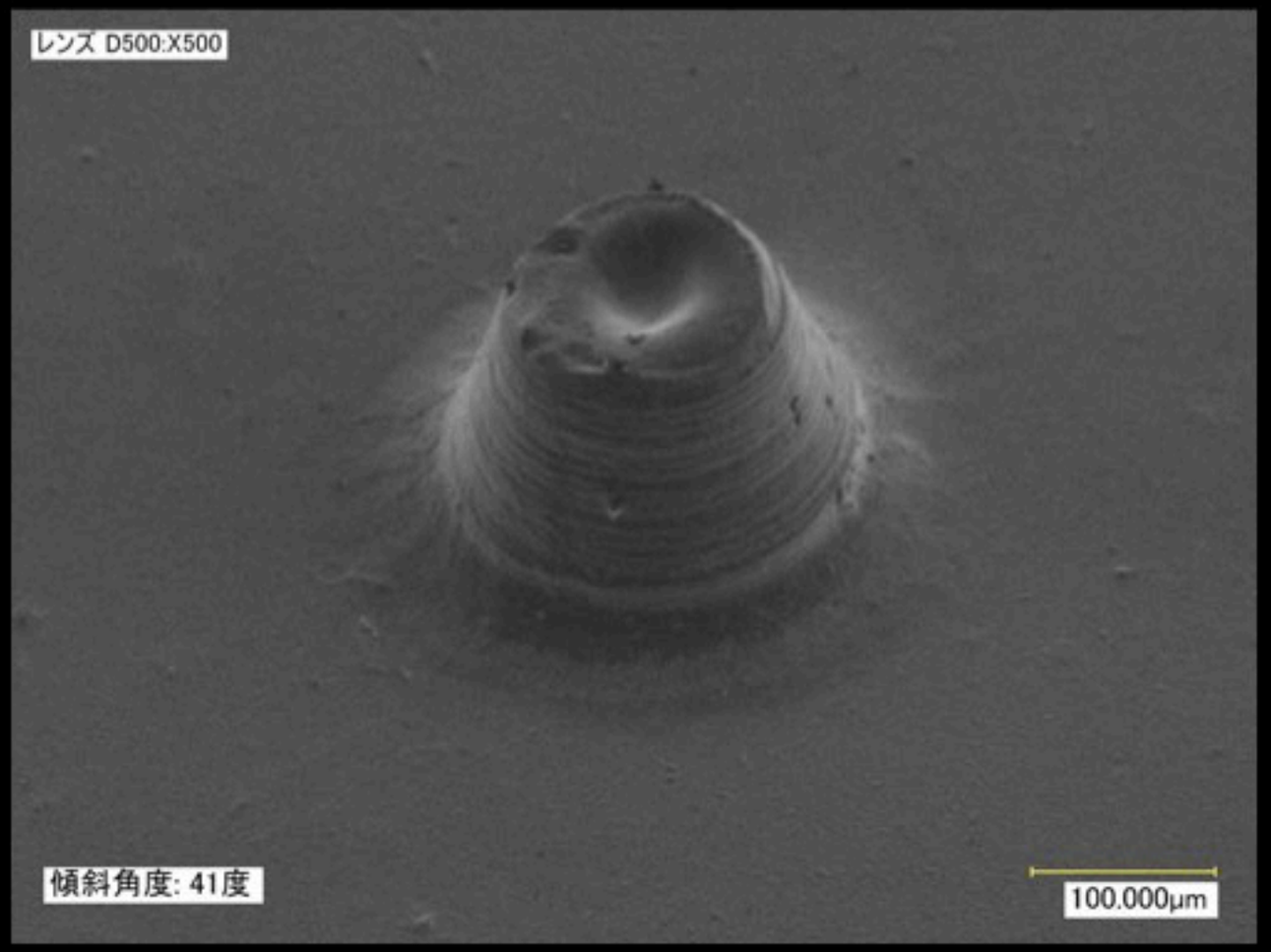




Clinical course after bFGF-microneedle application (thigh)



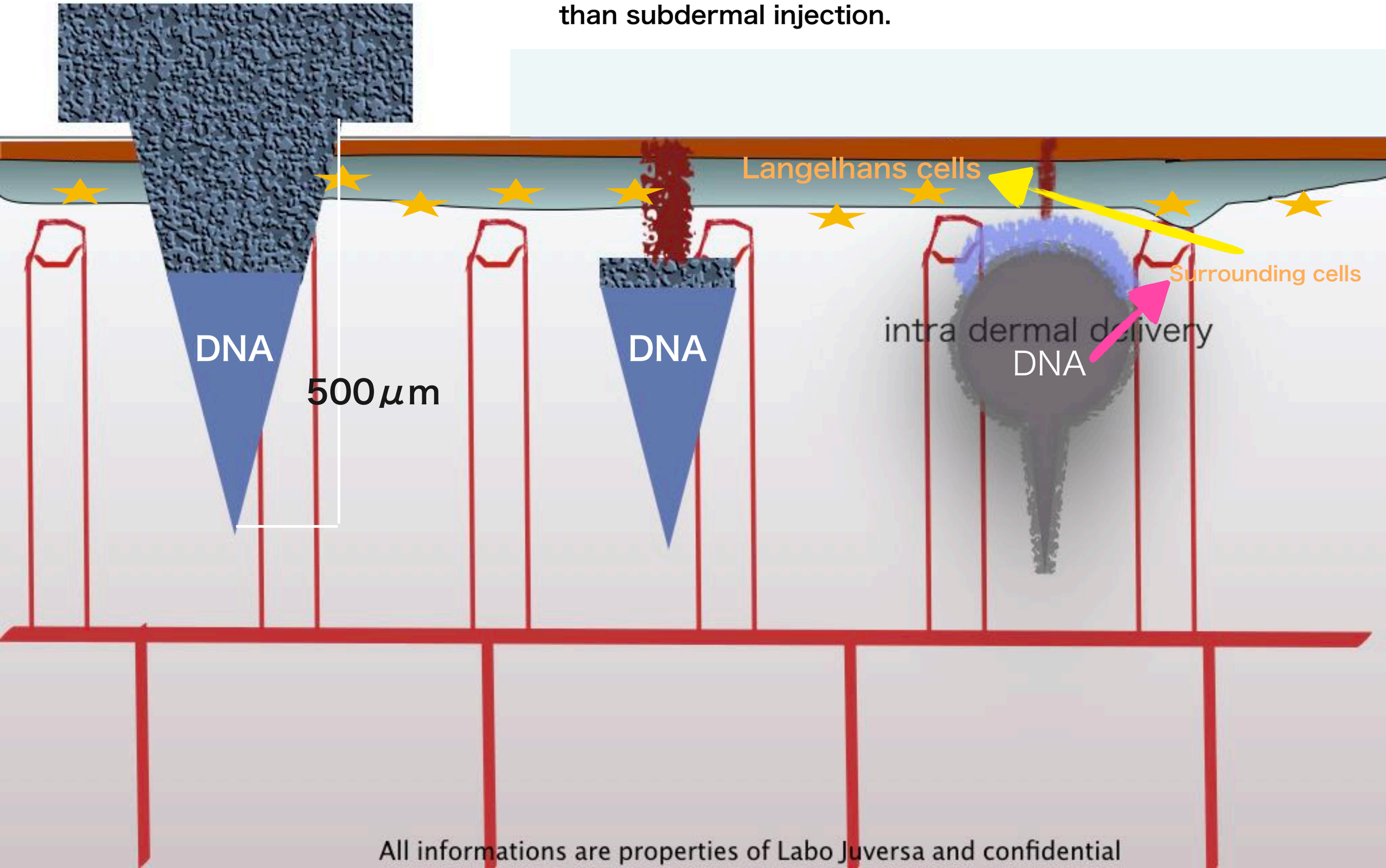
Before



After

Double layered microneedle for intra dermal delivery

Direct intra dermalmicroneedle application to i makes it possible to efficient immunization than subdermal injection.



The MERS DNA vaccine candidate is being developed using Inovio's DNA Medicines platform to deliver optimised synthetic antigenic genes into cells, where they are translated into protein antigens that activate an individual's immune system to generate robust targeted T-cell and antibody responses. Inovio's immunotherapies function exclusively in vivo, and have generated an antigen-specific immune response against targeted diseases in all clinical trials to date.

CEPI is also already working with with University of Queensland in a $10.6m collaboration to develop a "molecular clamp" vaccine platform, a potentially transformative technology that enables targeted and rapid vaccine production against multiple viral pathogens.

Finally, the new agreement with Moderna will see the company manufacture an mRNA vaccine against 2019-nCoV, to be funded by CEPI. The Vaccine Research Center (VRC) of the National Institute of Allergy and Infectious Diseases (NIAID), part of NIH, collaborated with Moderna to design the vaccine. NIAID will conduct IND-enabling studies and a Phase 1 clinical study in the U.S.

DNA vaccine and RNA plasmid vaccines are under developing.
Self-dessolving MN is ideal drug delivery system for plasmid to induce immunological response for Corona virus through Langelhans cells existing in epidermis and upper part of dermis.

The system is already completed and available!