COMPARISON OF THE TRANSDERMAL BALLISTIC DELIVERY OF MICRO-PARTICLES INTO HUMAN AND PORCINE SKIN

M.A.F. Kendall, F.V. Carter, T.J. Mitchell and B.J. Bellhouse
The PowderJect Centre for Gene and Drug Delivery Research, University of Oxford, UK

Abstract- **This paper describes a comparative investigation into the impact penetration characteristics of DNA-coated gold micro-particles into human and porcine skin. This work is aimed at establishing the link between the particle parameters required in delivering particles to the epidermis of pigs and humans. The particles are delivered to the skin using the PowderJect concept: a method that accelerates vaccines and drugs in micro-particle form to velocities sufficient to penetrate the skin and achieve a therapeutic effect. Devices are configured to deliver particles to predetermined velocities to both the *in-vivo* inguinal region of the pig and the *ex-vivo* skin from the human back and arm. Location of the gold particles within the tissue sites was assayed in histological sections taken from the tissue sites. The penetration results in pig and human tissue are analyzed and compared with calculations performed with a semi-empirical unified penetration model.**

***Keywords*- Biolistics, Skin Penetration, Particles, Stratum Corneum, PowderJect, Powder Injection.**

I. INTRODUCTION

At the University of Oxford and PowderJect Pharmaceuticals PLC, a unique form of needle-free transdermal vaccine and drug delivery technology has been developed. The principle behind this concept is to accelerate vaccines and drugs in micro-particle form to a velocity sufficient for them to penetrate the skin or mucosa to achieve a therapeutic effect [1].

In recent years, a component of research has been directed towards producing systems configured to deliver particles to the required layers within the epidermis [2]-[6]. The particle density, size and impact velocity requirements for the desired penetration characteristics have been explored by the delivery of model particles to excised human skin [7]-[8] and mucosal tissue [9]. In order to explore the therapeutic capabilities of the PowderJect concept prior to clinical trials in man, however, animal models such as the pig, rat and dog are regularly employed [10].

One application of particular interest is genetic vaccination, achieved by the delivery of DNA coated gold particles to dendritic cells within the epidermis of the skin. The pig animal model is used to provide essential immunology information for PowderJect configurations. In order to optimise physical impact conditions for such animal work, a firm understanding of the particle impact parameters in pigs is required. Furthermore, in negotiating the step from animal testing to clinical trials in man, the link in impact conditions for both cases needs to be more fully understood.

In this paper the penetration characteristics of DNA-coated gold particles into pig and human skin are compared. Controlled delivery devices were used to accelerate gold particles to both the *in-vivo* inguinal region of the pig and the *ex-vivo* skin from the human back. The inguinal region is most favored for delivery due to the thinness of the stratum corneum, the principal barrier to penetration, and the proximity of the draining lymph nodes. The penetration of the gold particles was then assayed in histological sections taken from the injection sites, with both particle diameter and penetration depth from the surface measured across the site. This data was then analyzed and compared to calculations from a unified penetration model.

II. METHODOLOGY

A. Particles and Delivery Devices

In order to investigate the delivery of small, high-density particles into the skin, devices that deliver particles with controllable and incremental velocities are required. The systems employed in this study are variants of the Contoured Shock Tube (CST) [5], [6] and [8]. Three CST device configurations were used to provide a distinct range in particle impact velocities of between 420 m/s and 640 m/s.

The radius of gold micro-particles used in this study were 0.89 ± 0.58 μm, 1.12 ± 0.56 μm and 1.52 ± 0.58 μm (mean ± SD) with an average density of 16.8 g/cm^3. Payloads were within the range of 0.5-1.5 mg.

B. Excised Human Skin Preparation

Excised human skin was used as a target for these experiments. Skin was harvested by dermatome from the back and arm of two cadavers (Caucasian, aged 70 and 72 years, and male). After excision, the skin was washed in sterile saline at room temperature. Residual liquid was allowed to drain off the skin before it was placed in Dulbecci's Modified Eagle's Medium (DMEM) at 4ºC for a minimum of 2 hours. It was then removed, packed and sealed into plastic envelopes before being frozen at 1ºC/min until -80ºC as which temperature it was stored. Upon thawing the skin was washed, then re-hydrated and reheated to physiologic conditions. Particles were injected with the skin

Report Documentation Page

Report Date	Report Type	Dates Covered (from... to)
25 Oct 2001	N/A	-

Title and Subtitle Comparison of the Transdermal Ballistic Delivery of Micro-Particles Into Human and Porcine Skin	**Contract Number**
	Grant Number
	Program Element Number
Author(s)	**Project Number**
	Task Number
	Work Unit Number
Performing Organization Name(s) and Address(es) The PowderJect Centre for Gene and Drug Delivery Research University of Oxford, UK	**Performing Organization Report Number**
Sponsoring/Monitoring Agency Name(s) and Address(es) US Army Research, Development & Standardization Group (UK) PSC 802 Box 15 FPO AE 09499-1500	**Sponsor/Monitor's Acronym(s)**
	Sponsor/Monitor's Report Number(s)

Distribution/Availability Statement
Approved for public release, distribution unlimited

Supplementary Notes
Papers from 23rd Annual International Conference of the IEEE Engineering in Medicine and Biology Society, Oct 25-28, 2001, held in Istanbul, Turkey. See also ADM001351 for entire conference on cd-rom, The original document contains color images.

Abstract

Subject Terms

Report Classification unclassified	**Classification of this page** unclassified
Classification of Abstract unclassified	**Limitation of Abstract** UU

Number of Pages
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resting on an absorbent dressing. After injection the tissue was snap frozen and embedded in tissue freezing media. It was then sectioned in a cryotome to a thickness of 12 μm. Sections were stained in Haematoxylin (0.5% Gills N° II), and Eosin (1% w/v), mounted in an aqueous mountant, coverslipped and observed under an optical microscope. Aqueous mountant was used in order to observe the gold particles residing in the stratum corneum. Microphotographs were taken using digital camera (Leica DC100), and used for the subsequent analysis.

C. Porcine Skin Handling

The inguinal region of the pig was washed and shaved for the *in-vivo* experiments. After injection and euthanising the pig, the target tissue was excised and placed in 10% formalin for fixing. The tissue samples were then trimmed of fat and further fixing was carried out using a Shandon Citadel 1000 Tissue Processor. The tissue processor runs on a twelve hour cycle during which time the skin specimens undergo twelve chemical treatments starting with 10% neutral buffered formalin (NBF) and six rinses in ethanol. The skin was then processed through xylene and melted paraffin wax before ultimately being mounted in paraffin wax for sectioning. Microtome sections of a nominal thickness of 4 μm were taken across the centerline of the injection site and stained using Haematoxylin and Eosin, as per human skin. The methods employed to observe the particles were identical to that for the human skin samples.

III. Results and Discussion

A. Sample Histology Sections

Fig. 1 (below) shows a typical image of a histological section of porcine and human skin after particle impact.

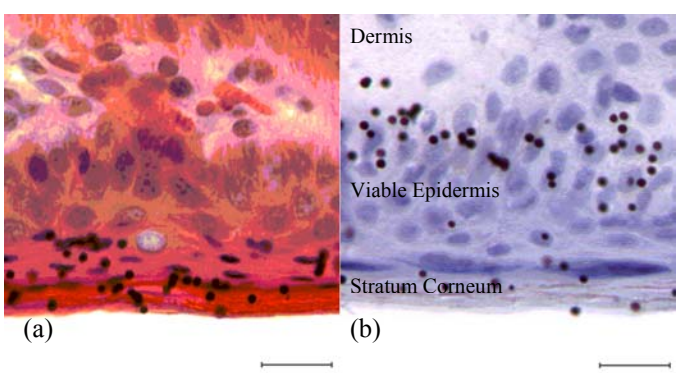

Fig. 1. Sample images of particle penetration into porcine (a) and human (b) tissue. Compartments of the skin are marked. The bar corresponds to a scale of 20 μm

From such images, particle radius and depth measurements were taken. The stratum corneum, viable epidermis and dermis of the skin section are labeled. For the porcine sample, the gold particles with a mean radius of 1.2 ± 0.2 μm impacted at a nominal velocity of 640 ± 50 m/s (mean ± SD). For the human skin sample, particles with a mean radius of 1 μm ± 0.2 μm impacted the skin with a mean calculated velocity of 580 ± 50 m/s (mean ± SD).

B. Raw Penetration Data

Over 3000 penetration measurements for impact into human and porcine skin were recorded. The data is shown below in Fig. 2, where the penetration depth of each particle is ranked against ρvr (the product of the particle density, impact velocity and radius). This term could be described as an indication of the impact momentum per cross-sectional area of the particle.

The raw data has also been equally grouped together according to the parameter ρvr for each of the skin types. Error bars are shown to illustrate the standard deviation for each collapsed data point. Here, porcine skin appears to be more resistant to the ballistic delivery of micro-particles than human skin.

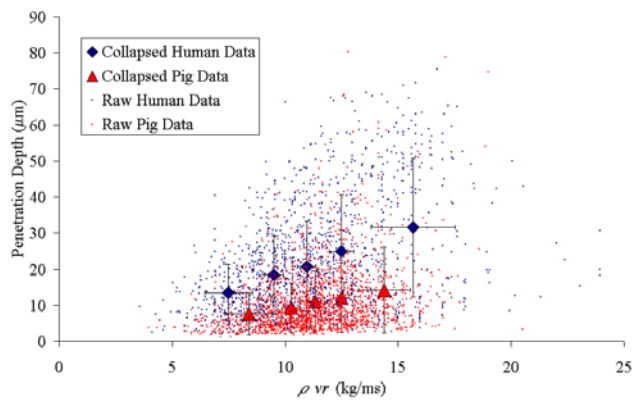

Fig. 2. Raw and collapsed penetration data into human and porcine skin. Penetration depth is ranked according to the term ρvr.

C. Comparison of Data with Models.

A theoretical analysis has been applied to the experimental data to further the understanding of the micro-particle penetration mechanisms. Such modeling helps provide insights into differences between porcine and human skin penetration. The favored model [11], describes the drag force D, acting on the particle as a function of particle and target material properties:

$$D = \frac{1}{2}\rho_t A v^2 + 3A\sigma_y \qquad (1)$$

where ρ_t is the target material density, A is the plan area of the particle, v is the velocity of the particle, and σ_y is the yield stress of the target. Here the first term represents the force required to accelerate the target material up to the speed of the particle, and the second term describes the force required

to yield the target material. Equation (1) may be integrated to yield theoretical penetration depths for given impact parameters, as shown by (2).

$$d = \frac{4\rho_p r_p}{3\rho_t}\left\{\ln\left(\frac{1}{2}\rho_t v_i^2 + 3\sigma_t\right) - \ln(3\sigma_t)\right\} \qquad (2)$$

where ρ_p is the density, r_p is the radius and v_i is the impact velocity of the particle. There is a dearth of data pertaining to the important mechanical properties of skin in the literature. This is particularly the case for the high strain rate (order 10^5 /s) conditions of ballistic penetration. For instance, a study investigating the yield stress of the stratum corneum under static loading [12] obtained measurements between 4.9 MPa and 22 MPa for relative humidity values of between 100% and 0% respectively. These values do not take into account the high strain rates that occur during particle impact. Significantly higher empirical values were chosen for the yield stress of human (45 MPa) and porcine skin (110 MPa).

The grouped penetration data are compared with calculations with the model for human and porcine skin samples in Fig. 4 and Fig. 5 respectively.

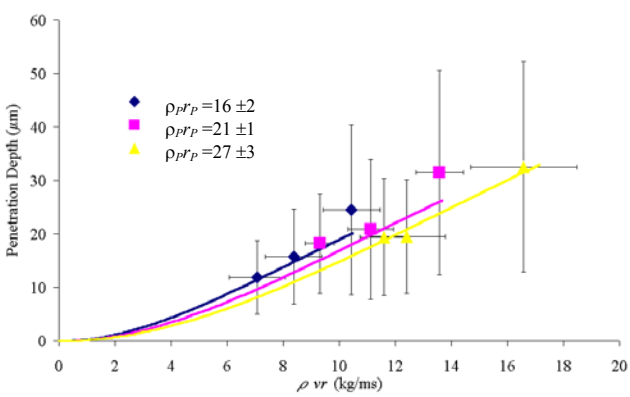

Fig. 4. Comparison between the grouped experimental data and theoretical model for human skin

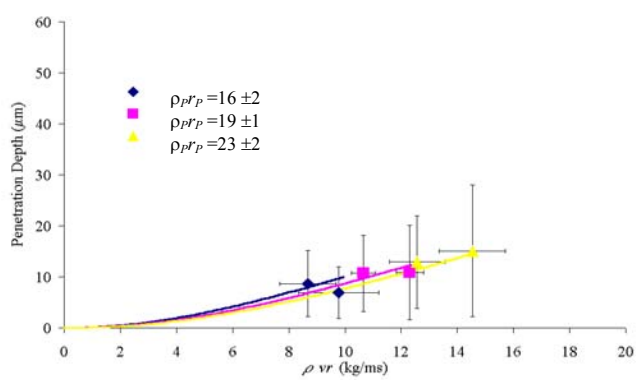

Fig. 5. Comparison between the grouped experimental data and theoretical model for porcine skin

In order to visualize the comparison of penetration depth as a function of three independent impact parameters ρvr (the density, radius and impact velocity of the particle), the data has been grouped into three bands of the product of the radius and density of the particle ($\rho_p r_p$). The non-linear variation of particle penetration with velocity has been illustrated for each group of $\rho_p r_p$.

Comparison of the experimental data in Fig. 4 and Fig. 5 suggests that the porcine skin is more resistant to penetration than human skin. This is supported by the difference in yield stress values (45 MPa for human and 110 MPa for porcine skin) chosen fit the theoretical models to both sets of experimental data. Despite the large error bars associated with the grouped data, the theoretical model appears to fit the data well, with the variations in impact parameters closely matched by the model. Although the authors have not identified in the literature any specific studies comparing the relative strengths of porcine and human skin, some general observations have been made. It is reported that the pig has a thicker and much more compact stratum corneum than that of human [13].

As the stratum corneum appears to constitute the major barrier to particle penetration, any differences in its strength or thickness will have a great effect on the ballistic delivery of micro-particles. It is believed that this higher relative strength of porcine stratum corneum accounts for the significant difference between the penetration into the two tissues.

In contrast with the literature [13], no significant difference in the thickness of the two animal species was found in this study. For the two human samples used the thickness were 8.2 ± 1.6 μm and 13.2 ± 1.9 μm (mean ± SD). The porcine stratum corneum was measured to have a thickness of 9.3 ± 1.7 μm. Therefore, it appears that for the tissue samples of the study, stratum corneum thickness variation is not the source of the differences in particle penetration depth between human and porcine skin.

It is unclear to what extent treatments to human skin prior to injection may alter key mechanical properties of the tissue. Previous studies [7] suggest that providing the skin is re-hydrated to physiological conditions then the biomechanical properties are not significantly altered. However, such assertions have not been tested in controlled comparative studies and different levels of hydration may be a source of the discrepancy in absolute penetration depths between human and porcine skin.

IV. Conclusion

The mechanical characteristics of the particle penetration into both human (back and arm) and porcine skin (inguinal region) have been investigated and compared. Contoured Shock Tube (CST) prototype devices were used as systems to deliver DNA coated Gold particles to the skin with controlled

impact conditions. Particle penetration depth was measured from histological sections of the skin using image analysis.

It was found that the penetration depth of gold particles in both the human and porcine skin was a strong function of the particle density, impact velocity and radius. Furthermore, it was established that for the samples used in this study, the porcine skin represented a greater barrier to particle penetration than the human skin. This finding was supported by a discrepancy between the yield stress values (45 MPa and 110 MPa for human and pig skin respectively) chosen to fit calculations with a Unified Penetration Model to the experimental data. Nevertheless, the result was obtained in human and porcine samples with similar stratum corneum thicknesses. One of the sources of the differences achieved in penetration depth may be in the preparation methods of the porcine and human skin samples. Future work will be directed in exploring these effects and providing a more comprehensive database to enable:

- Carefully configured studies with pig animal models targeted to a particular layer within the skin, and;
- An optimal transfer of operating conditions from animal model work to clinical trials in man.

ACKNOWLEDGMENT

This work was funded by PowderJect Pharmaceuticals and performed in collaboration with the PowderJect Vaccines division at Madison, USA. Thanks in particular go to Lee Roberts, Spencer Fast, Greg Guilfoil and Chris Lacey for supporting a collaboration on the pig histological work, the process development group Joe Burkholder and Sandra Hagen for preparing the gold powders, and Edna Gonzales for the porcine tissue processing and mounting of the samples.

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