An Experimental Study of Microneedle-Assisted Microparticle Delivery

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ABSTRACT: A set of well-defined experiments has been carried out to explore whether microneedles (MNs) can enhance the penetration depths of microparticles moving at high velocity such as those expected in gene guns for delivery of gene-loaded microparticles into target tissues. These experiments are based on applying solid MNs that are used to reduce the effect of mechanical barrier function of the target so as to allow delivery of microparticles at less imposed pressure as compared with most typical gene guns. Further, a low-cost material, namely, biomedical-grade stainless steel microparticle with size ranging between 1 and 20 μm, has been used in this study. The microparticles are compressed and bound in the form of a cylindrical pellet and mounted on a ground slide, which are then accelerated together by compressed air through a barrel. When the ground slide reaches the end of the barrel, the pellet is separated from the ground slide and is broken down into particle form by a mesh that is placed at the end of the barrel. Subsequently, these particles penetrate into the target. This paper investigates the implications of velocity of the pellet along with various other important factors that affect the particle delivery into the target. Our results suggest that the particle passage increases with an increase in pressure, mesh pore size, and decreases with increase in polyvinylpyrrolidone concentration. Most importantly, it is shown that MNs increase the penetration depths of the particles. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 102:3632–3644, 2013

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INTRODUCTION

Microparticle delivery systems (e.g., gene guns) have been used for transferring genes into cells and tissues (e.g. plant tissues) for some time.[1–6] Typically, the operation involves a microparticle accelerator, which can deliver gene-loaded microparticles into a target (e.g., biological cells) to achieve the desired mass transfer effect (e.g., gene transfection). The PowderJect delivery system is a case in point, which has been applied to exploit the microparticle gene transfer treatment.[7,8] In most cases, these delivery systems are based on the principle that biocompatible microparticles loaded with genes can be accelerated to a sufficient velocity so as to penetrate the barrier function of the target tissue and thereby achieve gene delivery.[9,10] However, cell and tissue damages are particular problems for these microparticle delivery systems, which are discussed further later.

It is obvious from previous research on microparticle-based gene delivery that knowledge of the velocity of the microparticles and its effects on particle penetration is one of the major research points in development of these systems. A number of researchers have studied the particle velocity for various designs of gene guns. For example, Quinlan et al.[11] have used a conical nozzle employed at 60 bar to accelerate polymeric microparticles of 4.7, 15.5, and 26.1 μm diameters to velocities of 350, 460, and 465 m/s, respectively. Kendall et al.[12] have used a converging–diverging nozzle, which has been shown to accelerate polystyrene particles of diameter 4.7 μm to a velocity of 800 m/s at the same pressure as used by Quinlan et al.[11] Such developments of the delivery systems can improve the velocity of microparticles to achieve a higher speed if compared with conical nozzles.[11] Mitchell et al.[13] have also studied the velocities of polystyrene particles (average size: 99 μm) for a light gas gun (LGG) proposed originally by Crozier and Hume[14] and gold particles (average size: 3.03 μm) for a contoured shock tube (CST). The particle velocity is shown to achieve 170, 250, and 330 m/s at pressure of 20, 40, and 60 bar for the LGG, respectively. The gold particles have been shown to achieve an average velocity of 550 m/s at 60 bar based on the CST. Liu et al.[15] have also used a CST to accelerate gold particles of diameter 2.7 μm to a velocity of 626 m/s at 60 bar pressure. Subsequently, Liu et al.[16] used polystyrene particles of 39 ± 1 μm diameter to study the particle velocity for CST and found improvements relative to the LGG, which is shown to achieve a velocity of 570 ± 14.7 m/s at 60 bar pressure. In recent years, Soliman et al.[17] have shown that a supersonic core jet can accelerate 1.8 and 5 μm diameters gold particles to velocities of 550 and 294 m/s at 30 bar pressure. O'Brien and Lummis[5] have also used gold particles of core diameters 40 nm and 1 μm to achieve maximum depths of 31 ± 6 and 50 ± 11 μm in mouse ear tissue by using a Helios gene gun.

Although very high velocities of the microparticles or/and gas may seem useful in delivering the particles deep into the target tissue, they may actually damage the target from their impacts. As such, it is logical that one controls both the velocity and the mass of the microparticles and gas that impact the target. This is somewhat reflected in a study by Belyantseva[18] who has used a pressurized Helios gene gun to accelerate DNA-coated gold particles (1 μm diameter) where the pressure is controlled at 14 bar. The author shows that this pressure is adequate for the penetration of the particles without excessive tissue damage. Xia et al.[19] have suggested that the pressure should be limited to around 14 bar to minimize damage for biolistic transfer to

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soft tissue. Uchida et al.[20] have fired plasmid DNA into cultured mammalian cells [e.g., human embryonic kidney cell (HEK293) and human breast adenocarcinoma (MCF7) cell] using a Helios gene gun, which shows that gene transfection is achieved in these cells but the cell damage occurs if the operating pressure in the gene gun is more than 200 psi (13.78 bar). O'Brien and Lummis[5] have cultured HEK293 cells and used them as targets for biolistic transfection using a gene gun. This work has shown that nanoparticles can be utilized as gene carriers similar to microparticles for biolistic transfection and lessen cell damage. These researches[5] show that cell damage can be reduced if particle size and operation pressure are reduced as they lower the particle impact force on the cells/tissue such as those observed by Uchida et al.[20] In most studies, the viable dermis layer of skin is considered as the target tissue for gene-loaded microparticle delivery as the penetration depth is limited by a number of factors.[11,21]

In the particles delivery process, the material of the particles is also of crucial importance. In order to deliver gene-loaded particles into cells effectively, high-density materials are generally preferred as they carry a larger momentum and are expected to penetrate more into the target tissue as compared with particles of low-density materials. The most common material of the particles is gold because of its high density, low toxicity, and lack of chemical inactivity. However, gold is an expensive material. In principle, other materials such as biomedical-grade stainless steel and polystyrene may be a good replacement for gold while reducing the cost due to the lower price of these materials in comparison with gold. However, these materials have lower density compared with gold and, as such, the momentum for these microparticles would be less for the same particles size and velocity. This implies that other factor is needed to break the resistance of the target tissue for the particles to enter easily while also enhancing the penetration depths. Microneedles (MNs), which can break the resistance of the target tissue almost painlessly,[22–26] seem to be a promising option in this regards. However, there is little or no study at the moment that demonstrates that MNs can be useful in the delivery of dry particulates particularly at lower pressures as compared with most current gene guns, which should be operated at very high pressure.[11,13,16] Previously, several studies have shown that the effectiveness of the MN-based drug delivery is limited by a wide varieties of variables, for example, MN height, spaces between the needles, patch size, insertion forces, tissue characteristics such as viscoelastic properties, materials of MNs, and so on, and as such, it is necessary to choose the MNs for specific application as well as the target tissue.[27–32]

In addressing these points in this paper, MNs have been used to enhance the penetration depths of low-density microparticles (dry particulates) using an experimental set up that mimic particle accelerator (e.g., gene guns) in its operation principle. As model particles, we use biomedical-grade stainless steel microparticles. Further, a ground slide is used to prevent the impact of high-pressure gas on the microparticle target as discussed in more detail in the next section. The use of the ground slide gives lower particle velocities compared with the CST under the same operating conditions, which aims to reduce the cell damage. However, the purpose of the microparticle gun is to accelerate the particles to a sufficient velocity, which can penetrate into a desired depth inside the target. For a MN-based injection system, this objective could be achieved by first applying solid MNs as they help in overcoming the tissue barrier.[33–35] In this study, solid MNs are used to create well-defined holes in the target, which remain open immediately after removing the MN. Hence, a number of microparticles should penetrate into the target via the holes to achieve the purpose of enhanced penetration depth. An increased penetration depth of microparticles should allow deeper tissue to be transfected if DNA/genes are coated on the microparticles. Therefore, the application of the MN-based particle delivery is a good improvement for particle injectors.

In addition to the aims discussed above, this paper aims to investigate the significance of various important factors, for example, the ground slide on the particle velocity for the MN-assisted microparticle injection. The microparticles are mixed with polyvinylpyrrolidone (PVP), compressed and bound as a cylindrical pellet for the purpose of this work. The pellet is mounted on a ground slide, which is accelerated along a barrel. The high-velocity pellet is separated by a mesh that presents a partial blockage to the flow. The work in this paper aims to determine the passage percentage and separated particle size. The paper also aims to study the effect of the MN on the microparticle penetration depth when they are fired into a homogeneous agarose gel, which is used a as a model target. Agarose gel has the advantages that it can be produced with a controllable mechanical property and its transparency provides a good quality to view the microparticle penetration using optical digital microscope. In agarose gel, the microparticles follow two routes of delivery. The first route is that a number of microparticles directly penetrate into the agarose gel without going through the holes created by MNs. The second route is that the microparticles are delivered through the pierced holes created by the MNs to enhance the penetration depth inside the agarose gel. In reality, the target skin for these microparticles may be different structurally and heterogeneous, and therefore the routes of the microparticle delivery may be affected by its individual layers. However, this is not a consideration in this study as we carry out the experiments in a controlled manner using homogeneous agarose gels. The detailed information on the MN-based injection system is described in section *Experimental Design*.

MATERIAL AND METHODOLOGY

Materials

Biocompatible stainless steel microparticles of high sphericity equaling approximately to 0.92 were bought from LPW Technology Ltd (Daresbury, UK). Detailed characterizations of these microparticles are presented in section *Characterization of the Microparticle*. PVP purchased from Sigma–Aldrich Company Ltd. (Gillingham, UK) was dissolved in ethanol (analytical grade, 99%, obtained from Fisher Scientific Ltd., Loughborough, UK) and used to bind the microparticles to form a cohesive mixture, which could be compressed into a pellet. Agarose powder (Sigma–Aldrich Company Ltd.) was used to prepare an agarose gel, which was used a target for the microparticles penetration experiments.

Photoelectric sensors (PS) were purchased from SICK Group (Waldkirch, Germany) to detect velocity of the microparticles pellets loaded onto a ground slide. Meshes of three different pore sizes were obtained from Streme Limited (Marlow, UK). A solid MN array (Adminpatch), which has 31 needles of 1500 μm