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# Multifunctional core-shell polymeric nanoparticles for transdermal DNA delivery and epidermal Langerhans cells tracking

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ABSTRACT

Skin is a highly immune-reactive tissue containing abundant antigen-presenting cells such as Langerhans cells (LCs), and thus is a favorable site for DNA immunization. This study developed a multifunctional core-shell nanoparticle system, which can be delivered transdermally into the epidermis via a gene gun, for use as a DNA carrier. The developed nanoparticles comprised a hydrophobic PLGA core and a positively-charged glycol chitosan (GC) shell. The core of the nanoparticles was used to load fluorescent quantum dots (QDs) for ultrasensitive detection of Langerhans cell migration following transdermal delivery, while a reporter gene was electrostatically adsorbed onto the GC shell layer of the nanoparticles. Results of fluorescence spectrophotometry, transmission electron microscopy, energy dispersive X-ray analysis, and X-ray diffraction measurement confirmed that the prepared nanoparticles had a core-shell structure with QDs in their core area. The surface charge of nanoparticles depended strongly on pH environment, enabling the intracellular release of the loaded DNA via a pH-mediated mechanism. Using a mouse model, this study demonstrated that bombardment of nanoparticles transfected DNA directly into LCs present in the epidermis; the transfected LCs then migrated and expressed the encoded gene products in the skin draining lymph nodes. These observation results suggest that the developed nanoparticle system is suitable for monitoring and fine-tuning important functional aspects of the immune system, in conjunction with the loaded fluorescence, and thus has potential for use in immunotherapy and vaccine development.



## 1. Introduction

Skin is a highly immune-reactive tissue containing abundant antigen-presenting cells such as Langerhans cells (LCs), particularly in the epidermis [1,2]; consequently, it provides a favorable site for DNA immunization. Previous human clinical studies on the gene gun have validated the concept of directly targeting LCs for delivering DNA-coated gold particles [3]. The coated DNA can be bombarded directly into the cytoplasm and nuclei of LCs; the activated LCs leave the epidermis via the lymph vessels and enter the T-cell area of the skin draining lymph nodes [4]. Transfected LCs express the encoded antigenic gene products and present the processed peptides to T cells to stimulate an immune response, and thus have potential for application in immunotherapy and vaccine development [5]. One disadvantage of gene-gun bombardments is that the accumulation of non-biodegradable gold particles used can cause adverse side effects [6].

Conventionally, monitoring of LCs has been carried out using fluorescent contact sensitizers [7]. Although this approach is useful and informative, LC marking has been transient because of fluorochrome dilution [5]. Semiconductor quantum dots (QDs) may be used as fluorescent probes to image and study biological processes [8,9]. When observed via two-photon microscopy, QD fluorescence is 100–1000 times brighter than conventional fluorophores and is maintained for long periods without photobleaching [10], permitting more precise tracking of LC migration from the skin to the lymph nodes. QDs have been used as stable fluorescent tracers for lymph node mapping in live animals [11].

Biodegradable polymers such as poly(D,L-lactic-co-glycolic acid) (PLGA) have been used to formulate QDs in the form of

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nanoparticles [12]. PLGA, a well-known biocompatible polymer, has been extensively adopted in drug delivery and tissue engineering [13]. However, the lack of functional groups available for modification on the surface of PLGA nanoparticles has limited their potential for surface tethering bioactive molecules, including DNA and RNA. Thus, various attempts have been made to modify the surfaces of PLGA nanoparticles by coating with surfactants or polymers [14].

This study describes the development of cationically-modified PLGA nanoparticles with a core-shell structure (CSNPs) for transdermal DNA delivery and epidermal LC tracking. The developed nanoparticles, comprising a hydrophobic PLGA core and a positively-charged glycol chitosan (GC) shell, can be delivered transdermally into the epidermis of a mouse model using a gene gun. GC is a derivative of chitosan and has been used as a carrier for drug-delivery [15–17]; it is hydrophilic, biodegradable, and low immunogenic [18]. The PLGA core of nanoparticles is used to load QDs (CSNPs/QDs) for ultrasensitive detection of LC migration from the skin to the lymph nodes, following bombardment of CSNPs/QDs into the skin. A reporter gene pEGFP-N2 is electrostatically adsorbed onto the GC shell layer of CSNPs/QDs, which can subsequently be released intracellularly via a pH-mediated mechanism. The expression of fluorescent protein EGFP in the migrated LCs in lymph nodes is visualized with a confocal laser scanning microscope (CLSM, Fig. 1).

**Table 1**
Particle size, polydispersity index, and zeta potential of PLGA nanoparticles (NPs), core-shell nanoparticles (CSNPs), and core-shell nanoparticles loaded with QDs (CSNPs/QDs) in deionized water ($n = 5$).

| | Particle Size (nm) | Polydispersity Index | Zeta Potential (mV) |
|---|---|---|---|
| NPs | 178.5 ± 9.6 | 0.11 ± 0.02 | −7.2 ± 0.7 |
| CSNPs | 208.5 ± 11.3 | 0.13 ± 0.04 | 29.6 ± 4.0 |
| CSNPs/QDs | 266.5 ± 10.8 | 0.12 ± 0.06 | 27.6 ± 3.0 |

## 2. Materials and methods

### 2.1. Materials

PLGA (lactide/glycolide molar ratio 75:25, inherent viscosity 0.17 dl/g) was obtained from BioInvigor (Taipei, Taiwan). Polyvinyl alcohol (PVA, MW = 30–70 kDa), GC (MW = 250 kDa) and dichloromethane (DCM) were obtained from Sigma–Aldrich (St. Louis, MO, USA). Furthermore, CdSe/ZnS QDs were purchased from Evident Technologies (Troy, NY, USA). The DNA (pEGFP-N2, 4.7 kb), containing a CMV promoter and an enhanced green fluorescence protein reporter, was obtained from BD Biosciences Clontech (Palo Alto, CA, USA).

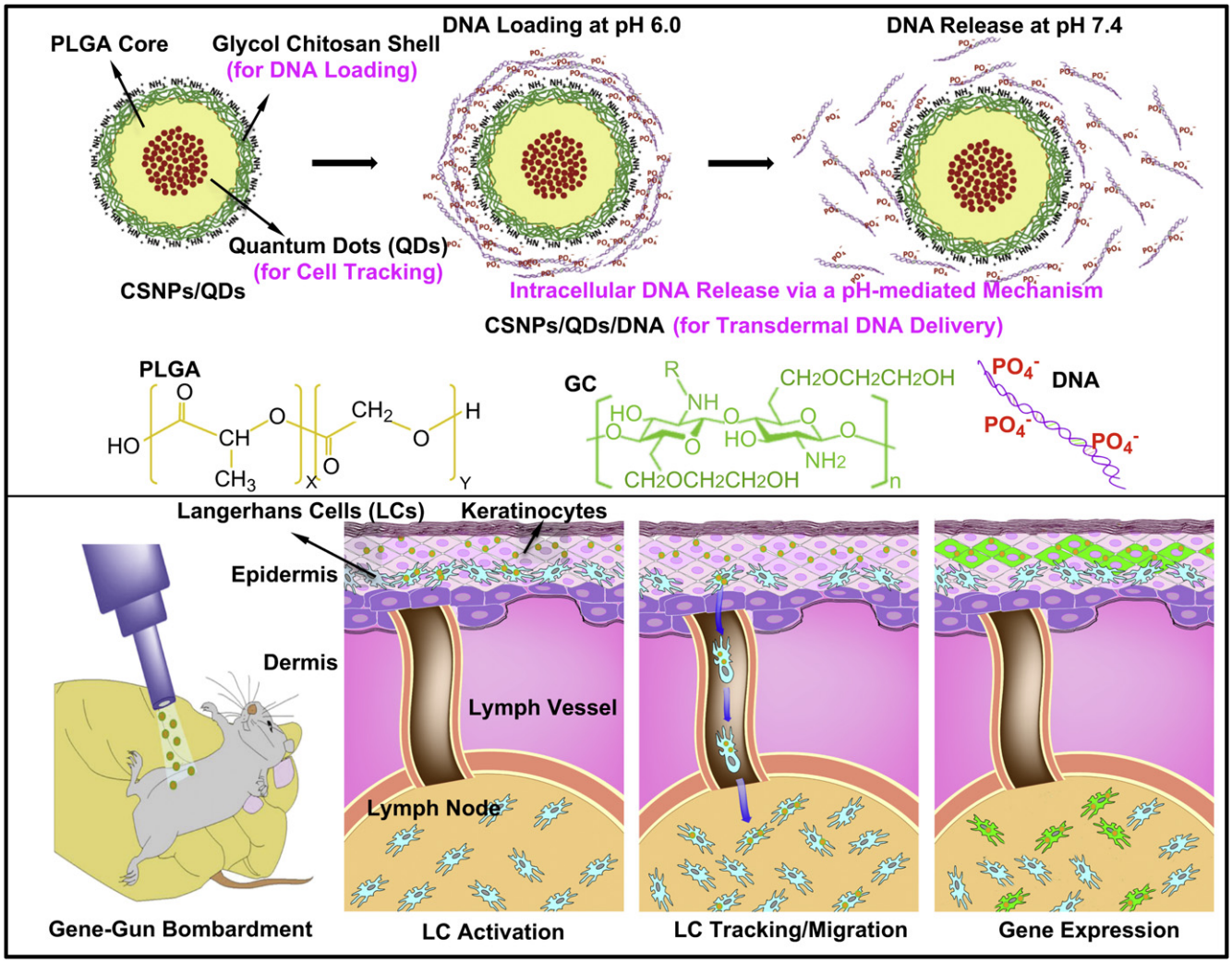


**Fig. 1.** Schematic illustrations of the concept of multifunctional core-shell polymeric nanoparticles designed in the study: transdermal DNA delivery, tracking of Langerhans cell migration, a pH-mediated DNA release mechanism, and gene expression in lymph nodes. CSNPs/QDs: core-shell nanoparticles incorporation with QDs; CSNPs/QDs/DNA: CSNPs/QDs loaded with DNA.