Potential of microneedle assisted micro-particle delivery by gene guns: A review

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Abstact

Context

Gene guns have been used to deliver deoxyribonucleic acid (DNA) loaded micro-particle and breach the muscle tissue to target cells of interest to achieve gene transfection.

Objective

This paper aims to discuss the potential of microneedle (MN) assisted micro-particle delivery from gene guns, with a view to reducing tissue damage.

Method

Using a range of sources, the main gene guns for micro-particle delivery are reviewed along with the primary features of their technology, e.g., their design configurations, the material selection of the micro-particle, the driving gas type and pressure. Depending on the gene gun system, the achieved penetration depths in the skin are discussed as a function of the gas pressure, the type of the gene gun system, and particle size, velocity and density. The concept of MN-assisted micro-particles delivery which consists of three stages (namely, acceleration, separation and decoration stage) is discussed. In this method, solid MNs are inserted into the skin to penetrate the epidermis/dermis layer and create holes for particle injection. Several designs of MN array are discussed and the insertion mechanism is explored, as it determines the feasibility of the MN based system for particle transfer.

Results

The review suggests that one of the problems of gene guns is that they need high operating pressures, which may result in direct or indirect tissue/cells damage. MNs seem to be a

promising method which if combined with the gene guns may reduce the operating pressures for these devices and reduce tissue/cell damages.

Conclusions

There is sufficient potential for MN-assisted particle develivery systems.

Key words: gene gun, microneedle, micro-particle, skin, penetration depth

1. Introduction

A gene gun, involving “particle bombardment” or “biolistic delivery” is a particle accelerator, which can deliver deoxyribonucleic acid (DNA) loaded micro-particles at sufficiently high velocities to breach the surface of target tissue and to penetrate to a depth to achieve gene transfection in the cells. A gene gun is considered to be a unique concept which was first used to deliver genetic materials into plant cells (Klein et al., 1987; Klein et al., 1992; Sanford et al., 1993; Svarovsky, 2008; Huang et al., 2011; O'Brien et al., 2011; Manjila et al., 2013). The technique has been used to transfer DNA-coated micro-particles to achieve gene transfection into many types of cells and organs (e.g., Meacham et al., 2013; Bennett et al., 1999), for example, mammals (Cao et al., 2013; Ettinger et al., 2012; Da’dara et al., 2002; Kuriyama et al., 2000; Sakai et al., 2000; Williams et al., 1991), plants (Kuriakose et al., 2012; Zuraida et al., 2010; Klein et al., 1992), artificially cultured cells (O’Brien and Lummis, 2011, 2006), fungi (Gu et al., 2011; Armaleo et al., 1990) and bacteria (Nagata et al., 2010; Smith et al., 1992). A number of commercial gene guns have been manufactured and used for *in vivo* gene transfection in plants, living animals, cultured cells, e.g., the PowderJect system developed originally by the University of Oxford (UK) (Bellhouse et al., 2006), the Helios gene gun by Bio-rad, Hercules, CA (USA) (Belyantseva, 2009), and the SJ-500 portable gene gun by Biotech instrument, New Jersey (USA). The Helios and portable gene guns are shown in Figure 1. In a research context, Da’dara et al. (2002) have used a Helios gene gun to delivery Sm23-pcDNA (an integral membrane protein) to mice to evaluate the immunogenicity of the protective efficacy of the DNA vaccination. Ahlen et al. (2013) have used a Helios gene gun to explore a method for monitoring hepatitis B and C viruses (HBV/HCV) specific to immune responses in mouse.

Loading the micro-particles with DNA (for example onto gold particles) is an important step in using this technology, as discussed by Zhao et al. (2012); O'Brien and Lummis (2006, 2011), Rao (2010), Satish, (2009), Svarovsky et al. (2008), and Thomas et al. (2001). However, a detailed discussion on these issues is outside the scope of this paper.

Such guns have been proposed for gene delivery in the treatment of some of the major diseases such as cancer (Nguyen-Hoai et al., 2012; Aravindaram et al., 2009; Han et al., 2002; Chen et al., 2002); these researchers have fired gene loaded micro-particles into mammalians or cultured cells instead of humans to study gene expression and cell damage. For example, Yoshida et al. (1997) used a gene gun to deliver DNA coated Au micro-particles into the liver of living rat at 250 psi (~17.2 bar) pressure, which resulted in a good gene expression but caused a number of cell deaths. The work also showed that cell damage was not obtained below 150 psi of pressure. Sato et al. (2000) have used various types of gene guns to transfer genes into live rodent brain tissue, which confirmed gene expression, but with mechanical damage to cells. Uchida et al. (2009) have fired plasmid DNA into cultured mammalian cells (e.g., human embryonic kidney (HEK293) cell, human breast adenocarcinoma (MCF7) cell) using a gene gun, which showed that transfection is achieved in the cells, but the cell damage occurs at operating pressures greater than 200 psi (~13.8 bar). O'Brien and Lummis (2011) have cultured HEK293 cells as a target for the biolistic transfection using a gene gun. Their work showed that nano-particles gave a similar performance to micro-particles for biolistic transfection, but created less cell damage.

The above works show that cell damage may be a problem for biolistic micro-particle delivery, due to significant gas and particle impactions on the tissue. This also means that while gene guns have been reported to be successful in many instances, they may have some disadvantages. Generally, for most gene gun systems, the maximum penetration of the micro-particle can breach the *stratum corneum* and end inside the epidermis layer of the tissue (Yager et al., 2013; Liu, 2006; Quinlan et al., 2001; Bennett et al., 1999). The epidermis layer is normally considered to be a target site for gene delivery due to its accessibility (Soliman et al., 2011b; Liu, 2006; Quinlan et al., 2001; Trainer and Alexander, 1997). However, a high

pressure is required for most gene guns, which might damage the tissue after the impaction of the pressurized gas on the skin.

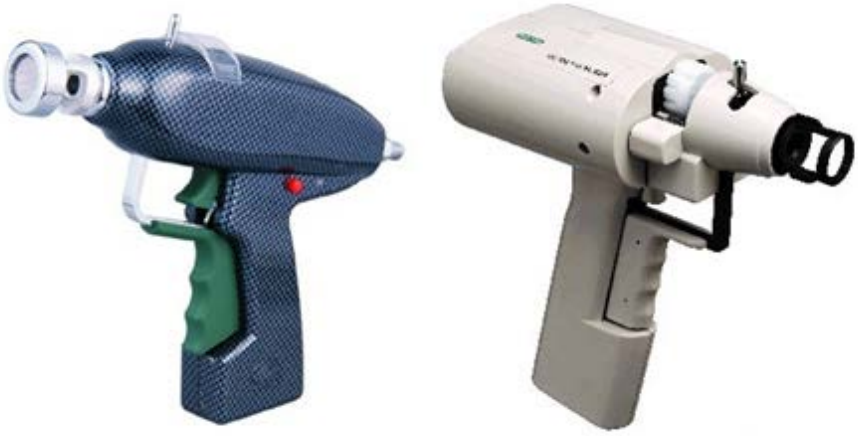


Figure 1: Left: The commercial hand-held gene gun of SJ-500 (Biopex, 2012), Right: the commercial Helios gene gun (Bio-rad, 2012)

Cell damage can be reduced by decreasing the particle size and/or operating pressure, as both reduce the impact forces. In order to satisfy these conditions and, possibly, increase the penetration depth of the micro-particle in the target, an innovative concept of combining MNs with a particle delivery system, namely MN-assisted micro-particle delivery, has been proposed recently by Zhang et al. (2013). The MNs are discussed in section 3 of this paper. The operating principle of the delivery in the approach of Zhang et al. (2013) is that a pellet of micro-particles is loaded on a ground slide which is accelerated by a pressurized gas to a sufficient velocity. The pellet is released after the ground slide reaches the end of a barrel in the system, and separates into micro-particles of a narrow size distribution by impaction on an open mesh; these separated micro-particles then penetrate into the desired target. The resistance of the target/skin to the penetration of the micro-particles is reduced by using MN to create a number of holes through which the micro-particles can enter, without the need for very high gas pressures.

MN assisted micro-particle delivery seems to be a promising approach for dry particulate delivery, but the potential of this method needs to be discussed thoroughly as it is still at an early stage of development. To this end, the main types of gene gun systems for micro-particle delivery and their operating principles are reviewed. The micro-particle material and size for

these gene guns are discussed, and the range of gas pressures and particle velocities are considered.

In this paper, MN arrays are discussed to understand how various geometries affect penetration of the target material and formation of holes, which remain after the needles are removed. This discussion provides relevant background knowledge and a sound foundation for further improvement of gene gun systems, with the help of MN assisted micro-particle delivery to achieve similar levels of penetration depth, but with less cell damage. To further understand the advantage of MN assisted micro-particle delivery, a comparison with other physical cell targeting approaches is discussed. Finally, some simplified models of the micro-particle delivery in the skin are described, which will be useful in predicting the penetration depths achieved by MN assisted gene gun delivery.

2. Main gene guns

2.1 Configurations and operating conditions

Based on the principles of the operation process and driving forces, we define that the gene guns can be divided into three types, namely: powder gene gun, high-voltage electric gene gun and gas gene gun.

A powder gene gun is the original device which uses an ignition gunpowder as a driving force to promote the movement of bullets of macro-projectiles, thus accelerating the micro-particles which are loaded onto the bullet (Huang et al., 2011; O'Brien and Lummis, 2007; Zhou, 1995; Klein et al., 1987; Sanford et al., 1987). An electric gene gun uses a high voltage (HV) to vaporize water droplets; the expanding gas is used to achieve the acceleration of micro-particles (Christou et al., 1990). A gas gene gun releases a high pressure gas to accelerate the micro-particles (or micro-particles loaded ground slide) to a sufficient velocity to breach the barrier of the target (Zhang et al., 2013; Soliman et al., 2011b; Liu, 2007; Kendall et al., 2002; Zhou, 2000).

Powder gene guns were originally developed to deliver genetic material coated micro-particles into plant cells (Klein et al., 1987; Klein et al., 1992; Svarovsky, 2008; O'Brien et al., 2011; Manjila et al., 2013). As shown in Figure 2, an explosion of gun powder accelerates

DNA-coated micro-particles attached onto the front surface of o macro projectile. Klein et al. used tungsten micro-particles of 4 μm average diameter, which could be accelerated to 400 m/s. However, the explosion of the gun powder is noisy and may cause cell/tissue damage.

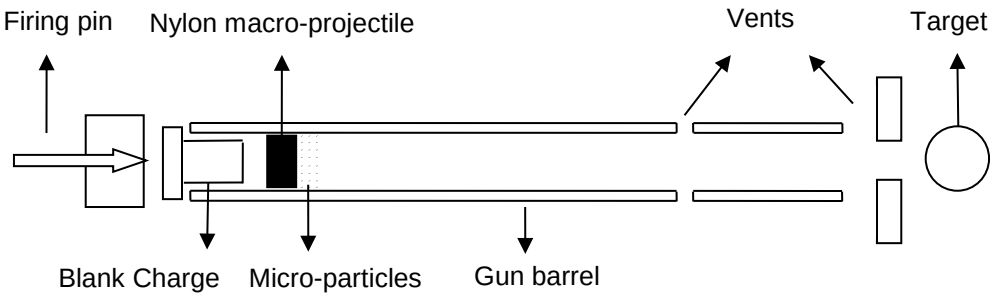


Figure 2: A schematic sketch of the powder gun (redrawn from Klein et al. (1987))

Zhou (1995) improved the design of Klein et al. (1987) and patented a powder gun (Figure 3) which is composed of a gun body, a gun barrel, a ground slide, a baffle plate and a baffle plate fixed pipe. An ignition of gunpowder, caused the bullet to accelerate and hit a ground slide, which then impacts on a baffle plate. Thus, the micro-particles gain an initial velocity to leave the ground slide, pass through the central hole of the baffle plate and penetrate into the tissue. The advantage of this powder gene gun is that the use of ground slide can reduce explosion damage to tissue. The base is designed as a shock absorber to reduce the recoil force, using a spring attached to the firing pin.

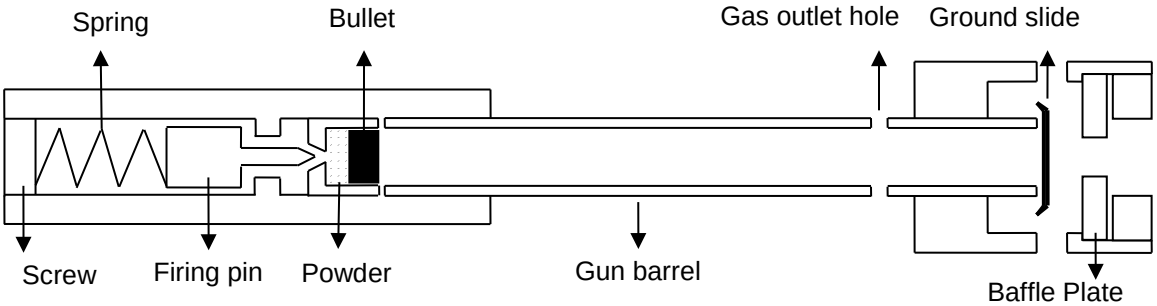


Figure 3: The layout of powder gene gun (redrawn from Zhou (1995))

Christou et al. (1990) have used a type of electronic gene gun to deliver DNA-coated gold micro-particles into soybean seeds; a schematic sketch of the high-voltage electric gene gun is shown in Figure 4. Yang et al. (1990) have applied an electronic gene gun to transfer genes

into the muscle, tissue and liver of a mouse, using 1-3 μm diameter golden particles. The disadvantage of their gun is that a high voltage up to 18 kV is required to deliver a micro-particle penetration depth of 125 μm. Recently, Ikemoto et al. (2012) used a type of high-voltage electric gene gun, namely, an electrospray device to accelerate liquid droplets in a high voltage (12 kV) to collide with DNA coated micro-particles and deliver them into living cells.

Gas gene guns can be classified into two different delivery methods. The first uses a high pressure gas to push the micro-particle coated ground slide to achieve the goal of particle acceleration. The disadvantage is that a very high operating pressure is required to achieve the desired micro-particle impact velocity. However, the advantage is that the ground slide prevents the released gas from impacting on tissue. The second method mainly uses a high pressure gas (e.g., N_2, He) to directly push the DNA-coated micro-particles to a sufficient velocity to breach the *stratum corneum*, penetrate into the epidermis/dermis layer of the skin or deeper tissues. The disadvantage is that very high gas pressure may damage the tissue and the muscles. The advantage is that the micro-particle can reach a desired velocity under lower operation pressure as the micro-particles have very small mass.

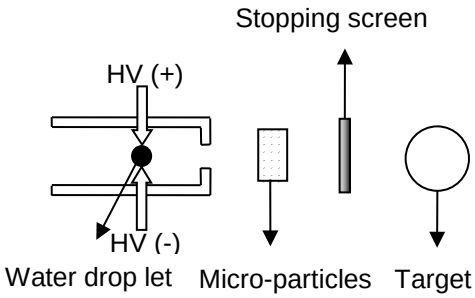


Figure 4: The schematic sketch of the high-voltage electric gene gun (redrawn from Christou et al. (1990))

Using the principles of the first delivery method, Zhou (2000) has invented a kind of high-pressure gene gun which consists of a casing, the compressed gas inlet pipe, emitting cavity and bombarding cavity. The schematic diagram of this gun is shown in Figure 5 which shows both the states of the gun before and after operation. The emitting cavity consists of an air storage house, membrane, bullet, baffle plate and sample holder. The membrane will be

ruptured when the air storage house reaches a certain pressure. High pressure gas is able to accelerate the bullet to the baffle plate. Since the bullet is blocked by the baffle plate, the coated particles will leave the surface of the bullet, go through the centre hole of the baffle plate and launch into the sample, thus completing the gene injection. In addition, the gas will be released from the vent hole. It has been claimed that this instrument has good stability, high efficiency, does not produce impurities, and particles can also attain a higher initial speed. However, it is generally applied for plant tissues.

Mitchell et al. (2003) have conducted many experimental and numerical studies on the light gas gun (LGG), as shown schematically in Figure 6. The LGG uses a high pressure helium gas to drive the micro-particle coated ground slide to a certain velocity. In this case, the polystyrene particles of 99 μm diameter have been used and the impact velocities of the particles have been shown to reach 170, 250 and 330 m/s under 20, 40 and 60 bar operation pressures.

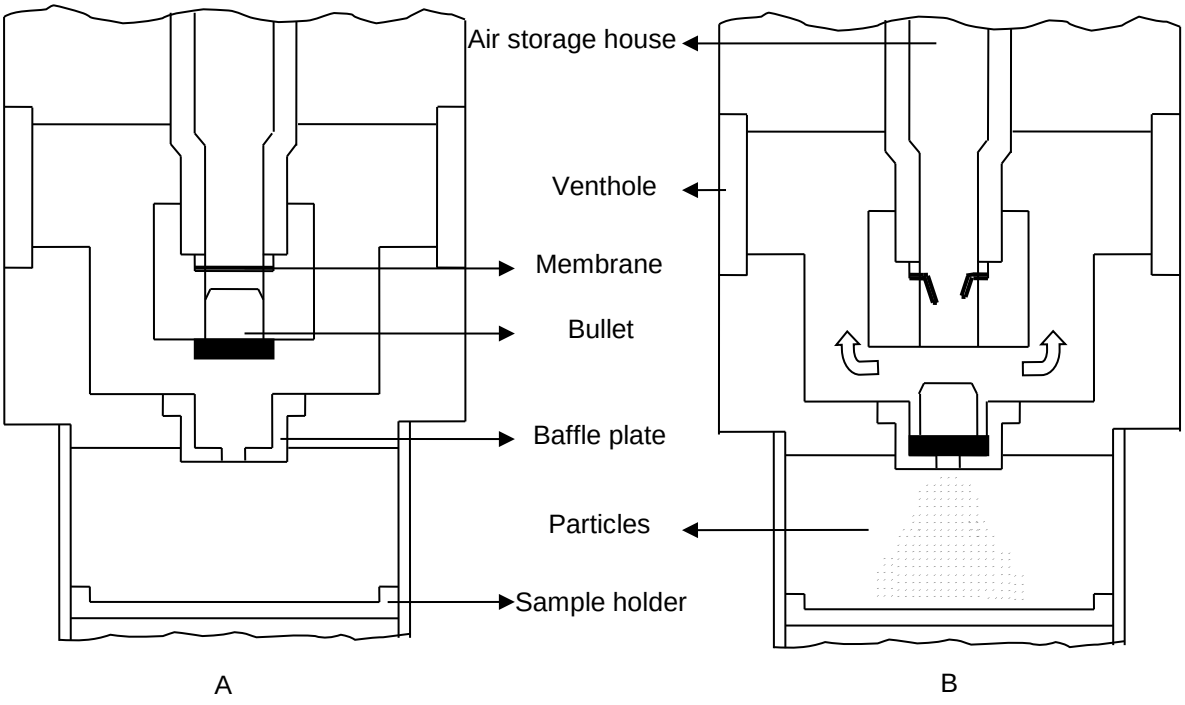


Figure 5: The schematic diagram and principle of high-pressure gas gene gun (redrawn from Zhou (2000))

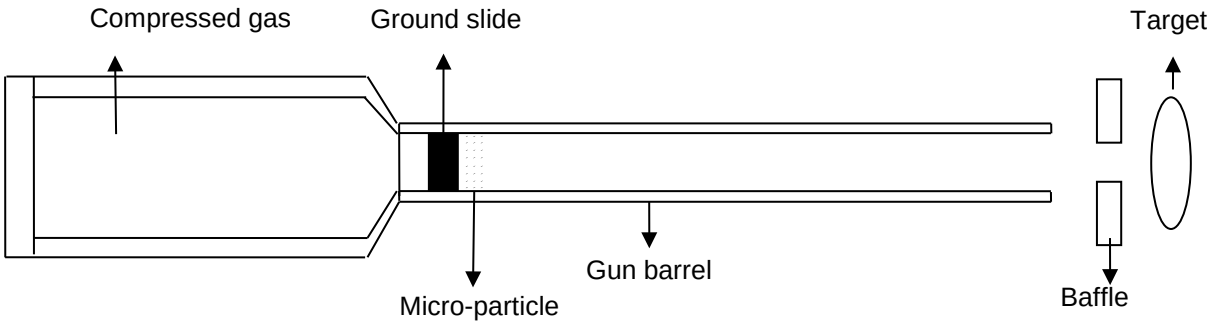


Figure 6: The schematic sketch of light gas gun (redrawn from Mitchell et al. (2003))

Williams et al. (1991) have studied a helium-driven gene gun which is somewhat similar to the design of Zhou's (2000), as shown in Figure 7. In this case, a membrane in the system breaks after the gas pressure reaches a certain value. The micro-projectiles are accelerated by the helium gas and separated well by a stopping screen. In addition, the large particle is blocked by a screen to avoid tissue damage. In this case, golden particles of 1 - 3 μm and 2 - 5 μm diameters have been fired and penetration depths of 150 μm and 200 μm have been obtained in a mouse liver, under 1300 psi operating pressure.

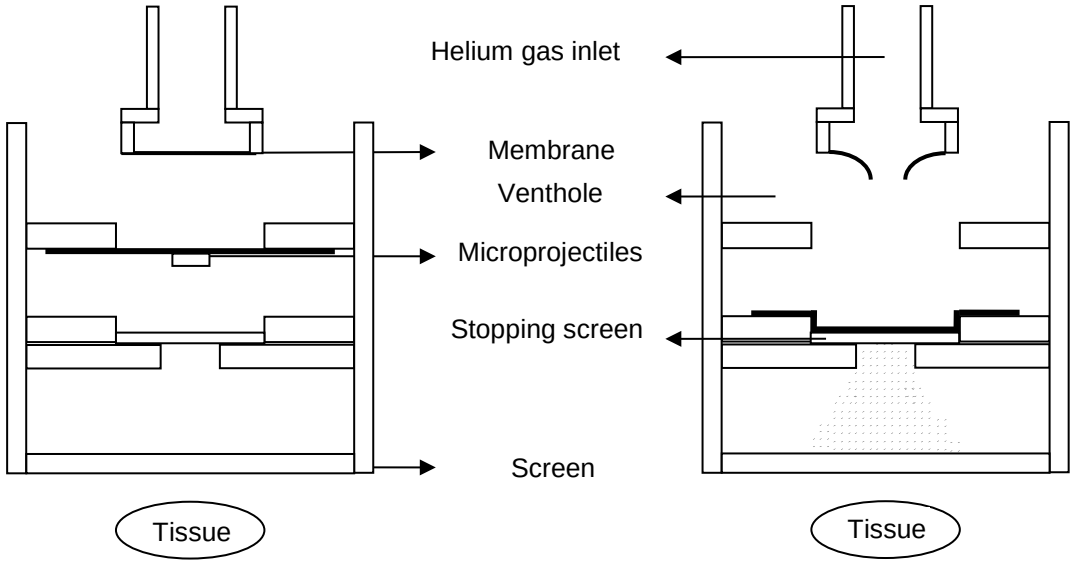


Figure 7: The schematic sketch of helium-driven gene gun (redrawn from Williams et al. (1991))

Kendall (2002) has reported a contoured shock tube which is shown in Figure 8. In this case, the compressed gas will pressurize the membrane, and the micro-particle will be accelerated

by a shock wave as the gas pressure rises to the point where the membrane ruptures. Liu et al. (2004a) and Liu (2006) have described an injection device, namely PowderJect (Figure 9), which uses helium gas as the source of momentum. A trigger actuates a mechanism to release helium gas, which expanded to accelerate the micro-particles to a sufficient momentum to pierce the outer layer of the target and into the cells of interest.

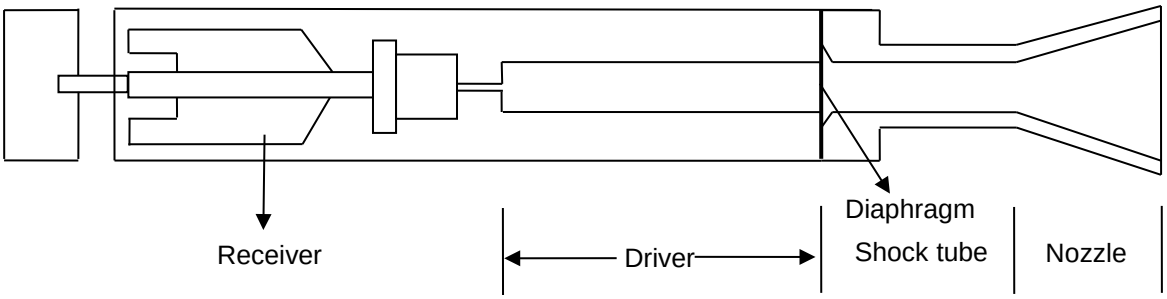


Figure 8: The schematic diagram of contoured shock tube (redrawn from Kendall (2002))

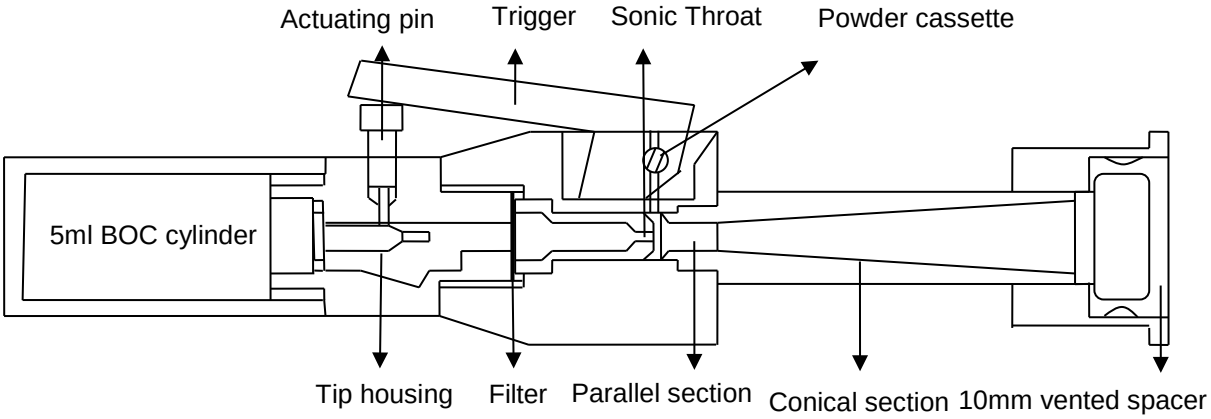


Figure 9: A schematic diagram of the PowderJect system (redrawn from Liu (2006))

Zhang et al. (2007) have introduced the principle of the Helios gene gun which contains acceleration, separation and deceleration stages. The process of micro-particle delivery is shown in Figure 10. This gene gun uses helium gas to accelerate DNA-coated micro-carriers which are separated by a stopping screen. The separated micro-particles exit from the gene gun at high speed, penetrate the tissue to the targeting area, enter into the cell and hit the nucleus membrane.

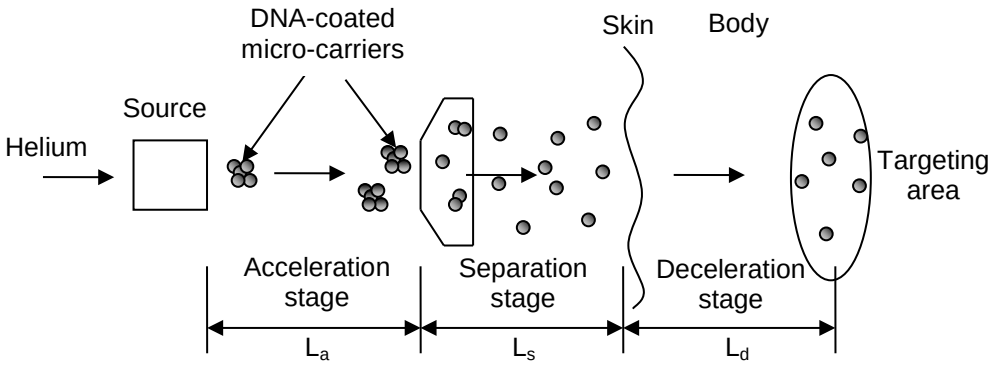


Figure 10: Gene transfer stages of the biolistic gene gun (redrawn from Zhang et al. (2007)); L_a, L_s and L_d are the distance of acceleration, separation and deceleration stages.

In addition, Zhou (2007) has created a special liquid gene sprayer (Figure 11) which is different from the above three gene gun types. It has been used to deliver liquid-form medication into the human body and consists of an interconnected casing and magazine. The magazine comprises of a hollow cylinder and sliding piston rod set; emission holes are arranged at the front end of the cylinder and the front end of the casing is fixed to rear end opening. An energy-storage driving mechanism consists of a spring and impeller and set in the internal cavity of the casing. The impeller moves backward to press the spring to store the energy, and pushes forward by a driving force from the released spring. This device uses a small volume energy storage device to inject the required amount of biological gene, and does not need any separate air supply equipment; it is easy to handle and carry. It uses the liquid as the DNA particle's carrier and hence golden particles are not required in this device. The DNA particles will be suspended in the liquid. Before using this device, the head of the hollow cylinder is inserted into the DNA particle coated liquid, and the sliding piston rod is pulled out to extract the liquid and load the gun. Then the spring is compressed very tightly, storing energy (see Figure 11a), which is released to accelerate the impeller and sliding piston rod; then the DNA particle coated with liquid are pushed out from the emission hole and penetrate into the tissue of plant or human to the target cells of interest. The state of the liquid gene gun after injection is shown in Figure 11b.

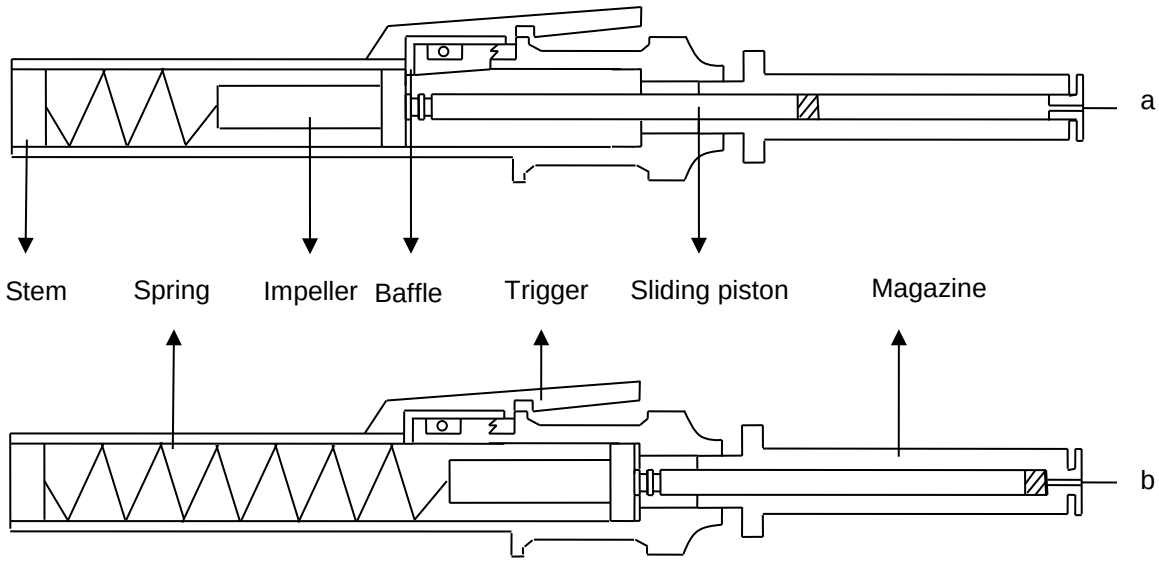


Figure 11: The liquid gene sprayer: A: The liquid gene gun at energy storage state, B: The state of liquid gene gun after injection (redrawn from Zhou (2007))

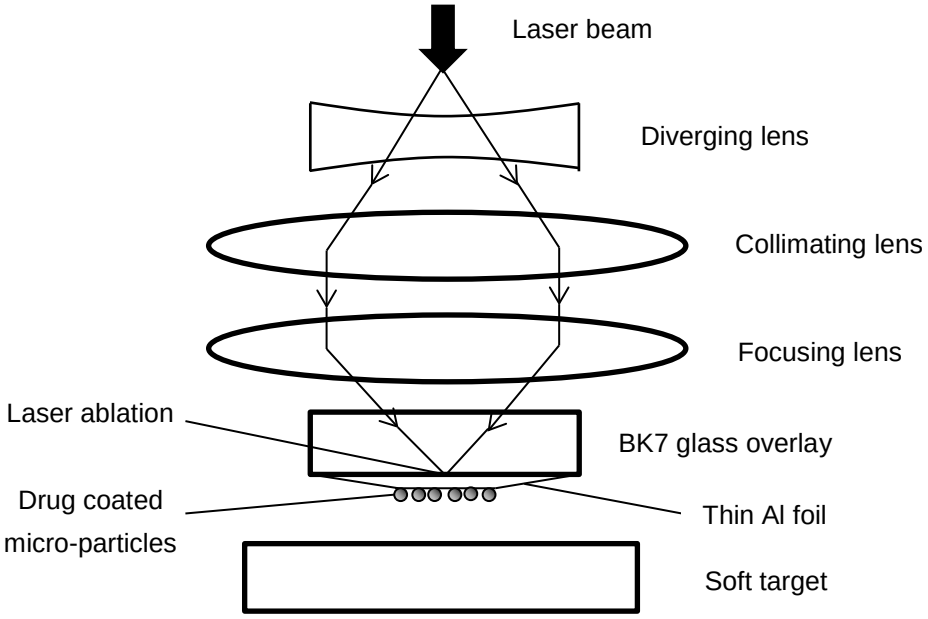


Figure 12: The schematic of the laser plasma jet (redrawn from Menezes et al. (2012))

Recently, Menezes et al. (2012) have designed an advanced laser plasma jet (see Figure 12) to deliver DNA-coated micro-particle. The operating principle is that a laser beam is fired and ablates a thin aluminum foil, using lenses for focusing. The laser ablation is confined by the BK7 glass overlay to improve performance. Thus, it causes the foil to evaporate into an ionized vapor and the sudden blow-off causes a shock wave to breach the foil to accelerate DNA

coated micro-particles. The device provides micro-particles impact velocities of up to 1100 m/s, which is faster than other gen guns (e.g. CST, LLG). However, this technique is costly due to the use of laser ablation.

2.2 Micro-particle materials and size

The materials and size of the micro-particles which are used in gene guns have significant importance on the operation of the system, e.g. by determining the routes and extent of particle penetration into tissue. In general, the routes of the micro-particle penetration in the tissue are normally divided into two types, which are the extracellular and intercellular routes (Bryan et al., 2013; Soliman, 2011; Mitchell et al., 2003). As presented in Figure 13(a-b), the penetration routes of the micro-particles in tissue depend on the particle size. The extracellular route is followed for large particle delivery, e.g., for epidermal powder immunization (Soliman, 2011; Hardy et al., 2005). An illustration of the range of particle material and sizes for the relevant gene gun systems is listed in Table 1. It shows that the extracellular route is normally followed for the less dense materials (e.g., stainless steel, polystyrene and glass) with diameters ranging from 15 to 99 μm. Hardy et al. (2005) have reported that particle diameters ranging from 25 to 100 μm are expected to follow the extracellular route, as their momentum is insufficient to breach the target barrier of target, due to the combination of relatively low density and small size. It has been recommended that stainless steel or polymer micro-particles should be used for extracellular routes, due to their biocompatibility and low cost (Soliman, 2011; Sung et al., 2011; Singh and Dahotre, 2007; Binyamin et al., 2006; Disegi et al., 2000).

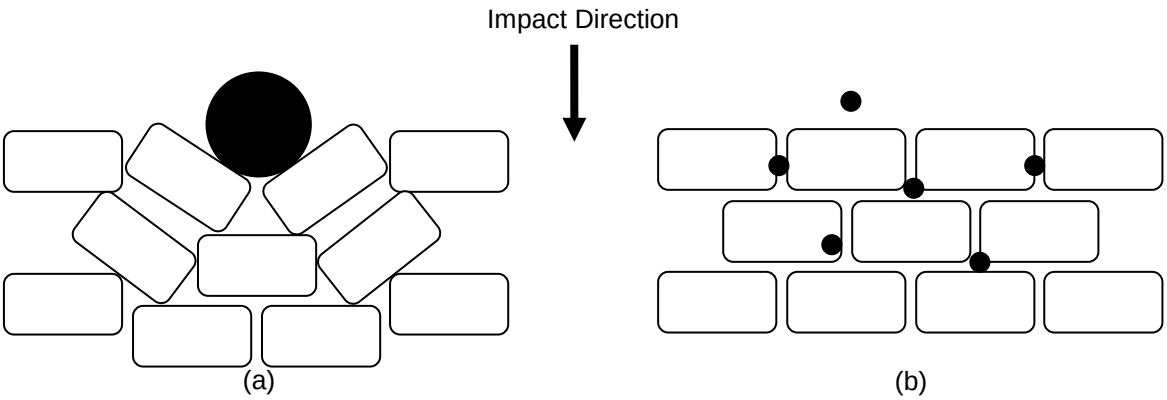


Figure 13: Schematic diagram of the extracellular and intercellular failure mechanisms (a): extracellular failure mechanism for large particles (b): intercellular failure mechanism for small particles (redrawn from Mitchell et al. (2003)).

In contrast, the intercellular route (e.g. for DNA immunization), uses smaller size, but much more dense gold or tungsten micro-particles (Soliman, 2011; Mitchell et al., 2003). In order to deliver DNA into cells effectively, dense materials are preferred which are prepared into micro-particles of diameters ranging from 0.6 to 6 μm (Soliman, 2011; Rao, 2010; Hardy et al., 2005) which are also smaller than the cell diameters. High-speed micro-particles breach the skin and may penetrate through the individual cell membranes. It is well known that the most recommended material of these micro-particles for gene gun system is gold due to its high density, low toxicity and lack of chemical reactivity (Valenstein, 2012; Rosi et al., 2006; Macklin, 2000). However, tungsten micro-particles have also been used as micro carriers in gene gun systems, due to their lower cost. Tungsten particles have some disadvantages for genetic transformation, such as non-biocompatibility and toxicity (Bastian et al., 2009; Yoshimisu et al., 2009; Russell et al., 1992). Recently, Hou et al. (2013) have used titanium dioxide (TiO_2) for biolistic micro-particle delivery due to their biocompatibility (Singh and Dahotre, 2007) and low density (2 g/cm^3) which may reduce the cell damage after particle impaction. The particle impaction may cause cell damage (O'Brien and Lummis, 2011; Sato et al., 2000), which is a significant area for gene gun research.

2.3 Gas pressure and particle velocity

Up to now, gene gun systems have been widely used to deliver DNA loaded micro-particles into cells for research of DNA transfection, e.g., CST (Rasel et al., 2013; Liu, 2008; Truong et

al., 2006; Liu et al., 2006; Kendall, 2002) and Helios gene gun (O'Brien and Lummis, 2011; Belyantseva, 2009). Helium and compressed air gases are often used as driving forces to accelerate micro-particles for gene gun system. Especially, helium gas is recommended for most gene gun systems due to its non-toxic, low density, lack of chemical inactivity and high compressibility factor (Marrion et al., 2005), which allow the particles to reach higher velocities (Tekeuchi et al., 1992). Compressed air is often used as a substitute for helium due to its lower cost.

The gas pressure is a major factor which should be considered for gas gene gun systems. It directly affects the velocity of the micro-particles, e.g., Liu et al. (2008) demonstrated that 1.8 μm diameter gold micro-particles can reach a velocity of 580, 650, 685 and 710 at 3, 4, 5 and 6 MPa, respectively. In addition, the velocity is also related to the micro-particle size and density: particle velocity is increased from an increase in operating pressure and a decrease in particle size.

334 **Table 1**: Illustration of the particle material and size for the relevant gene gun systems

Type of gene gun	Material of particles	Average diameter of micro-particles (μm)	Reference
Powder particle gun (PPG)	Tungsten	4	Klein et al. (1987)
Helium-driven apparatus (HDA)	Tungsten	3.9	Williams et al. (1991)
	Gold	1-3, 3-5	
Conical nozzle (CN)	Polymeric	4.7, 15.5 and 26.1	Quinlan et al. (2001)
Converging-diverging nozzle (CDN)	Polystyrene	4.7	Kendall et al. (2004a)
Pneumatic gun (PG)	Gold	0.47 ± 0.15, 1.1 ± 0.1	Rinberg et al. (2005)
	Silicon	2 – 18	Zilony et al. (2013)
	Gold	1.6	
Light gas gun (LGG)	Stainless steel	25	Mitchell et al. (2003)
Contoured shock tube (CST)/LGG	Polystyrene	15.5, 25.2, 48 and 99	
CST	Gold	3.03	Truong et al. (2006)
	Polystyrene	15 and 48	
	Glass	46	
	Gold	2.7 and 3.5	
	Polystyrene	39 ± 1	Liu et al. (2006)
Helios gene gun	Gold	0.6, 1.0 and 1.6	Uchida et al. (2009)
		1	O'Brien and Lummis (2011); Kuriakose et al. (2012); Cao et al. (2013)
Biolistic PDS/1000 Helium system (BioRad, USA)	Gold	0.6, 1.0 and 1.6	Zuraida et al. (2010)
BioWare low pressure gene gun	Gold	1	Yen and Lai (2013)

Along with the development of gene gun technology, the achievable particle velocity and penetration depth in the target vary between gene gun systems. For example, Quinlan et al. (2001) have applied a conical nozzle to accelerate polymeric micro-particles of 4.7, 15.5 and 26.1 μm diameters, to reach velocities of 350, 460 and 465 m/s at 60 bar pressure, respectively. A contoured nozzle has been tested by Quinlan et al. (2001) who employed 60 bar to accelerate polymeric micro-particles of 4.7 μm diameter to a velocities of about 1000 m/s, but 26.1 μm diameter of particles only reached 740 to 810 m/s. The velocity of polymeric micro-particles of 15.5 μm diameter only reaches 330 m/s at 60 bar for the LGG system (Mitchell et al., 2003). Kis et al. (2011) have concluded that the particle velocity reached about 700 m/s for CST (Liu et al., 2006) and range from 200 to 800 m/s for converging-diverging nozzles (Liu et al., 2004b; Kendall, 2002; Quinlan et al., 2001). Recently, Menezes et al. (2012) have operated an advanced laser plasma jet to drive gold micro-particles of 1 μm diameter to achieve an average velocity of 1100 m/s within a distance of only 10 mm. The particular achievements (e.g., particle velocity, operating pressure and penetration depth) for various gene gun systems are listed in Table 2.

From the above studies, it can be concluded that the operating pressures for gene gun systems generally vary from 20 to 60 bars. Xia et al. (2011) have suggested that the pressure should be held below about 13 bar to minimise damage from the impaction of pressurized gas on soft tissue. Uchida et al. (2009) achieved gene transfection is cells but noted damage if the operating pressure is over 13 bar. These reports demonstrate that cell death is unavoidable for many of the current generation of gene gun systems. Mitchell et al. (2003) show that golden micro-particles of 3.03 μm diameter employed at 60 bar pressure in the CST only reach a maximum penetration depth of 60 μm in the canine buccal mucosa. Normally, the viable epidermis layer of skin is the target area for gene gun systems. Mitchell et al.'s (2003) results show that micro-particles require a still higher velocity to penetrate through the *stratum corneum*, which means higher operating pressures are necessary. However, Mitchell et al. (2003) also show stainless steel micro-particles of 25 μm diameter can achieve 124 μm penetration depths in the canine buccal mucosa at 20 bar pressure (see Table 2). But O'Brien and Lummis (2011) show that cultured cells are damaged by gold micro-particle of 1 μm diameter, when operating at 3.4 bar pressure using a Helios gene gun. They indicate that

366 stainless steel micro-particles of 25 μm diameter impact on the tissue at a velocity of 170 m/s
367 will damage the target tissue and cells. Thus, a new concept of applying MN to micro-particle
368 delivery, which may reduce the cell damage, is discussed in section 4.2.
369
370 **Table 2**: Illustration of the materials and size of micro-particles used in gene gun systems

Gene gun	**Material and average diameter of micro-particles**	**Operating pressure (bar)**	**Velocity at impact (m/s)**	**Target and maximum Penetration depth in target**	**Reference**
PPG	Tungsten, 4 μm	N/A	430	Onion, 40 μm	Klein et al. (1987)
HAD	Gold, 1 - 3 μm	90	N/A	Mouse liver tissue, ~ 130 μm	Williams et al. (1991)
CN	Polymers, 4.7, 15.5 and 26.1 μm	60	350, 460 and 465	N/A	Quinlan et al. (2001)
LGG	Stainless steel, 25 μm	20	170	Canine buccal mucosa, 124 μm	Mitchell et al. (2003)
CST	Gold, 3.03 μm	60	550	Canine buccal mucosa, 60 μm	
CST	Gold, 1 ± 0.2 μm	40	580	Human skin, 66 μm	Kendall (2002)
CST	Polystyrene, 39 μm	60	570 ± 14.7	N/A	Liu et al. (2006)
Helios Gene Gun	Gold, 40 nm and 1 μm	5	N/A	Mouse ear tissue, 50 ± 11 μm and 31 ± 6 μm	O'Brien and Lummis (2011)
Laser plasma jet	Gold, 1 μm; Tungsten, 1 μm	N/A	1100	N/A	Menezes et al. (2012)

371
372

3. MNs

3.1 Types and configurations of MNs

Henry et al. (1998) are widely regarded as the first to have developed a method of transdermal drug delivery using MNs, which has gradually developed for various applications of drug delivery. MN arrays are minimally invasive device that bypass the outer layer of skin, namely the *stratum corneum*, to achieve enhanced transdermal drug delivery (Olatunji et al., 2013; Donnelly et al., 2012; Prausnitz and Langer, 2008). MN are normally separated into two categories, namely, solid and hollow (Koelmans et al., 2013; Han et al., 2008; Qi et al., 2007). Each of these can be made of different materials and used for various functionalities, depending on their designs. The most common materials used for fabricating MN are metal, silicon, and polymer (Kim et al., 2012; Memon et al., 2011; Zhao et al., 2006). The primary metals used for MNs are stainless steel (Kim et al., 2012; Quan et al., 2010; Bal et al., 2008; Martanto et al., 2004) and titanium (Kim et al., 2010; Fermandez et al., 2009; Parker et al., 2007). Metal MNs have the advantages of low cost, tougher hardness, ease of penetration into the tissue and they are not easily broken in the tissue. Silicon and silica materials have better biocompatibility than metallic materials, but they are expensive (Chen et al., 2013, 2008). Furthermore, they break up more easily and fragments may be left inside the tissue after MN removal (Memon et al., 2011; Zhao et al., 2006). However, silicon is the first material used to fabricate MNs for pre-treatment of skin prior to patch application, e.g., Henry et al. (1998) have used solid conical silicon MNs (Figure 14a) with a height of 0.15 mm, inner diameter of 80 μm and tip diameter of 1 μm to increase skin permeability and to provide an effective delivery of drugs to diffuse through the skin. Mikszta et al. (2002) used a silicon MN array (see Figure 14b) for gene delivery in skin.

Polymeric materials are a cheap option which can exhibit biocompatibility and biodegradability, but the hardness is generally lower (Nayak and Das, 2013; Oh et al., 2008; Han et al., 2007; Park et al., 2005). Various types of polymer have been used for fabricating MNs, such as poly-glycolic acid (PGA) (Park et al., 2006, 2005) and polycarbonate (PC) (Han et al., 2007). Park et al. (2005) have fabricated a PGA solid MN array with needle length of 1500 μm, base diameter of 200 μm tapering to tip diameter of 20 μm. An example of solid polymer MN array is shown in Figure 15a. Recently, Donnelly et al. (2011) have used poly (vinyl) alcohol (PVA) to

fabricate solid MN arrays with a needle height of 600 µm, base width of 300 µm, and MN interspacing of 300 µm. As can be seen from Figure 15 (b), MNs have been fabricated into uniform conical-shaped needles.

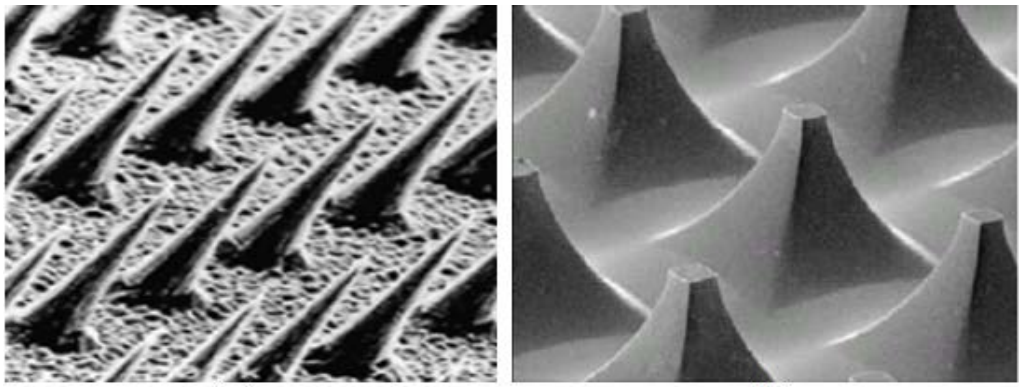


Figure 14: (a). Solid conical MN arrays (Henry et al., 1998) (b) Silicon MN array used for gene delivery in skin (Mikszta et al., 2002).

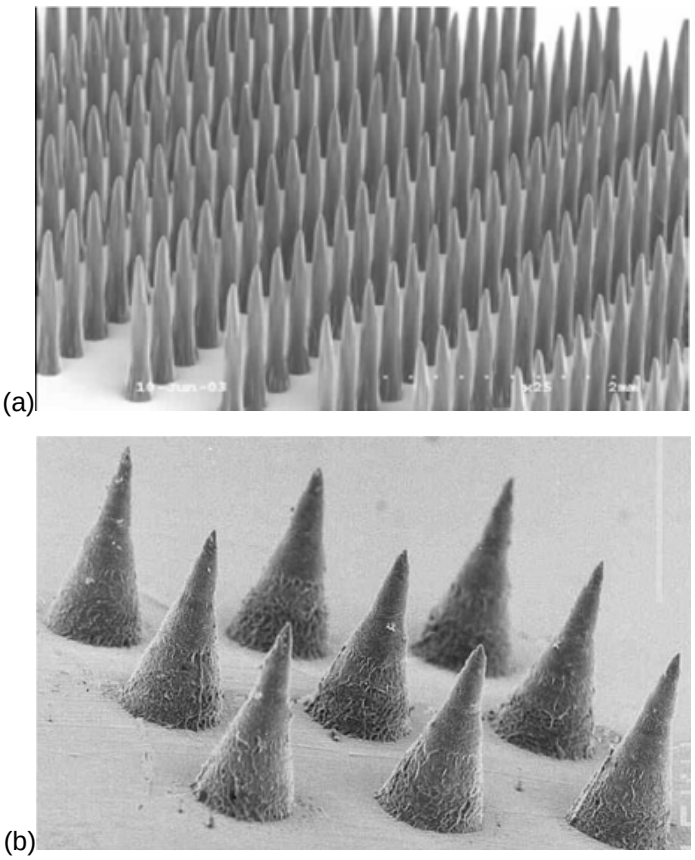
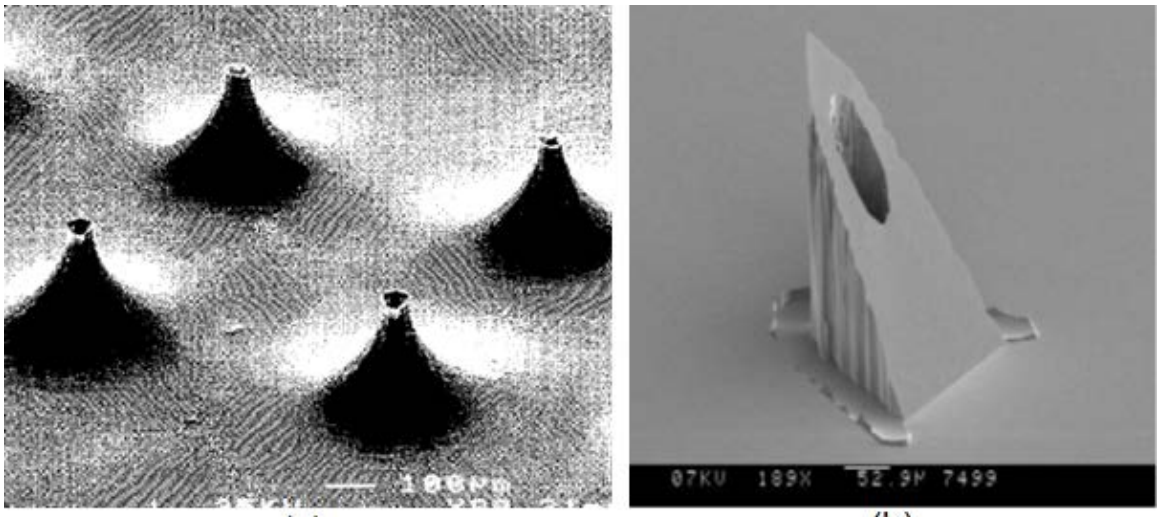


Figure 15: (a): Solid MN array made with the help of PGA (Park et al., 2005) (b): Solid MN array made with the help of PVA (Donnelly et al., 2011)

Hollow MN arrays are normally used for fluid infusion of liquid drug and nanoparticles into the skin (Han et al., 2008). However, they are not widely used due to their cost and complex fabrication methods (Zhu et al., 2012). An example of the hollow MN array designed by Stoeber and Liepmann (2000) is shown in Figure 16a. It is a hollow conical MN array with a height of 200 μm and a channel diameter of 40 μm. McAllister et al. (2000) have improved Henry et al.'s (1998) design, by inserting a hole in the centre of each MN to fabricate a hollow silicon MN array.



(a) (b)

Figure 16: (a) Hollow conical MN arrays on the right (Stoeber and Liepmann, 2000) (b) hollow silicon MN array with sloping side walls (Gardenier et al., 2003)

Subsequently, Gardenier et al. (2003) have designed a hollow silicon MN array with sloping side wall, which is shown in Figure 16b. The length of the needles varies between 150 and 350 μm, with a base diameter of 250 μm (measured at the widest section) and a maximum hole width of 70 μm. The centre of the hole is positioned 40 μm from the tip of the needle. Davis et al. (2004) have used a hollow metal MN array (Figure 17) with a tip diameter of 75 μm, base diameter of 300 μm, wall thicknesses of 5 μm and height of 500 μm to measure the required force for the insertion of MNs into the tissue. They reported that a force ranging between approximately 0.1 – 3 N is sufficient to penetrate a single MN into the tissue.

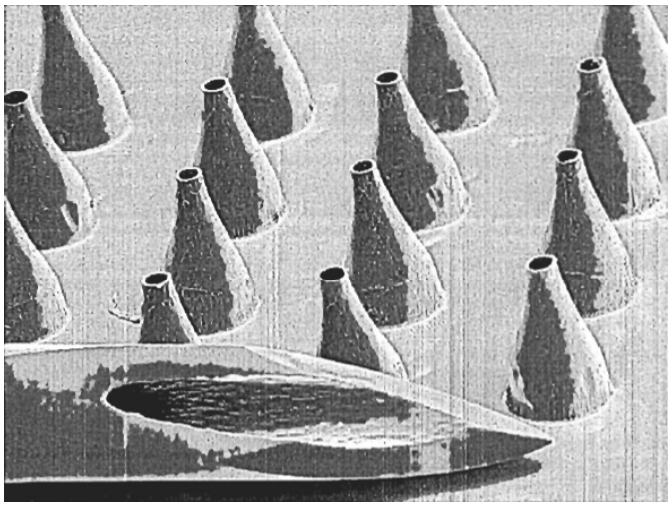


Figure 17: Hollow metal MNs (Davis et al., 2004)

Until now MNs have been developed as a minimally invasive means to deliver genes via the transdermal route (Tuan-Mahmood et al., 2013; Coulman et al., 2009; Henry et al., 1998), e.g., Chabrai et al. (2004) have successfully used micro-fabricated silicon MNs for non-viral gene delivery without causing pain. Recently, Zhang et al. (2013) have proposed the use MNs with a gene gun system to assist the micro-particle delivery in the skin. In particular, solid MNs can create holes in the tissue to provide an environment for the penetration of high-speed micro-particles. Furthermore, hollow MNs may allow a number of micro-particles to go through the hollow needles and penetrate to a greater depth into deeper tissue layers to be gene transfected. However, the waste of micro-particles is likely to be higher as the hollow MNs have blockage problems.

There have been a number of studies which report on the effects of MNs types and configuration on drug delivery (Han and Das, 2013; Olatunji et al., 2012; Al-Qallaf and Das, 2009a,b, 2008; Al-Qallaf et al., 2009a,b, 2007; Davidson et al., 2008). The current contribution will focus on the most relevant MNs types and configurations; reviews of other aspects related to the application of MN may be found elsewhere (Olatunji and Das, 2011, 2010).

3.2 MN insertion in skin

MNs can overcome a target surface to provide an advantageous condition for micro-particle delivery (Zhang et al., 2013). Human skin consists of two distinct macroscopic layers called the dermis and the epidermis (Marks et al., 2006; Parker, 1991; Phipps, 1988) which are shown in

Figure 18. The epidermis layer consists of the *stratum corneum* (SC), the *stratum basale* (or *stratum germinativum*), *stratum sinosum*, and the s*tratum granulosum* (Gerard et al., 2011; Marks et al., 2006; Holbrook, 1994). The top layer of the skin is the SC which is the major barrier for entry of foreign substances. The thickness of the epidermis varies with gender, age, ethnicity and the regions of the body, but has an average thickness of between 20 and 100 µm (Matteucci et al., 2009; Schaefer and Redelmeier, 1996). The thickness of the *stratum corneum* also varies with the above conditions, but the average thickness is between 10 and 20 µm (Mohammed et al., 2012; Holbrook et al., 1974).

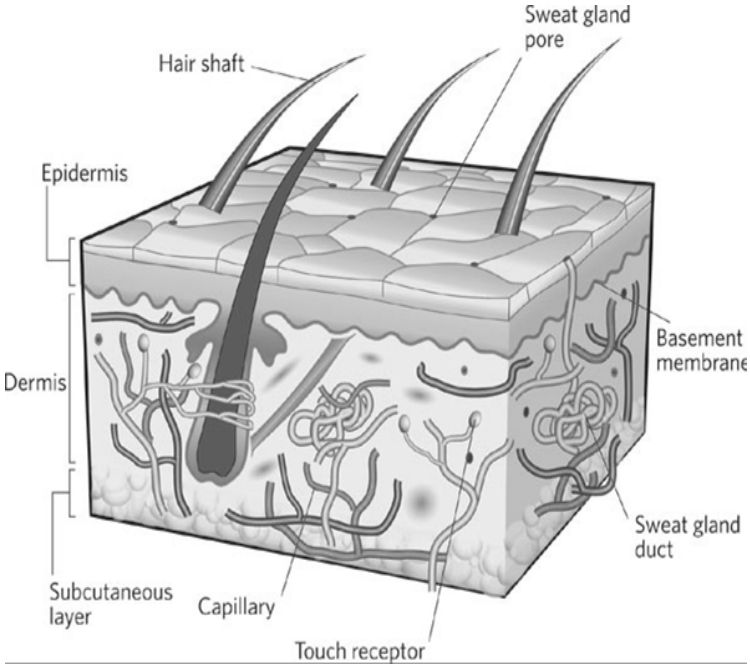


Figure 18: The structure of the skin (MacNeil, 2007)

Normally, MN insertion is painless as it simply penetrates the skin surface without reaching the dermis layer, which contains the nerves ending. (Gupta et al., 2011a; Silpi et al., 2011; Donnelly et al., 2010a; Henry et al., 1998). Pain sensation depends on the MN design which is generally small enough to avoid significant damage to the nerves in the tissue (Shah et al., 2011; Palastanga et al., 2006; Kaushik et al., 2001). Also, as the pain sensation is related to the MN design, an increased size increases the likelihood of stimulating the nerves (Sachdeva and Banga, 2011; Shah et al., 2011; Gupta et al., 2011a,b). There are a number of clinical studies which show that MNs insertions into skin are painless, or the pain is undetectable. For example, Mikolajewska et al. (2010) have used polymeric MN cones with needle height of 644

μm, base diameter of 217 μm tapering to tip diameter of 41 μm to progress the skin pre-treatment and report that the MNs insertion is a painless process. Previously, Haq et al. (2009) have used several MN arrays with lengths of 180 and 280 μm and compared the pain responses of a number of subjects for these MNs with the pain responses from hypodermic needles. They indicated that a hypodermic needle is painful after insertion into skin, and the pain response of MN insertion is less and decreases with a decrease in needle height.

The irregular surfaces and viscoelasticity of skin causes difficulty with MN insertion. In addition, the skin is generally folded after the insertion of MNs, which may cause MNs to pierce partially, depending on the MN length (Verbaan et al., 2008). Thus, there is a need to understand the required force to insert a given MNs in the skin; the depth of MN penetration into the skin is directly related to the used force for penetration (Olatunji et al., 2013; Donnelly et al., 2010b). In addition, the depth of MN penetration in the skin is dependent on the length of MNs (Badran et al., 2009).

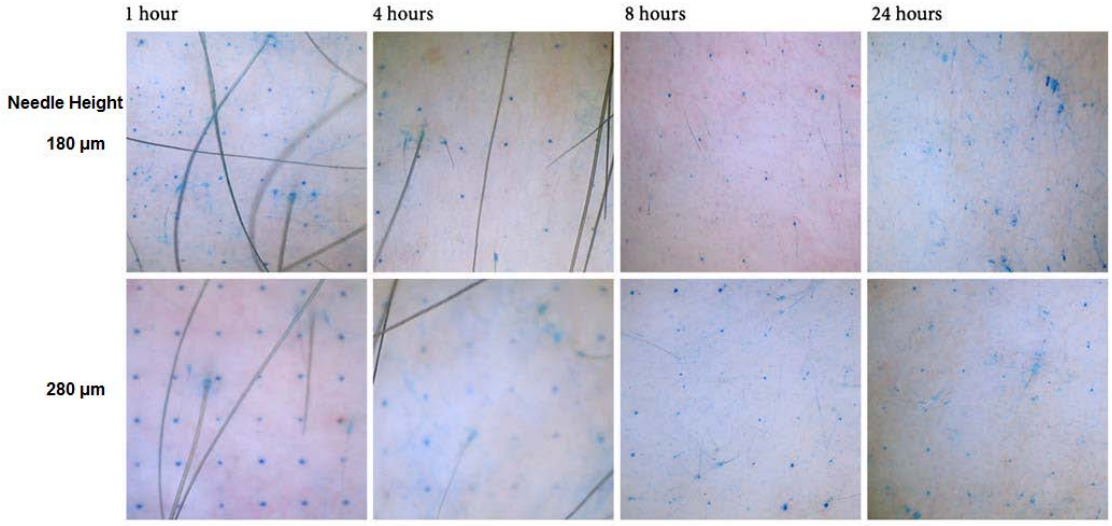


Figure 19: Methylene blue staining of MN holes on the human skin (needle height: 180 and 280 μm) (Haq et al., 2009)

Several studies of MN insertion in skin show that the holes remain in the skin after the removal of the MNs (e.g., Haq et al., 2009), as shown in Figure 19. Previously, McAllister et al. (2003) have applied a cylindrical MN of 20 μm diameter to perform staining experiments, which indicates that a hole will remain after the removal of MNs. Martanto et al. (2004) have shown that a number of visible holes remained when MNs were applied with needle lengths of 1000

μm and width of 200 μm by 50 μm on a rat skin. In addition, McAllister et al. (2003) have reported a residual MN hole of radius 6 μm following insertion of MNs with radius of 10 μm, which means that the holes shrink to ~60 percent of the diameter of the MNs. While inserting the MNs, it can also be seen that the entire MN length cannot be inserted into the skin completely, and the depth of penentration of MN in skin is related to its length and the application force. These factors directly affect the size of pierced holes before and after the removal of MNs, Donnelly et al. (2010b) have used optical coherence tomography (OCT) to detect the effect of MN height and application force on the depth of penentration inside the porcine skin. As shown in Figure 20a, the penetration depth is increased significantly by an increase in MN height and application force. In addition, the application force presents a positive effect on the penetration depth of the MN inside skin, as shown in Figure 20b: the pore width is increased by an increase in needle height and application force, when the base width is kept constant. However, there is a clear gap left between the MN base plate and the skin surface. Donnelly et al. (2011) have further used OCT to obtain 3D views of MN embedded in the human skin. They reported that a MN with needle height of 600 μm and base diameter of 300 μm penetrated approximately 460 μm into the human skin with a clear gap of 136 μm between the MN base plate and skin. They also indicated that the width of the pierced holes in the skin was about 265 μm in diameter. These reports demonstrate that MN assisted micro-particle delivery is expected to realize a greater penetration depth of micro-particles in the skin.

4. Potential of MN assisted micro-particle delivery

4.1 MN assisted micro-particle delivery

As mentioned earlier, cell and tissue damages are particular problems for the biolistic gene transfection (Obrien et al., 2011; Uchida et al., 2009; Thomas et al., 2001; Sato et al., 2000; Yoshida et al., 1997). Reduction in the operation pressure and in the particle size can minimize the cell damage, but these also decrease the momentum of the micro-particles. This minimizes the penetration depth of the micro-particles in the tissue and may cause a failure of the DNA transfection.

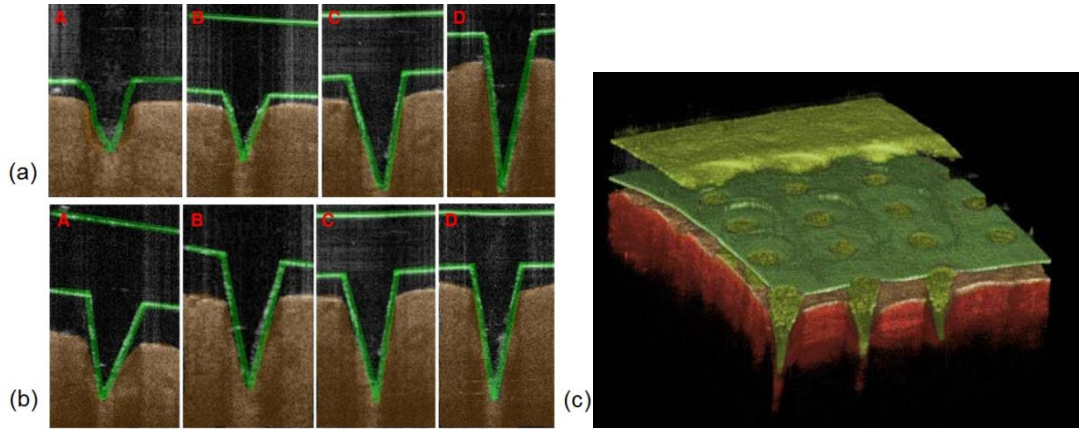


Figure 20: (a): A 2D OCT image for the investigation of the effect of the MN height on the penetration depth inside the porcine skin (A: 280 µm; B: 350µm; C: 600µm; D: 900µm) (Donnelly et al., 2010b) (b): A 2D OCT image to analyse the effect of the application force on the penetration depth of MN inside the porcine skin (A: 4.4 N; B: 7.0 N; C: 11 N; D: 16.4 N) (Donnelly et al., 2010b) (c): A 3D OCT image showing MN insertion in the skin (needle height: 600 µm; base width: 300 µm; spacing: 300 µm) (Donnelly et al., 2011).

Based on a consideration of reducing the operating pressure, a new concept of combining MNs with gene guns for micro-particle delivery has been presented by Zhang et al (2013). As presented in Figure 21, the concept may be developed from the light and Helios gas gun systems. The operating process consists of particle acceleration, separation and deceleration stages. For the acceleration stage, a pellet of micro-particles is loaded onto the ground slide which is accelerated by pressurized gas. The pellet is then separated into individual micro-particles by impaction onto a mesh stopping screen in the separation stage. The separated micro-particles spray forward through a conical nozzle with a uniform velocity and distribution to breach the skin tissue to target the cells of interest.

As shown by Zhang et al (2013), by using an array of MNs it is possible to overcome the effect of the skin on the particle penetration. One of the many advantages of this new concept is that the pressurized gas will be released from the vent holes (see Figure 21), which is likely to avoid the damage of the target from the impaction of pressurized gas on tissue. In addition, the use of the ground slide slows down the velocities of micro-particles to reduce the impact force on tissue to minimize the cell damage. As required, the velocity is controllable by changing the driving pressure of compressed gas, if higher micro-particle velocities are necessary. Even if the micro-particles cannot reach the desired penetration depth due to insufficient momentum,

the use of MNs allows a number of micro-particles to penetrate further to achieve the purpose of gene transfection in the desired depth of the tissue. However, the disadvantage of this concept is that a very high impact velocity of micro-particles is not easy to achieve, because the ground slide has significant mass.

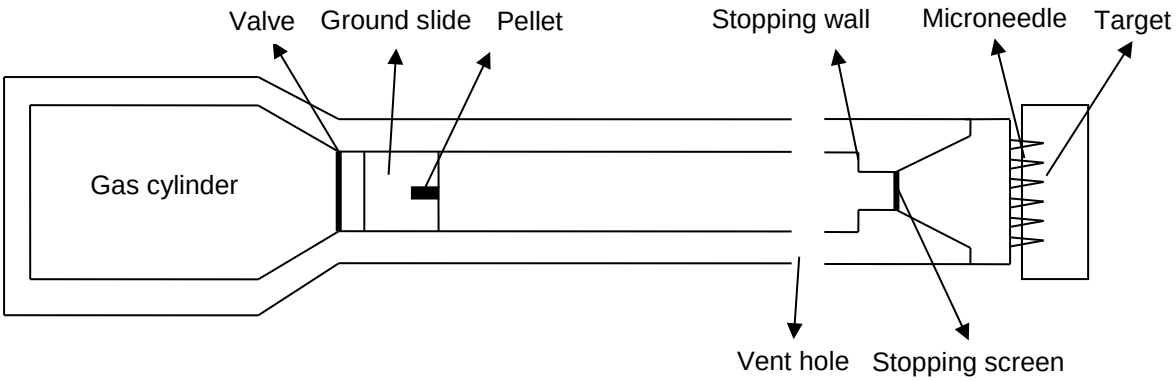


Figure 21: A possible structure of MNs assisted micro-particle delivery system

The solid MN used by Zhang et al. (2013) aimed to create holes on the skin to allow a number of micro-particles penetrate through the pierced holes and increase the penetration depth. Zhang et al. (2013) also indicate that some agglomerated micro-particle may be present in the target; the size of the agglomerates is controllable and decreases with a decrease in mesh size and binder concentration. Results (see Figure 22) have shown that a number of micro-particles are able to penetrate through the pierced holes (created by the MNs) and reached a greater penetration depth inside the target, demonstrating the feasibility of MN assisted micro-particle delivery. In principle, the maximum penetration depth of micro-particles is affected by the particle size, density and operating pressure which determine the momentum of the micro-particles and hence the impact of the particles on the target. An increased length of the pierced holes enhances the particle penetration depth due to a decreased resistance when micro-particle travel in the hole. The length of the pierced holes depends on the height of MN.

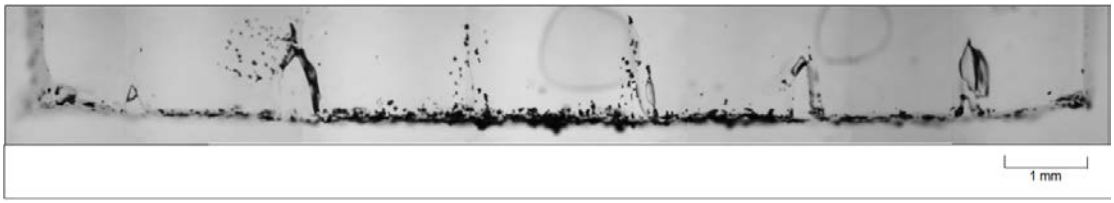


Figure 22: Optical microscope image of stainless steel micro-particle penetration into agarose gel (Zhang et al., 2013)

4.2 Effects of physical approaches to drug delivery

With the development of transdermal drug delivery, several physical technologies have been developed, particularly in needle free gene gun systems. In order to understand the advantages of MN assisted micro-particle delivery for the drug/gene delivery, a comparison with other physical cell targeting approaches is presented in this section. Figure 23 illustrates a schematic of four physical cell targeting approaches, which include diffusional delivery (Figure 23a), solid MN assisted micro-particle delivery (Figure 23b), hollow MN assisted micro-particle delivery (Figure 23c) and needle free biolistic micro-particle injection (Figure 23d). The route of the diffusion delivery (Figure 23a) is that the molecules permeates through the aperture of the SC and diffuse into the target (Glenn et al., 2003). It is a method which operates without damage to the skin.

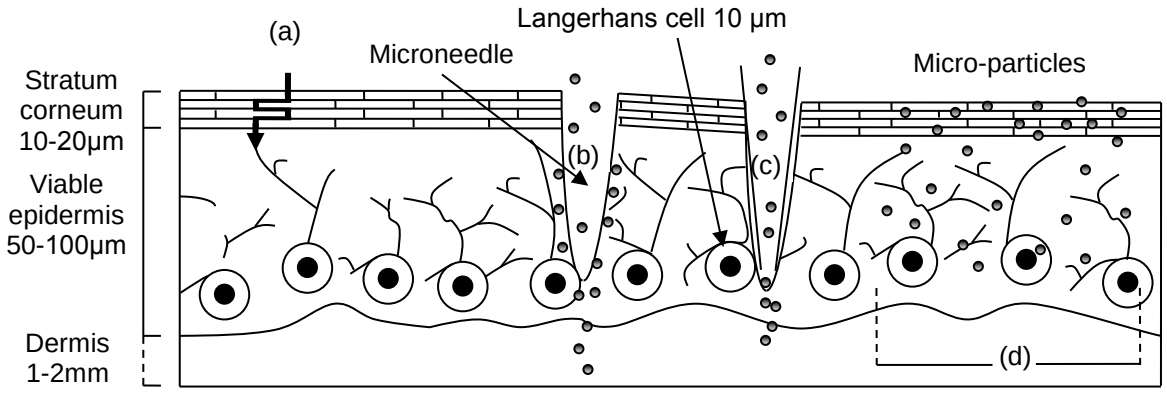


Figure 23: A schematic cross-section of the skin: (a) the normally diffusion route (b)route of solid MN assisted micro-particle delivery (c) route of hollow MN assisted micro-particle delivery (d) route of normal micro-particle penetration using a gene gun system (Kendall, 2006)

In recent years, needle-free biolistic micro-particle delivery (Figure 23d) provides a great improvement for transdermal gene delivery. The principle of this technique is that DNA is

loaded onto micro-particles which are accelerated to a sufficient velocity to pierce the skin and travel to a certain depth to achieve the DNA transfection in the viable epidermis layer. It can be seen from Figure 23d that micro-particles penetrate to greater depth than diffusion delivery. In addition, biolistic micro-particle delivery is painless as the micro-particles settle within epidermis without reaching the nerves (Quinlan et al., 2001). There are some disadvantages for biolistic micro-particle delivery such as micro-particle penetration causes a significant cell death in the skin due to impaction (O'Brien and Lummis, 2011; Raju et al., 2006; Sato et al., 2000). In addition, pressurized gas may damage the skin surface, if the pressure is over than 200 psi (Belyantseva, 2009; Uchida et al., 2009; Yoshida et al., 1997).

As mentioned earlier, the method of MN assisted micro-particle delivery requires use of a MN to overcome the skin surface to deliver micro-particles to a greater depth. The projected routes are presented in Figure 23b-c. In Figure 23b the penetration depths of micro-particles are greater than for needle-free biolistic micro-particle delivery, due to the pierced holes providing a low resistance path for micro-particle penetration. Figure 23c shows hollow MN assisted micro-particle delivery, which should allow particles to penetrate further in the skin via the hollow needles. However, the disadvantage of MN assisted micro-particle delivery is that the process may be painful if the micro-particles are deliver into dermis which have nerves ending in that layer.

Overall, it is obvious that needle-free biolistic micro-particle injection present more efficiency than diffusion delivery. Ziegler (2008) has shown that acceptable DNA vaccination requires the coated micro-particle to penetrate the skin surface with around 20-100 μm penetration depth. It indicated that needle-free biolistic micro-particle injection achieves a more efficient pharmaceutical effect than diffusion delivery. Further, MN assisted micro-particle delivery may deliver micro-particles deeper than the needle-free gene gun system in the skin to allow deeper tissues to be transfected. This it has been demonstrated that MN assisted micro-particle delivery may achieve a further enhanced DNA transfection in the target.

4.3 Modelling micro-particle delivery in skin

The process of micro-particle delivery is normally divided into two stages, which are the particle acceleration and penetration stages. In the modelling, the acceleration stage should be considered along with the driving source (gas pressure) which accelerates the micro-particle to a sufficient velocity to pierce the skin surface. The velocity varies with the gene gun system design, particle density and size. For the penetration stage, the effect of the skin is the major resistance to prevent the micro-particle delivery. Micro-particle delivery requires breaching of the SC and piercing into the epidermis layer (Yager et al., 2013; Soliman et al., 2011b; Liu, 2006; Quinlan et al., 2001; Bennett et al., 1999; Trainer and Alexander, 1997). The impact velocity, particle size and density, target density and yield stress are the major variables affecting the penetration depth.

Normally, the micro-particle acceleration stage involves gas and particle flow for gas gene gun systems, e.g., CST, PowderJect and Helios Gene gun. The flow is defined as symmetric and fully turbulent in the device (Liu, 2006; Soliman et al., 2011a). A model which has been widely adopted to model micro-particle flow is based on a balance of forces using Newton's second law and Stokes' law. For example, Liu (2006) has focused on simulating the velocity distribution in the converging (conical) section of a venturi system which is developed from a gene gun, namely, the PowderJect system (PowderJect Research Ltd., Oxford, UK) (Bellhouse et al., 1999, 2003, 2006). The particle velocity has been simulated based on a balance between the inertia of micro-particles and other resistance forces acting on the particles. Zhang et al. (2007) have used the MATrix LABoratory (MATLAB, The MathWorks Inc., Natick , USA) (Shampine et al., 1997) to simulate three different stages of the particle delivery in the gene gun, namely, acceleration, separation and deceleration stages. In their work, the particle velocity is analyzed on the basis of Newton's second law in the acceleration stage; an energy conservation law is applied to describe the separation of micro-carriers into micro-particles in the separation stage, and Stokes' law is applied to model the penetration of micro-particles in the deceleration stage into a viscous target. Soliman et al. (2011b) have used a commercial turbo-machinery flow simulator, namely, FINE^{TM}/Turbo (NUMECA International, Brussel, Belgium) to simulate the behaviour of gas and particle flow in a supersonic core jet in a gene gun. This work used Newton's second law to mimic the particle

trajectories and determine the penetration depths of micro-particles in the skin. As discussed below, a number of studies have shown that the penetration depth depends on the momentum of micro-particles which again depend on the particle size, density and velocity.

For the penetration stage, various studies have separated the resistance force on the micro-particle into a yield force (F_y), frictional resistive force (F_f) and resistive inertial force of the target material (F_i) (Soliman et al., 2011b; Liu, 2007; Mitchell et al., 2003; Kendall et al., 2001; Dehn, 1987). The force balance equation is shown below:

$$m\frac{dv}{dt} = -(F_i + F_f + F_y) \quad (1)$$

From this the stopping distance can be calculated as

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

Based on this force balance equation, the theoretical penetration depth (equation 2) is obtained and adopted for the modelling. This model has been widely adopted in several studies. For example, Soliman et al. (2011b) have modelled the delivery of golden particle of diameters 1.8 and 5 μm using 3 MPa pressure and have shown that penetration depths of 95μm and 135μm can be achieved for particle of diameters 1.8 and 5 μm, respectively. Kendall et al. (2001) have analysed the golden particle penetration by using equation 2 for particle penetration in human and porcine skins. In addition, predictions from the theoretical model have been shown to agree well with the experimental results by Kendall et al. (2001). More recently, Soliman et al. (2011b) have also implemented equation 2 in a theoretical model which is implemented using FINE/Turbo code to calculate the penetration depth of gold micro-particle inside the skin.

5. Conclusion

The background of the gene gun system for micro-particle delivery is reviewed in this paper. A number of gene gun systems have been listed and the operating principles along with their advantages and disadvantages have been studied briefly. In addition, the recommended gas type, particle material and size for these type engineering systems are discussed. The range of particle velocities and applied operating pressures for several gene gun systems are described, which indicated that cell/tissue damage is a major problem for biolistic

694 micro-particle delivery, due to the impaction of pressurized gas and high-speed micro-particles
695 on the target tissue. In addressing this point, a new concept is proposed of MN assisted
696 micro-particle delivery, which combines a gene gun system with MN to enhance the
697 penetration depth of micro-particles. This technique may reduce the cell damage from
698 pressurized gas and reduce the impact velocity of micro-particle. In order to further understand
699 the MN assisted micro-particle delivery, a number of MN designs have been discussed, paying
700 attention to key characteristics that affect biolistic delivery.
701
702 A number of researchers have studied the MN insertion in the skin which suggests that holes
703 remained on the skin after the removal of MNs. These indirectly show the feasibility of the MN
704 assisted micro-particle delivery to enhance the penetration depths of micro-particles inside the
705 target. Based on the above research works, the detailed penetration route of MN assisted
706 micro-particle delivery is also discussed and compared with other physical approaches on
707 drug delivery. Finally, various models of micro-particle delivery for different gene gun systems
708 are described to understand the theoretical principles of micro-particle penetration and which
709 may be used for modelling of MN assisted micro-particle delivery.
710
711 **6. Conflict of Interest**
712 Authors declare no conflict of interest
713
714 **7. References**
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