Microneedle Assisted Micro-Particle Delivery from Gene Guns: Experiments Using Skin-Mimicking Agarose Gel

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ABSTRACT: A set of laboratory experiments has been carried out to determine if micro-needles (MNs) can enhance penetration depths of high-speed micro-particles delivered by a type of gene gun. The micro-particles were fired into a model target material, agarose gel, which was prepared to mimic the viscoelastic properties of porcine skin. The agarose gel was chosen as a model target as it can be prepared as a homogeneous and transparent medium with controllable and reproducible properties allowing accurate determination of penetration depths. Insertions of various MNs into gels have been analysed to show that the length of the holes increases with an increase in the agarose concentration. The penetration depths of micro-particle were analysed in relation to a number of variables, namely the operating pressure, the particle size, the size of a mesh used for particle separation and the MN dimensions. The results suggest that the penetration depths increase with an increase of the mesh pore size, because of the passage of large agglomerates. As these particles seem to damage the target surface, then smaller mesh sizes are recommended; here, a mesh with a pore size of 178 μm was used for the majority of the experiments. The operating pressure provides a positive effect on the penetration depth, that is it increases as pressure is increased. Further, as expected, an application of MNs maximises the micro-particle penetration depth. The maximum penetration depth is found to increase as the lengths of the MNs increase, for example it is found to be 1272 ± 42, 1009 ± 49 and 656 ± 85 μm at 4.5 bar pressure for spherical micro-particles of 18 ± 7 μm diameter when we used MNs of 1500, 1200 and 750 μm length, respectively. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:613–627, 2014

Keywords: gene gun; stainless steel micro-particles; micro-needle; penetration depth; agarose gel; particle size; biocompatibility; biomaterials; transdermal drug delivery; skin

INTRODUCTION

Gene guns have been shown to be useful for the delivery of DNA vaccines into tissues.[1–5] These delivery systems are primarily accelerators of micro-particles, which deliver DNA-loaded micro-particles into target tissues to achieve the desired gene transfection.[2,6–8] The micro-particles are generally required to penetrate to certain depths within the target to carry out the desired effect of gene delivery and, as such, the penetration depth of the micro-particles is one of the major variables studied in gene delivery research. Ziegler[9] has indicated that an acceptable DNA delivery requires that the micro-particles penetrate into the target skin tissue by approximately 20 ± 100 μm. However, the top layer of the skin, that is the *stratum corneum*, limits the penetration depths for the particles[10,11] because of its resistance to particle motion. Furthermore, whatever the particles achieve in terms of penetration depths in the target tissue, depends on a number of key variables such as the operating pressure, particle size, properties of the target tissue and so on.[12–16]

In general, the micro-particles follow two routes of penetration into the target tissue, which are the extracellular and intercellular routes.[16] The extracellular route is followed during delivery of large particles, when the tissue is damaged between the cell boundaries. Soliman[17] has suggested that particles which have larger diameters, for example 15–100 μm, are expected to penetrate by extracellular failures of the tissues. In this case, an increased size of lower density micro-particles can achieve sufficient momentum to breach the target layer and penetrate further to the desired depths inside the target tissue.[18,19] Because of their biocompatibility and low cost, biomedical grade stainless steel and polymeric micro-particles are considered to be good choices to replace high-density gold particles. For example, polymeric micro-particles of 15.5 and 26.1 μm diameters have been delivered at 6 MPa in a conical nozzle by Quinlan et al.[20] Kendall[21] has also used converging–diverging nozzle to deliver polystyrene micro-particles of 26.1 and 39 μm average diameters at 4 MPa to velocities of 365 and 350 m/s, respectively. Truong et al.[22] have used polystyrene particles of 15 and 48 μm diameter at 6 MPa in a contoured shock tube (CST). Liu and Kendall[23] have operated with polystyrene particles of diameter 40 μm at 6 MPa to study the particle velocity for a CST. Mitchell et al.[16] have used stainless steel micro-particles of 25 μm diameter and concluded that the particles can penetrate up to 150 μm into excised canine buccal mucosa at a velocity of 170 m/s. Polystyrene particles of 15.5, 25, 48 and 99 μm diameters have also been operated at 2, 4 and 6 MPa pressures in the light gas gun (LGG) by Mitchell et al.[16]

On the basis of these previous studies, it can be concluded that the diameters of low-density micro-particles (e. g., polystyrene and stainless steel) which have been used in gene delivery typically ranged between 15 and 100 μm. Furthermore, the operating pressures for particle delivery fall in the range between 2 and 6 MPa, which may be considered to be high in many devices. Xia et al.[24] have indicated that 200 psi (1.4 MPa) should be the maximum pressure for biolistic transfer of micro-particles to tissue without any damage to

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the target material. Traditionally, heavy metal micro-particles including tungsten[25–28] and gold[2,29,30] coated with DNA have been used for targeting tissues. These elements have high densities and are well suited for particle bombardment. However, tungsten particles have several disadvantages such as non-biocompatibility, DNA degradation and toxicity to cells.[31–33] Gold particles carry the disadvantage of being very expensive. Cell damage is another problem for the biolistic micro-particle delivery. Sato et al.[34] have used various types of gene guns to transfer genes into live rodent brain tissue, which confirmed mechanical damage on cells from micro-particles delivery. However, cell damage decreases from a decrease in particle size and operating pressure.[2,24,35]

Addressing the points above, a method of delivering micro-particles is explored in this work using a model experimental rig, which mimics a typical gene gun for delivery of micro-particles. A model experimental rig is preferred over a gene gun as it allows control and monitoring of important operating variables. A polytetrafluoroethylene (PTFE) made ground slide is used in the current rig, which prevents the impact of pressurised gas onto the target skin and slows down the velocity of micro-particles while achieving the purpose of minimised cell damage. The rig also makes use of the application of the micro-needle (MN) to overcome the effect of the barrier of micro-particle target, allowing a number of micro-particles to reach the deeper area of the target tissue via the holes created by MNs. Micro-particles of biocompatible stainless steel, which have a lower density compared with gold and tungsten and are cheaper than gold, are used in this work.

The mechanisms of MN insertion in the skin and, in particular, its application in creating well-defined holes in the skin have been studied for some years. For example, McAllister et al.[36] have observed that holes are created in skin indicating that there is an amount of residual strain that remains after the MNs have been removed. They have used a cylindrical MN of 20 μm diameter to perform staining experiments which indicated that the holes remain after removal of the MNs. Davis et al.[37] have used a conical hollow MN of 720 μm length and 30–80 μm tip radius to insert into the skin to study the holes created after the removal of MN. In addition, Martanto et al.[38] have used a MN array with a needle length of 1000 μm and width of 200 μm by 50 μm to create visible holes on a rat skin for drug delivery. Kalluri and Banga[39] have applied conical MNs of 559 ± 14 μm length, 213 μm base width and 4 μm tip radius on the skin and reported that they create micro-channels of 60 μm surface diameter and 160 ± 20 μm depth.

The above studies on gene guns show some situations where the gene guns could be coupled with MNs for improved delivery of micro-particle delivery from gene guns in the practice. In a recent review paper, Zhang et al[40] have discussed the potential uses of these coupled systems in detail and therefore they are not discussed in length. This paper is focused on developing a MN-based system for micro-particle penetration. For the purpose of this paper, agarose gel is chosen as a target, as it is a homogeneous and semi-clear material, providing the convenience to measure the micro-particle penetration depth by a digital optical microscope. Furthermore, changing the agarose concentration allows alteration of the viscoelastic properties of the target from one experiment to another, which is difficult to achieve in the case of real tissue, for example porcine skin. In our experiments, agarose gel with viscoelastic properties which mimicked porcine skin is used to study micro-particle penetration. In addition, this paper is aimed at studying the penetration depth in relation to important variables which affect the particle penetration, for example operating pressure, particle size and MN length, using the skin-mimicked concentration of agarose and others.

MATERIAL AND METHODOLOGY

Materials

Irregular shaped and spherical micro-particles made of biocompatible stainless steel were purchased from Goodfellow Cambridge Ltd. (Huntingdon, UK) and LPW Technology Ltd. (Daresbury, UK), respectively. Detailed characterisation of the micro-particles is introduced in section *Characterisation of the micro-needles*. Agarose powder was purchased from Sigma–Aldrich Company Ltd. (Gillingham, UK). Porcine ear skin samples were obtained from a local butcher.

Stainless steel meshes, used for micro-particle separation, were bought from MeshUK, Streme Limited (Marlow, UK). Two different MN arrays (AdminPatch MN 1200 and 1500), which are 1200 and 1500 μm long, were purchased from nanoBioSciences limited liability company (Sunnyvale, California). In addition, an in-house stainless steel MN array which is made of 750 μm long was used in this study. The characterisation of each MN array is explained in section *Characterisation of the MN*.

Experimental Design

A detailed description of a MN-based micro-particle delivery system has been introduced in a previous study by Zhang et al.[41] Generally, the system comprises an acceleration, a separation and a deceleration stage. In such a system, a pellet of micro-particles is accelerated by a pressurised gas to a sufficient velocity in the acceleration stage. It is then separated into a number of small particles by impaction onto a mesh in the separation stage. Finally, the separated particles penetrate the target which is the final deceleration stage. To achieve the aims of this paper and carry out an in-depth study of the penetration depth of the solid micro-particle, an improved version of the experimental rig[41] is used in this work. Figure 1a shows the sections corresponding to the acceleration, separation and deceleration stages. The improvement has been made in the deceleration stage which contains the target material for the particles to penetrate. For the purpose of this paper, a sliced test tube (described below) has been placed in the deceleration stage to hold in place the agarose gel, which acts as a target for the micro-particles. Both ends of the glass tube are open, which make it convenient to remove the agarose gel without damage, following a penetration test.

In this work, a setup modified from Zhang et al.[41] is used. It is made by using a sliced test tube (see Fig. 1b) which allows the observation of particle penetration without the need to slice the gel. It is based on a PTFE mold which is placed inside the sliced test tube, as shown in detail in Figure 1b. A test tube is sliced into approximately 1 cm thick sections where both sides are kept open. The mold is then inserted into a tube piece. The void space in the mold contains the agarose gel. The mold can separate into two parts, providing a convenient method for the removal of the gel. On the basis of the application of the mold, the agarose gel is prepared into uniform pieces of 1 cm thickness with smooth surfaces on both sides to provide a

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