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Using fluid mechanics for targeting genes and drugs to the skin for better vaccines

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Abstract

Millions of people die each year from infectious disease, and many more are affected by allergies. A major stumbling block to the full use of improved immunotherapies (e.g. vaccines) against these problems is our limited ability to deliver genes and drugs to the required sites in the body. Specifically, effective methods to deliver genes and drugs into outer skin and mucosal layers (sites with immunological, physical and practical advantages that cannot be targeted via traditional delivery methods) are lacking. This paper investigates this particular challenge for physical delivery approaches using fluid mechanics. The skin structural and immunogenic properties are examined in the context of the physical cell targeting requirements of the viable epidermis. Selected current physical cell targeting technologies engineered to meet these needs are examined: needle and syringe; diffusion patches; liquid jet injectors; microneedle arrays/patches; and biolistic particle delivery. The focus then moves to biolistic particle delivery: we first analyse engineering these systems to meet demanding clinical needs.

Introduction

The provision of safe and efficient routes of delivery of immunotherapeutics to the immunologically sensitive dendritic cells in the skin (and mucosa) has the potential to enhance strategies in the treatment of major disease. Examples of these include DNA vaccines and the immunotherapy of allergies. The application of physical methods to achieving this goal presents unique engineering challenges in the physical transport of immunotherapeutic biomolecules (e.g. polynucleotides) to these cells.

In this paper, the physiology, immunology and material properties of the skin are examined in the context of the physical cell targeting requirements of the viable epidermis. Selected cell targeting technologies engineered to meet these needs are briefly presented. The operating principles of these approaches are described, together with a discussion of their effectiveness for the non-invasive targeting of viable epidermis cells and the DNA vaccination against major diseases. The focus then moves to one of these methods, called biolistics, that ballistically delivers millions of microparticles coated with biomolecules to outer skin layers. The engineering of these devices is presented, beginning with earlier prototypes before examining a more advanced system configured for clinical use.

Engineering of physical approaches for the targeting of skin and mucosal cells

Within the viable epidermis, the location of Langerhans cells—as a delivery target for DNA vaccination—is tightly defined by:

- a vertical position at a consistent suprabasal location [1];
- a spatial distribution in the horizontal plane evenly distributed throughout the skin ; and

- a constitution of 2% of the total epidermal cell population.
- How can these and other epidermal skin cells be targeted? Despite its recognized potential, the viable epidermis has only recently been viewed as a feasible cellular targeting site with the emergence of new biological and physical technologies (discussed in detail in [2]). The challenge is the effective penetration of the SC and precise targeting of the cells of interest.

Mechanical properties of the stratum corneum physical barrier

The SC is a semi-permeable barrier that—owing to its variable mechanical properties—is challenging to breach, in a minimally invasive manner, to target the viable epidermal cells below. Mechanically, the SC is classified as a bio-viscoelastic solid and shows highly variable properties. Obvious differences include the huge variation in thickness and composition with the skin site and the age of an individual [3,4]. However, there are more subtle and equally important variations in SC properties to consider when configuring targeting methods.

As one example, the SC mechanical breaking stress is strongly influenced by the ambient humidity/moisture content [3]—the relative humidity range from 0% to 100% results in a decrease in excised human SC breaking stress from 22.5 MPa to 3.2 MPa. Similarly, an increase in ambient temperature also results in an SC breaking stress decrease by an order of magnitude. These and other sources of variability in the SC mechanical properties present challenges in configuring approaches to breach, in a minimally invasive manner, the SC and effectively deliver polynucleotides or antigens to the underlying cells.

Physical cell targeting approaches

Many *physical* technologies are being developed to overcome some limitations of biological approaches using needle-free mechanisms to breach the SC barrier to facilitate drug and vaccine administration directly to epidermal cells. Figure 1 illustrates schematically key physical targeting approaches relative to the scale of typical skin and the Langerhans cell layer of interest.

Needle and syringe (Figure 1a). For the illustration of the most common physical delivery method, a small gauge needle and syringe is shown in half-section in Figure 1a. Although this approach easily breaches the SC, precise targeting of the Langerhans cell-rich viable epidermis cannot be practically achieved. Hence, the needle and syringe is used for intramuscular injection. This inefficient, indirect targeting of dendritic cells with DNA has resulted in modest immune responses [5]. Other disadvantages of the needle and syringe include risks due to needle-stick injuries and needle phobia.

(a) *Diffusion/permeation delivery (Figure 1b).* Perhaps the least invasive method of breaching the SC is by permeation *through it*, driven by diffusion from patches applied to the skin [6]. However, currently, the general view is that this mode of delivery is best suited to smaller biomolecules (<500 Da [6])—considerably smaller than oligonucleotides and antigens. This

view is being challenged, with a recent study showing that very large recombinant antigens of ~1 MDa can be delivered to elicit systemic responses by diffusion from patches. The transport of larger biomolecules through the SC can be further enhanced by simple approaches, including tape stripping with an adhesive tape, brushing with sandpaper or the application of depilatory agents. Amongst the more advanced technologies are electroporation, ablation by laser or heat, radiofrequency high-voltage currents, iontophoresis, liposomes, sonophoresis and microporation. Many of these approaches remain untested for complex entities such as vaccines and immunotherapies. Permeation through the SC can also be enhanced by the coating of plasmid DNA on nanoparticles (~100 nm) for DNA vaccination.

(b) Liquid jet injectors (Figure 1c). An earlier physical needle-free approach with an initial interest in the mid-twentieth century was the high-speed liquid jet injector [7]. This technique has seen a recent resurgence, with liquid delivered around the Langerhans cells in gene transfer and DNA vaccination experiments [7], and the delivery of drugs [8]. As shown in Figure 1c, current liquid jet injectors typically disrupt the skin in the epidermal and dermal layer. To target exclusively the viable epidermal cells, such as Langerhans cells, the challenge of more controlled delivery needs to be addressed.

(c) Microneedle arrays/patches (Figure 1d). Researchers have overcome some of the disadvantages described by fabricating arrays of micrometre-scale projections to breach the SC and to deliver naked DNA to several cells in live animals [9]. Similar microprojection devices are used to increase the permeability of drugs and “conventional” protein antigen vaccines. Figure 1d shows that, unlike current liquid jet injectors, these microneedles can accurately target the viable epidermis. Furthermore, they are as simple to use as patches, whilst overcoming the SC diffusion barrier to many molecules. Moreover, compared with both the needle and syringe and liquid jet injectors, these microneedle methods are pain-free because of epidermal targeting. By drawing upon a range of manufacturing techniques, McAllister *et al.* [10] have shown that these microneedle arrays can be fabricated cost effectively from a range of materials, including silicon, metal and biodegradable polymers. These collective advantages make microneedle patches a very promising method of delivering oligonucleotides practically and cost effectively to epidermal cells for DNA vaccination. Figure 2 shows an example of the micro-nanoprojection patch device used for this purpose within our research group.

(d) Biolistic microparticle injection (Figure 1e). Currently, the most established physical method of DNA vaccination is biolistic microparticle delivery, otherwise known as gene guns. In this needle-free technique, pharmaceutical or immunomodulatory agents, formulated as particles, are accelerated in a high-speed gas jet to sufficient momentum to penetrate the skin (or mucosal) layer and to achieve a pharmacological effect. Biolistic DNA vaccination is achieved by the delivery of gold microspheres coated with plasmid DNA coding for specific antigens to epidermal dendritic cells [11]. Contoured Shock Tube, was conceived and developed [12]. The devices operate with the principle of delivering a payload of microparticles to the skin with a narrow range of velocities, by entraining the drug payload in a quasi-one-dimensional, steady supersonic flowfield.

In experiments with simple prototype CST devices, it was shown that the desired gas flow was achieved repeatedly. Importantly, further work with particle payloads measured a variation in free-jet particle velocity of $\pm 4\%$. In this research, measurements were made with Particle Image Velocimetry (PIV).

An embodiment of the CST configured to meet clinical needs is shown in Fig. 3, with the key components labelled. The device was fabricated from biocompatible materials and the device wall thickness was kept relatively constant to meet autoclave sterilization requirements.

To reduce the overall system length, the bottle reservoir (which operates by an actuation pin) is located within the driver annulus. A challenge of this co-axial arrangement was to maintain integrity of transonic gas flow within the driver initiated after diaphragm rupture. This challenge was met by carefully contouring the driver and obstacle of the mounting arrangement. Possible fragments from opening of the aluminium gas bottle are contained by a sealed filter at the bottle head.

The powdered pharmaceutical is enclosed and sealed by a cassette created by the inclusion of additional diaphragms upstream of the particle payload. In this case, the cassette houses two jets designed to mix the particles into a cloud, hence reducing the dependence on the initial particle location. Therefore, a nominally uniform spatial distribution of particles is released within the quasi-steady flow through the shock tube and nozzle. Repeated *in vitro* and *in vivo* experiments show that polycarbonate diaphragm fragments do not damage the target.

Elements of the silencing system are also shown in Fig. 3. The primary shock initiated by diaphragm rupture, reflected from the target, is identified as the main source of sound to be attenuated. This shock is collapsed into compression waves by a series of compressions–expansions induced by an array of orifices and saw-tooth baffles, resulting in appropriate sound levels for the operator and patient.

The device lift-off force is also to be well within user constraints. A peak lift-off force of 13 N is achieved by the careful selection of endbell contact diameter, silencer volume, flowrates through the reservoir and silencer geometry. This peak was for only a very short time within a gas flow lasting only ~200 μs (with a helium driver gas). The point of contact between the device and skin target was selected to maintain a target seal and to minimize the lift-off force, whilst not adversely affecting the impact velocities of the particles. The effect of silencing was also minimized by maintaining a supersonic gas flow transporting particles through the nozzle—so changes in the nozzle boundary condition were not fed upstream.

The range of impact conditions for the CST platform was achieved by the selection of appropriate helium/nitrogen mixtures within the gas bottle driver/driven area ratios.

Figure 1e shows a representative schematic diagram of microparticles in the skin following biolistic delivery, mirroring the observed microparticle distributions in human skin [13] and porcine skin [3].

Careful engineering analysis and design of the gas–particle dynamics of hand-held biolistics devices have led to advanced systems with nominally uniform and repeatable microparticle impact velocity distributions. Owing to the range in microparticle size, there is a broader than ideal impact momentum per unit area distribution for the microparticles—a parameter shown to be important for targeting specific skin layers [3] and the mucosa.

Because of this momentum variation, and the discussed large variability in tissue mechanical properties, a significant fraction of the microparticle payload does not breach the SC, as shown in Figure 1e. Importantly, Figure 1e also shows that many microparticles *do* reach the viable epidermis, allowing the triggering of the direct transfection of Langerhans cells as well as

keratinocytes; this enables the antigen to be processed via the exogenous pathways, thereby generating a balanced immune response [2] for DNA vaccination. Interesting recent work has shown that the action of ballistic particle penetration can lead to significant cell death in the skin [14]. These findings suggest that indirect, cross-priming transfection mechanisms take place in the skin, and these are the subject of further investigation. The overall goal of this and our other ballistics research is to optimize the delivery of microparticles and local biological effects to provide a further enhanced and consistent physical DNA vaccination platform technology.

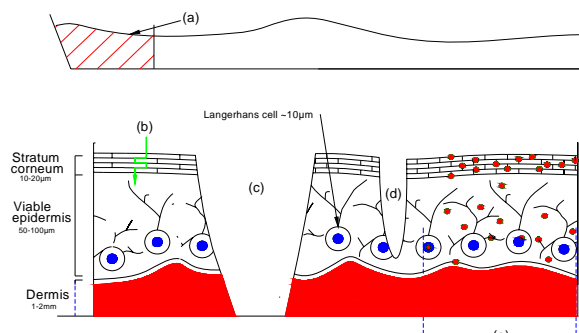


Figure 1 A schematic cross-section of the skin showing Langerhans cells. Five physical cell targeting approaches are also shown. (a) A half-section of a small gauge needle and syringe; (b) route of diffusion from patches; (c) penetration from a liquid jet injector; (d) a hole from a microinjector; and (e) distribution of microparticles following ballistic injection.

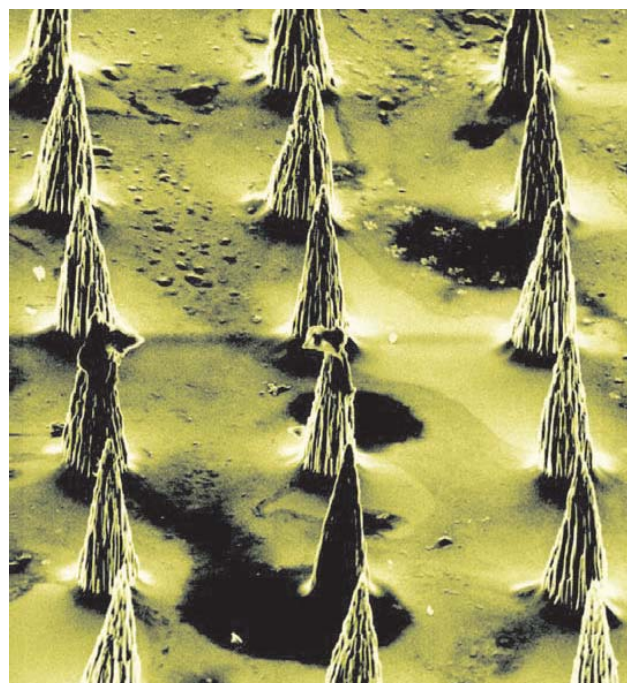


Figure 2. A scanning electron micrograph of a micro-nanoprojection patch following application to human skin.

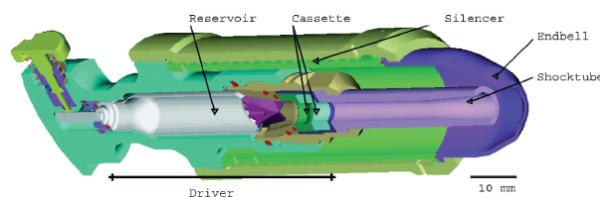


Figure 3. A contoured shock tube (CST) prototype configured for clinical ballistic delivery

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