

Introducing
Breakthrough for Vaccine
Transdermal Simple Injection System
“Medical Micro Needle”

Labojuversa Inc.

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

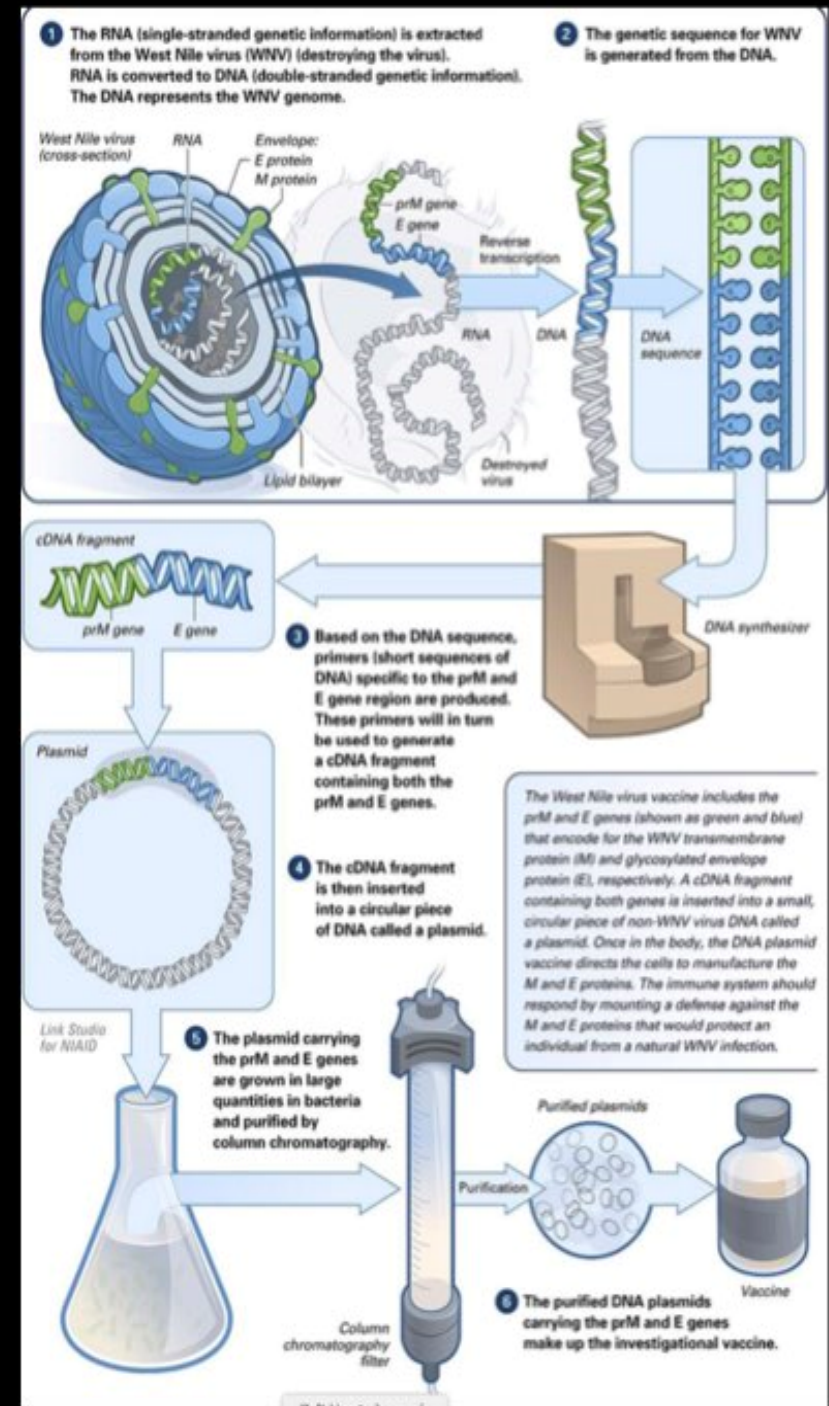
[DNA vaccination](#)

[T-cell receptor](#) peptide vaccines

Targeting of identified bacterial proteins

from Wikipedia

DNA vaccination



from Wikipedia

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

Drug delivery

- 1, Intra venous administration (IV)
- 2, Intra muscular administration (IM)
- 3, Sub dermal administration
- 4, Intra dermal administration
- 5, Sub epidermal administration
- 6, Intra epidermal administration
- 7, External application (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.

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.

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]



from Wikipedia

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

Delivery [[edit](#)]

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

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](#);^[26] 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]}

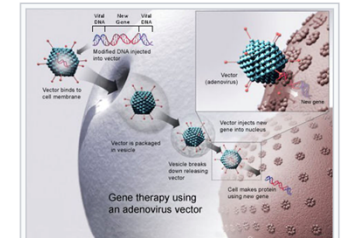
Dosage [[edit](#)]

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,^[3] [biodegradable](#) microspheres,^{[29][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]



DNA vaccine and Gene therapy techniques are similar.

from Wikipedia

ANSWER FROM DR. ICHIRO ONO

Over 20 years research and development efforts he has come up with this breakthrough transdermal drug delivery system, Micro Needle, which will replace conventional drug delivery and system. The entirety of MN system is patented in the key countries with proprietary trade secret.

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**

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

1. Length of microneedle: susceptible of the purpose, i.e. for epidermis, dermis, sub dermis or general administration through vessels at papillary dermis.
2. Shape and modifications on tip; diameter, hardness and coating.
3. Impact applicator for secure and quick drug delivery.
4. Numbers of layers; Mono layer, double layers or multilayers.
5. Variation of drug can be contained as well as target of the skin being possible.
6. Methods of fabrication.

Shapes of microneedle

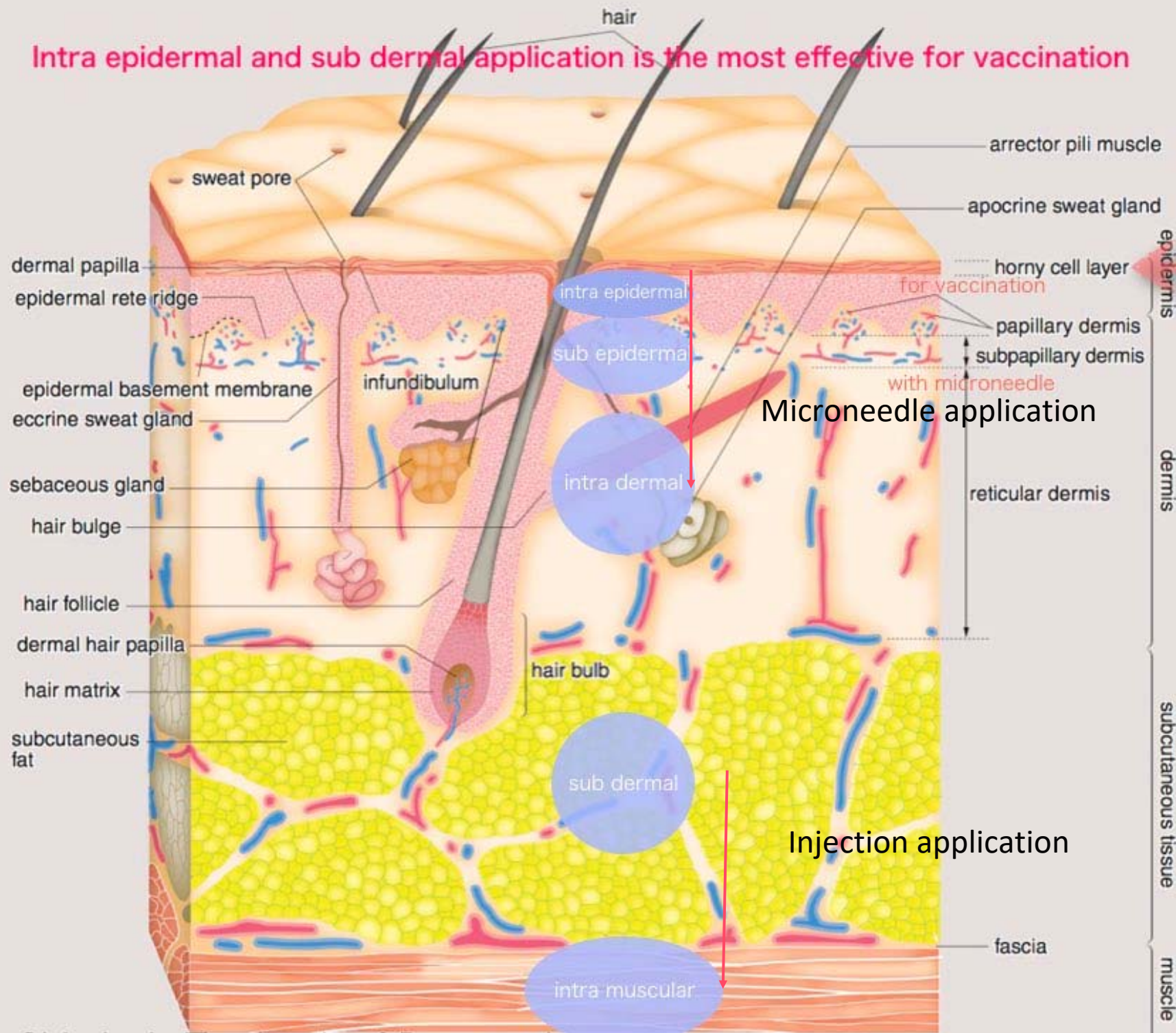


round shape



square shape

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



Strong barrier

High molecular substance dose not penetrate through horny layer.

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/mm² to 1,000/mm². 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.

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.

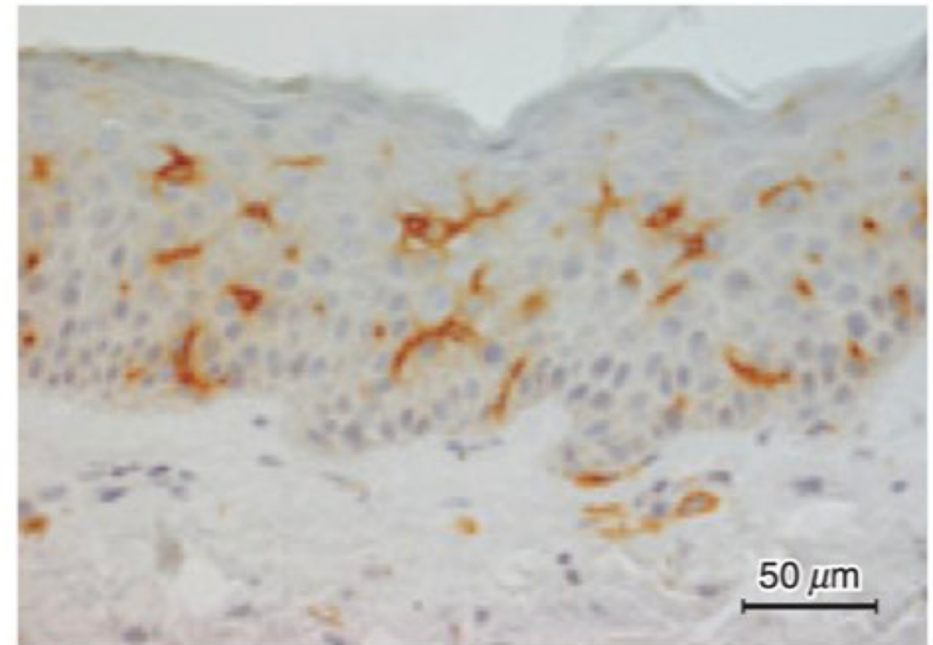


Fig. 1.20 Langerhans cell (immunostaining against CD1a).

Critical mass for designing and fabricating medical use microneedle

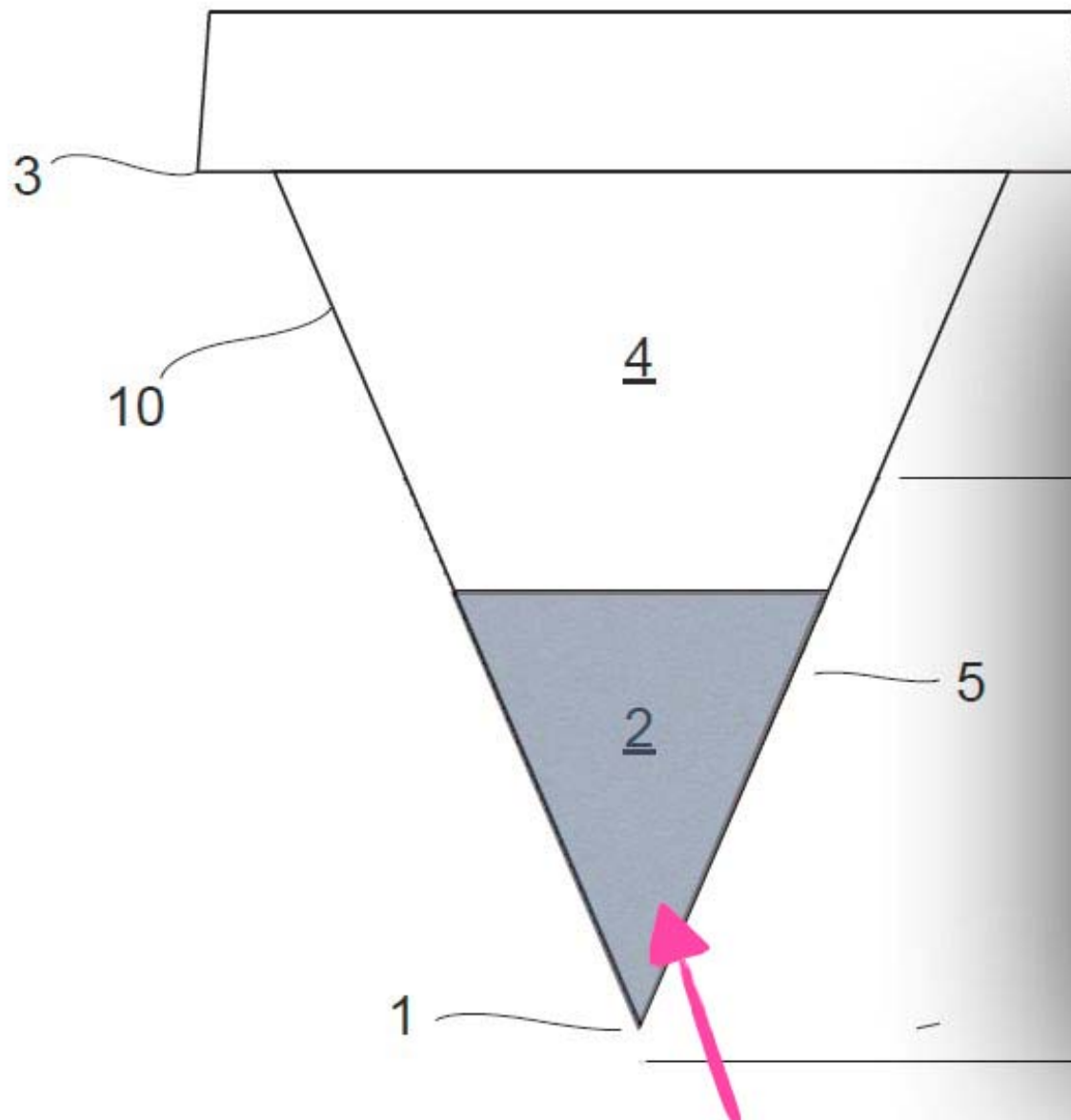
- Length of microneedle.
- What part of the microneedle for drug.
- Depth of the target. Epidermis, dermis or general administration?
- Selective delivery to where; epidermis, dermis, sub dermis, intra muscular or intra vascular through vessels exist in papillary dermis.
- Sharpness and hardness of the microneedle tip.
- What kind of company is suitable to fabricate microneedle patches and deliver them?

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

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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



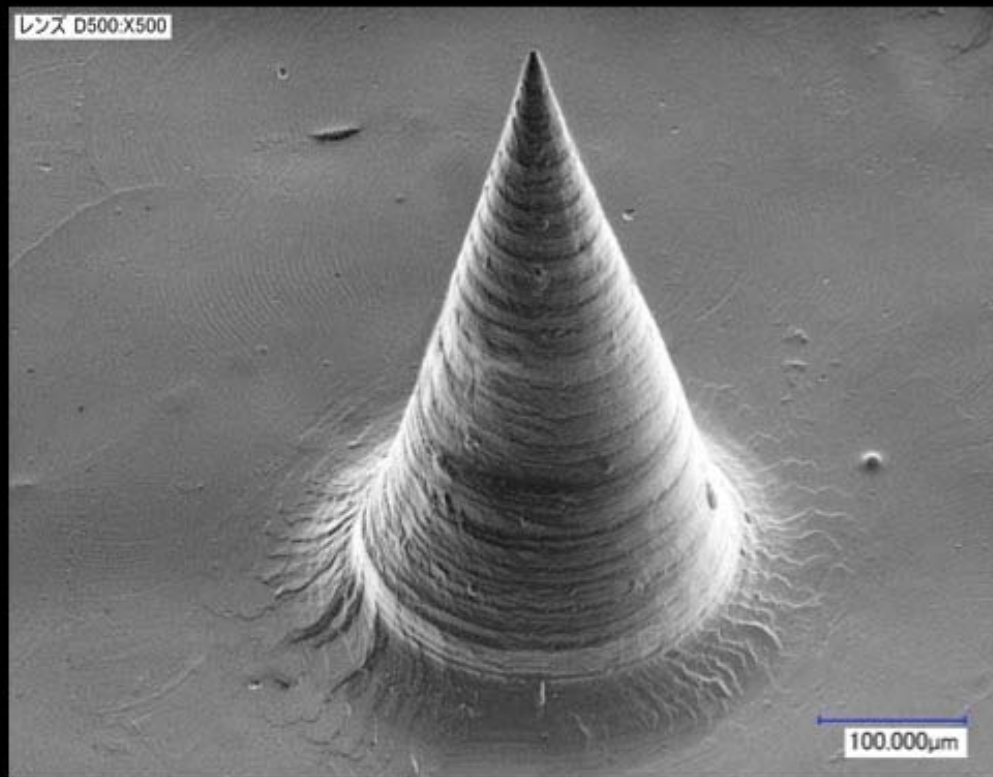
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Due to the inherent elasticity and irregular surface topography of the skin, it remains a major challenge to the reproducibility of MN penetration. Therefore, in order to achieve uniform and reproducible MN penetration into skin, an external source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

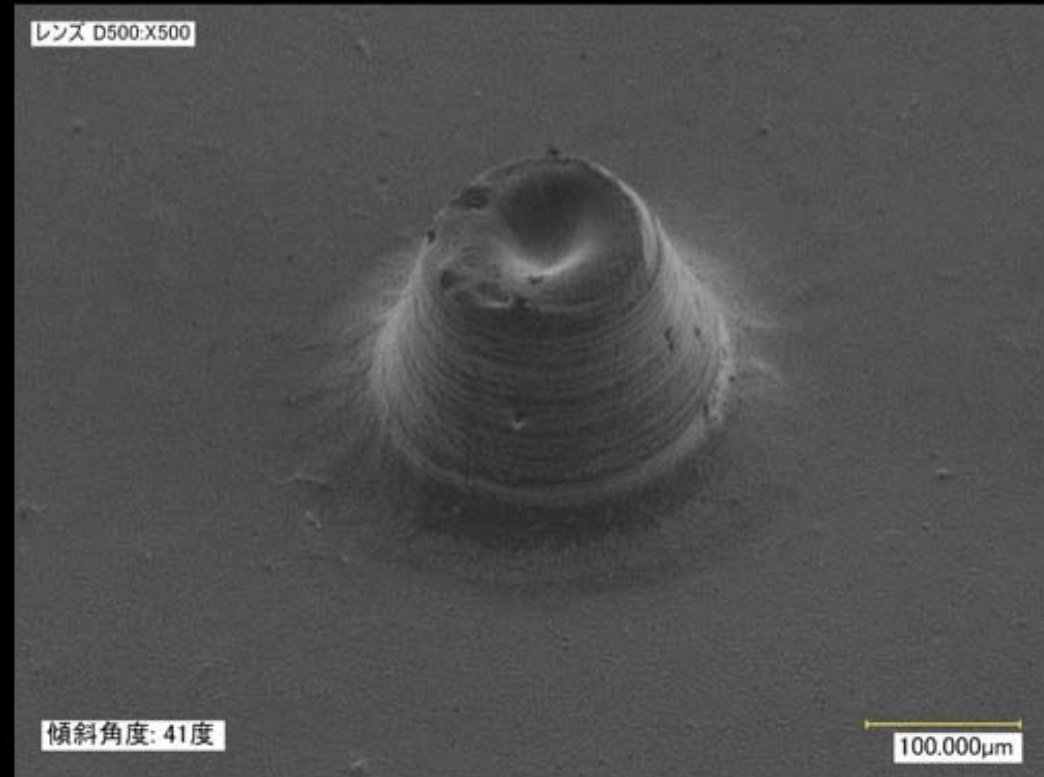


Clinical course after bFGF-microneedle application (thigh)

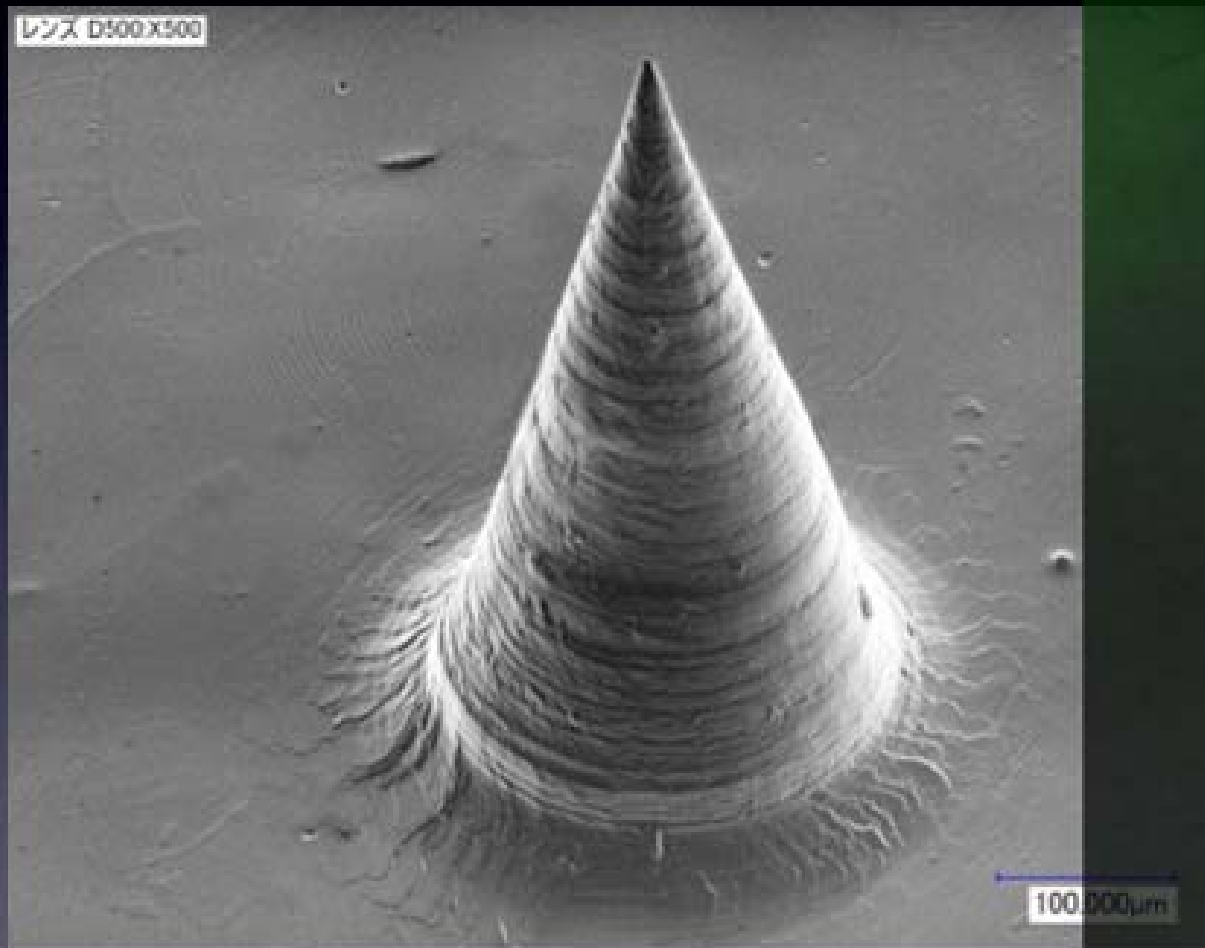
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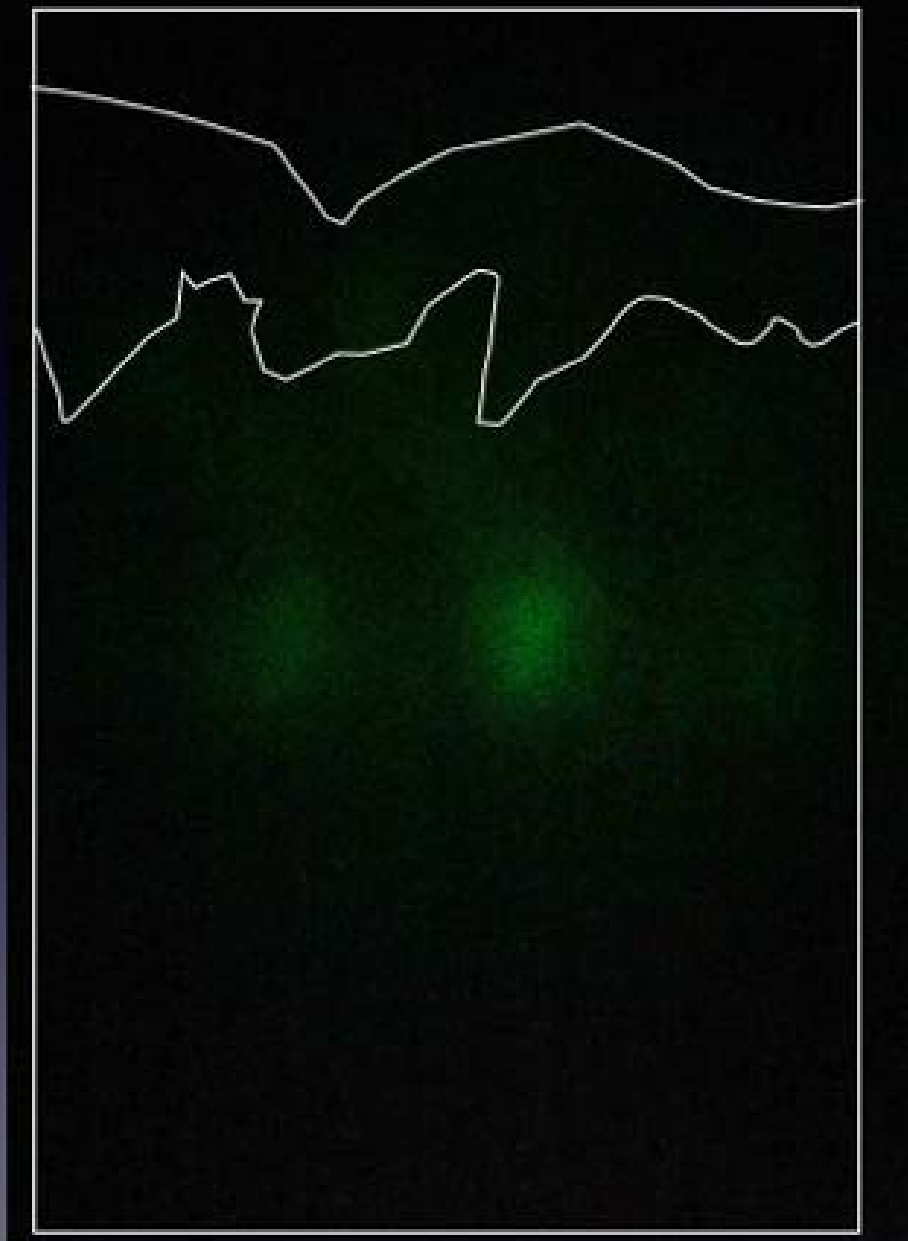
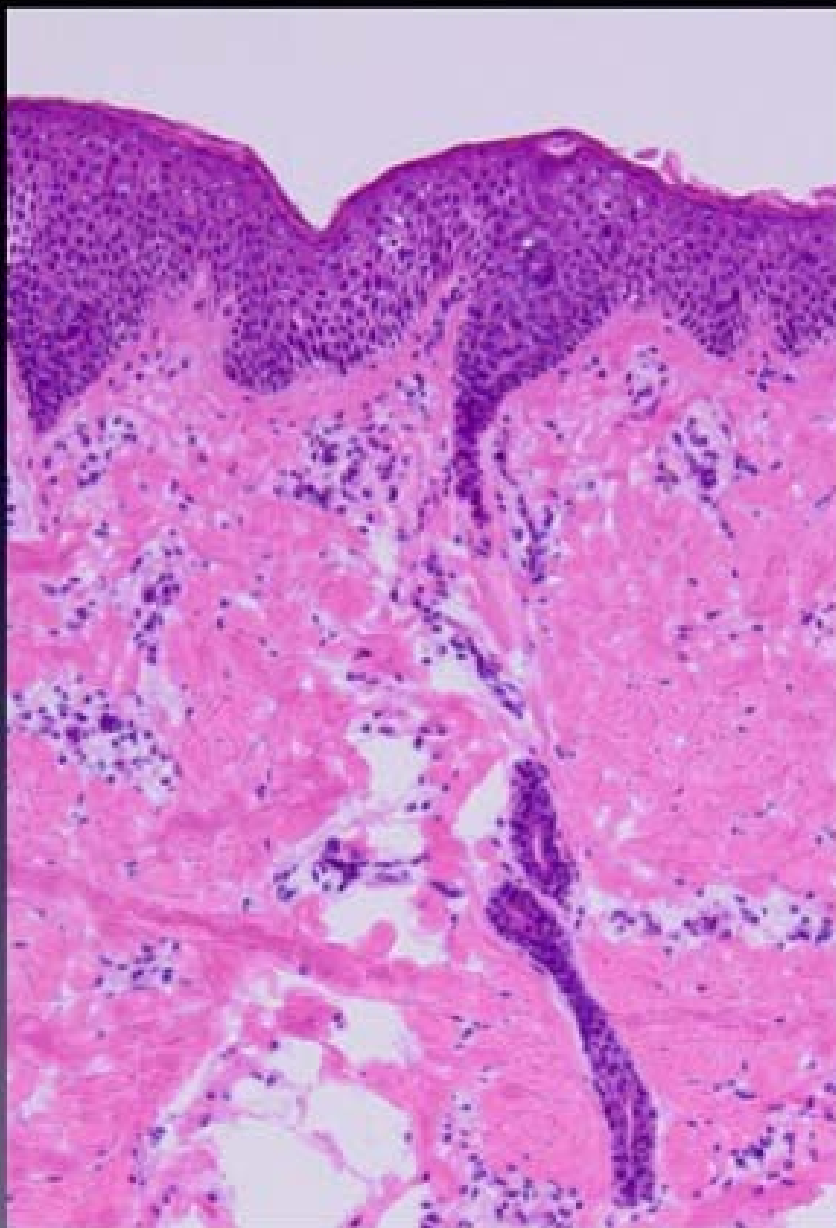


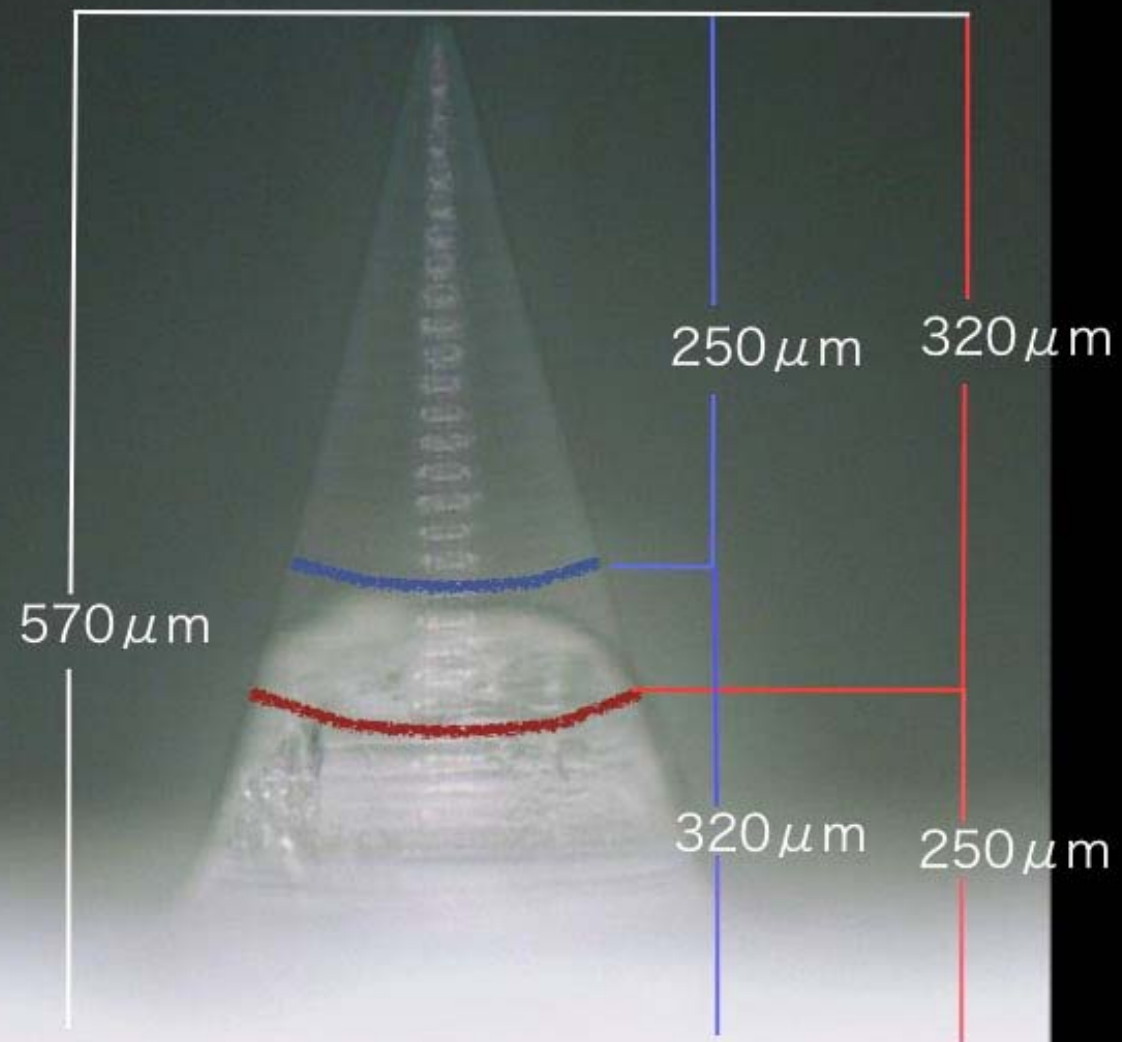
Before



After



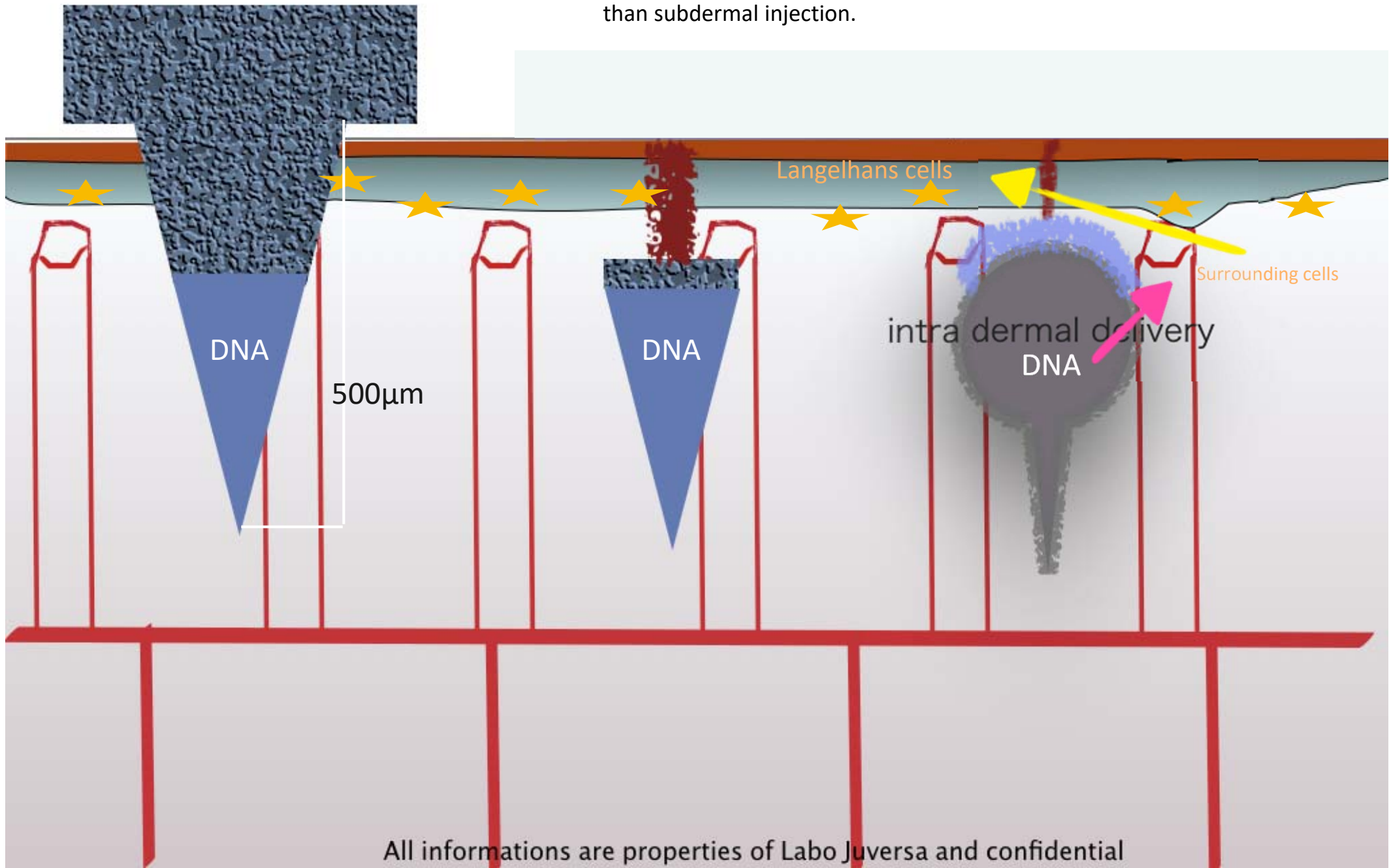




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Double layered microneedle for intra dermal delivery

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



Our system has been established through human clinical study for more than ten years using 20,000 patches, and also proved to be deliver one hundred percent of drug loaded to the tips of the microneedles in six hundred μ m length, which stimulate Langerhans cells in epidermis to induce antigen with small amount of vaccine.

Contact; flywing@cb3.so-net.ne.jp