Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Journal of Pharmaceutics 275 (2004) 13–17
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Potential use of nanoparticles for transcutaneous vaccine delivery: effect of particle size and charge

A.K. Kohli, H.O. Alpar*

School of Pharmacy, Centre for Drug Delivery Research, University of London, 29-39 Brunswick Square, London WC1N 1AX, UK

Received 20 January 2003; received in revised form 27 October 2003; accepted 31 October 2003

Abstract

The aim of this study was to investigate the effect of size and charge on the permeation of nanoparticles through the skin as the first step in designing a transdermal vaccine delivery system. Fluorescent particles ranging in size and charge were applied to the surface of full thickness pig skin in a diffusion chamber and the receptor fluid was assayed to determine permeation. Fluorescence microscopy was used to visualise the skin after experiments. The results showed that only 50 and 500 nm particles that were negatively charged were able to permeate the skin. This provides evidence of the potential of nanoparticles as delivery vectors for antigens and DNA for the purpose of transdermal vaccination protocols. The results would indicate that negative particles with sufficient charge may be ideal carriers for this purpose.

Keywords: Skin; Transdermal; Permeation; Nanoparticles; Vaccine; Adjuvant

1. Introduction

The benefits of vaccination have been observed globally for many years. Successful outcomes of initiatives such as those to eradicate smallpox, polio and the world health organisations' "Expanded Programme on Immunisation" (Dittmann, 2001) are the tip of an iceberg which remains still under exploited. A major drawback with current vaccination regimes lies in the method of administration, which generally utilises the parenteral route. Factors such as the need for trained personnel, needle-stick injuries, needle disposal and cost all complicate the wide-spread use of this method in less developed nations and areas of the population which may be financially deprived. As a consequence alternative routes of vaccine administration are being sought. Such routes include administration via mucosal surfaces which include the nasal, oral and vaginal route. In recent years the skin has also been added to the list of sites studied for the purpose of non-invasive vaccination.

The skin has been shown to be an active immune organ (Bos, 1997) and several authors have now displayed its ability to stimulate an immune response. In a series of papers, Glenn et al. showed that with the use of enterotoxin or cholera toxin as adjuvants, both mucosal and systemic responses can be elicited to co-administered antigens (Glenn et al., 1996, 1998, 1999; Hammond et al., 2001). This success has been extended to the delivery of DNA, in combination with chitosan and liposomes to which both humoral and cell-mediated responses have been elicited (Shi et al.,

* Corresponding author. Tel.: +44-207-753-5928; fax: +44-207-753-5942.
E-mail address: oya.alpar@ulsop.ac.uk (H.O. Alpar).

0378-5173/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2003.10.038

1999; Cui and Mumper, 2001, 2002). In our own laboratories we have shown that the application of *Yersinia pestis* in chitosan-pluronic carriers is capable of generating a significant response that can also be boosted by the nasal route (Somavarapu et al., 2001). The success of the aforementioned studies is thought to be due to the antigen presenting Langerhans cells, which lie in the viable epidermis, below the stratum corneum (Bos, 1997) and have the ability to stimulate an immune response with the uptake of nanomolar concentrations of antigen (Tuting et al., 1998; Banchereau and Steinman, 1998). The stratum corneum is the principle barrier to penetration through the skin, owing to its "brick and mortar" structure of proteins and intercellular lipids. Various methods have been employed to overcome this barrier to deliver drugs and antigens ranging from physical processes such as iontophoresis, electroporation and sonophoresis to chemical permeation enhancers and the use of delivery vectors such as liposomes, transferosomes and nano/microparticles.

Our work aims to investigate the delivery of nanoparticles through the skin to study the effect of charge and size on permeation in vitro.

2. Materials and methods

Pig skin (Wingate Institute, UK) was used as a model membrane owing to its close resemblance with human skin with regards to permeability (Hammond et al., 2000).

Skin was frozen as soon as it was excised from the animal and stored at −20 °C until use. Prior to use the subcutaneous fat was removed using forceps and scissors and was inspected for damages using light microscopy after staining with haematoxylin and eosin before being used for diffusion experiments. The samples were placed in a modified diffusion cell (Fig. 1). The cell was based on the ORNL chamber used by Holland et al. reviewed in Kemppainen and Reifenrath (1990). A static chamber was selected to allow accumulation of the permeant in the receptor compartment.

Phosphate buffered saline (PBS) was used as the receptor fluid and the cell was heated to 32 °C using a water jacket. The tissue was allowed to acclimatise to the conditions of the receptor phase by placing it in PBS at 32 °C for 30 min. This was then applied between the two phases taking care not to induce any air bubbles. Following this a 0.5 ml aliquot of receptor fluid was removed from a sampling port by forcing PBS into the system from a syringe which led into the receptor phase. Since the system was air tight, this allowed for the sampling of receptor fluid into another syringe positioned at the other side of the compartment. On applying the donor solution another sample was withdrawn, with subsequent aliquots taken at 1, 2, 3, 4 and 6 hourly intervals. The donor phase consisted of a 1% (v/v) dilution of fluorescent particles in water. The particles tested were 50, 100, 200 and 500 nm latex particles that were positively charged (Interfacial Dynamics Corporation, Oregan, USA) negatively charged and neutral (Polysciences, Inc., Warrington, USA). The concentration of particles permeating was established by detecting the fluorescence using a Wallac Victor^{2TM} multilabel counter in each of the samples withdrawn.

The concentrations obtained were corrected for the autofluorescence of the skin and the dilution of each sample due to the addition of replacement fluid after each aliquot was also corrected for.

Each section of skin was frozen at the end of the permeation experiments in liquid nitrogen. They were subsequently sectioned vertically using a cryotome (Bright instu, Cambridge, UK) and inspected using fluorescence microscopy (Nikon Microphot FXA, UK).

3. Results and discussion

The permeation experiments showed that 50 and 500 nm particles that were negatively charged were able to permeate the skin. The remaining particles that were tested did not show any permeation.

Fig. 2 shows the delivery of the particles that showed permeation through the skin, i.e., 50 and 500 nm negatively charged particles. The results show that there is no real difference in the magnitude of permeation between the two sizes except on first applying the particles. Fig. 3 shows the permeation observed of the fluorescent latex particles that were able to permeate the skin. The micrograph of the vertically sliced skin after the application of 50 nm particles shows an accumulation of fluorescence in the stratum corneum and in the viable epidermis. There are no visually defined particles owing to their small size. In contrast the micrograph of the permeating 500 nm

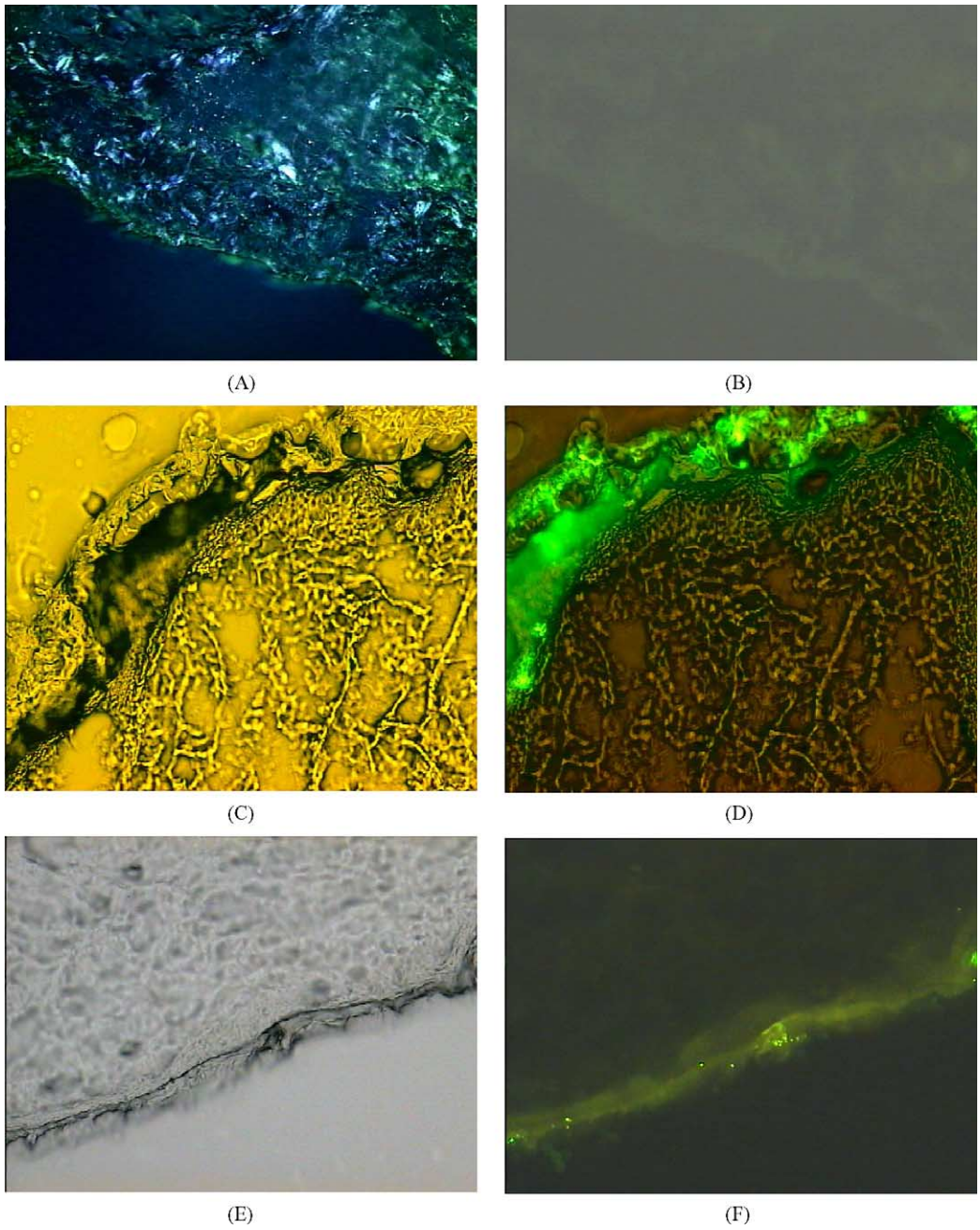

Fig. 1. (A) Vertical cross-section of the skin with no particles applied, (B) fluorescent micrograph of vertical cross-section of skin with no particles applied, (C) vertical cross-section of the skin following application of 50 nm carboxylate particles, (D) fluorescent micrograph of cross-section of skin following application of 50 nm carboxylate particles, (E) vertical section of the skin following application of 500 nm particles, (F) fluorescent micrograph of cross-section of skin following application of 500 nm particles.

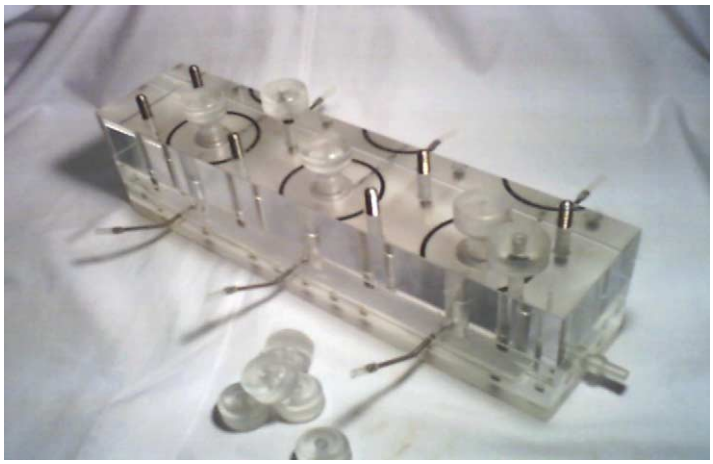

Fig. 2. Diffusion chamber constructed for this study.

spheres shows discrete nanoparticles in the stratum corneum and viable epidermis. Therefore, although it appears that there is more fluorescence in the 50 nm micrographs this is likely to be a consequence of the poor resolution of these particles under the fluorescent microscope. Hence, these micrographs must not be interpreted as quantitative measures of particle permeation, but as a means of illustrating that the process of permeation is occurring in these samples.

It is recognised that the major barrier to delivery through the skin is the stratum corneum. From this it is assumed that those particles that permeate this layer encounter little resistance and so are quantified in the receptor phase of the diffusion unit, explaining why there is no fluorescence in the lower layers of the skin on the micrographs. Additionally at the end of each experiment any particles that were in the lower layers would have continued to diffuse to the posterior surface of the skin and would be removed when the samples were washed to remove any excess solution.

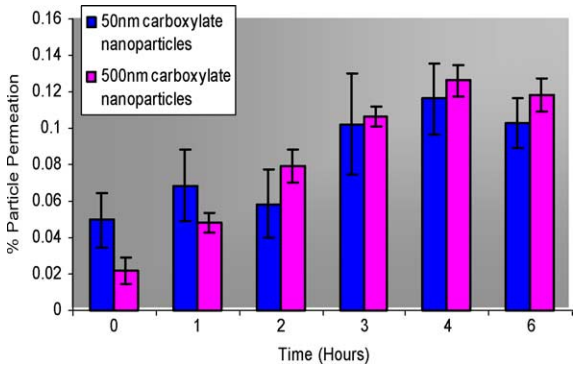

Fig. 3. Permeation of 50 and 500 nm negatively charged particles through full thickness pig skin over a 6 h time period ($n = 5$).

The particles that permeated were both negatively charged and did not follow a size dependency. Positively charged and neutral particles of all sizes and negatively charged 100 and 200 nm particles did not penetrate. We believe this is due to the charge of the particles playing an important role in overcoming the skin's barrier.

It is suggested that 50 and 500 nm nanoparticles that are negatively charged allow a high charge density of contact with the skin; 50 nm through their large surface area stemming from their small size and 500 nm from a high number of charged groups within the latex copolymer that are required to charge such a large particle. The net effect of these characteristics may lead to a greater concentration of charge from the 50 and 500 nm particles per unit area of skin as compared to the other particle sizes.

The observed permeation of particles is contradictory to the permselectivity of the skin which due to its negative charge favours the crossing of cationic species (Marro et al., 2001). We speculate the permeation seen in the present of study to be a result of repulsive forces between negatively charged lipids within the skin and particles at the surface. These forces may result in the temporary initiation of channels within the skin allowing for particle permeation. On the basis of these findings it is proposed that there maybe

a threshold charge which must be reached to allow adequate repulsion of lipids to permit permeation of species through the skin explaining why only 50 and 500 nm particles were measured in the receptor chamber. This favourable permeation of negative particles is supported by Cui and Mumper (2001, 2002) who showed that negatively charged chitosan nanoparticles were able to elicit higher immune titres and cytokine proliferation compared to positive particles when applied to the skin. This phenomenon was not able to be explained by the authors and it is difficult to establish if this is due to the favourable permeation of negative species or a superior stimulation of the immune system by these particles. The results observed in this study were proposed to be due to permeation via the hair follicles and sweat glands. Studies have shown that the administration of plasmid DNA can result in antibody and cellular responses to the encoded antigen (Shi et al., 1999; Cui and Mumper, 2001, 2002). Fan et al. (1999) showed that in hairless mice no immune response to the delivered plasmid was elicited. Taken together these studies indicate that the permeation of plasmid may be through the hair follicles. Alternatively work using PLGA microparticles as delivery vehicles for anti-viral drugs has showed that particles were able to permeate into the epidermis showing permeation through the stratum corneum (Ga de Jalon et al., 2001). This was displayed by fluorescence micrographs; however the same study was unable to show the presence of particles in the receptor compartment.

The work carried out by the other groups discussed shows that in addition to permeation via the hair follicles particles can also permeate through the skin. The findings of this work indicate that the charge of particles plays an important role in this penetration, although it is difficult to provide any conclusions as to the mechanism by which this occurs. Despite this, since particles were shown to permeate in the study, we believe that this provides evidence for the potential of particulate delivery of vaccines through the skin.

Acknowledgements

We gratefully acknowledge David McCarthy for the microscopy work, Lionel Catch for building the diffusion cell and Satyanarayana Somavarapu for insightful discussions.

References

Banchereau, J., Steinman, R.M., 1998. Dendritic cells and the control of immunity. Nature 392, 245–252.

Bos, J.D., 1997. Skin Immune System, 1st ed. CRC Press, Boca Raton.

Cui, Z., Mumper, R.J., 2001. Chitosan-based nanoparticles for topical immunization. J. Control. Release 75, 409–419.

Cui, Z., Mumper, R.J., 2002. Topical immunization using nano-engineered genetic vaccines. J. Control. Release 81, 173–184.

Dittmann, S., 2001. Vaccine safety: risk communication—a global perspective. Vaccine 19, 2446–2456.

Fan, H., Lin, Q., Morissey, G.R., Khavari, P.A., 1999. Immunization via hair follicles by topical applications of naked DNA to normal skin. Nat. Biotechnol. 17, 870–872.

Ga de Jalon, E., Blanco-Prieto, M.J., Ygartua, P., Santoyo, S., 2001. PLGA microparticles: possible vehicles for topical drug delivery. Int. J. Pharm. 226, 181–184.

Glenn, G.M., Rao, M., Matyas, G.R., Alving, C.R., 1996. Skin immunization made possible by cholera toxin. Nature 391, 851.

Glenn, G.M., Scharton-Kersten, T., Vassell, R., Mallet, C.P., Hale, T.L., Alving, C.R., 1998. Cutting edge: transcutaneous immunization with cholera toxin protects mice against lethal mucosal toxin challenge. J. Immunol. 161, 3211–3214.

Glenn, G.M., Scharton-Kersten, T., Vassell, R., Matyas, G.R., 1999. Transcutaneous immunization with bacterial ADP-ribosylating exotoxins as antigens and adjuvants. Infect. Immun. 67, 1100–1106.

Hammond, S.A., Alving, C.R., Walwender, D., Glenn, G.M., 2001. Transcutaneous immunisation: T cell responses and boosting of existing immunity. Vaccine 19, 2701–2707.

Hammond, S.A., Tsonis, C., Sellins, K., Rushlow, K., Scharton-Kersten, T., Glenn, G.M., 2000. Transcutaneous immunization of domestic animals: opportunities and challenges. Adv. Drug Deliv. Rev. 43, 45–55.

Kemppainen, B.W., Reifenrath, W.G., 1990. Methods for Skin Absorption. CRC Press, Boca Raton.

Marro, D., Guy, R.H., Delgado-Charro, M.B., 2001. Characterization of the iontophoretic permselectivity properties of human and pig skin. J. Control. Release 70, 213–217.

Shi, Z., Curiel, D.T., Tang, D-C., 1999. DNA-based non-invasive vaccination onto the skin. Vaccine 17, 2136–2141.

Somavarapu, S., Eyles, J.E., Flick-Smith, H.C., Alpar, H.O., 2001. Cross-talk between *trans*-cutaneous and nasal routes of immunization. Proc. Ann. Congr. Br. Soc. Immunol. 104 (1), 1.14.

Tuting, T., Storkus, W.J., Falo Jr., L.D., 1998. DNA immunization targeting the skin: molecular control of adaptive immunity. J. Invest. Dermatol. 111, 183–188.