Journal of Controlled Release 179 (2014) 11–17

Contents lists available at ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

Polyplex-releasing microneedles for enhanced cutaneous delivery of DNA vaccine

Nak Won Kim [a], Min Sang Lee [a], Kyu Ri Kim [b], Jung Eun Lee [a], Kyuri Lee [a], Jong Sung Park [a], Yoh Matsumoto [c], Dong-Gyu Jo [a], Haeshin Lee [b], Doo Sung Lee [d], Ji Hoon Jeong [a,*]

[a] School of Pharmacy, Sungkyunkwan University, Suwon 440-746, Republic of Korea
[b] The Graduate School of Nanoscience & Technology (WCU), Department of Chemistry, KAIST, Daejeon 305-701, Republic of Korea
[c] Department of Molecular Neuropathology, Tokyo Metropolitan Institute for Neuroscience, Musashidai 2-6, Fuchu, Tokyo 183-8526, Japan
[d] School of Chemical Engineering, Sungkyunkwan University, Suwon 440-746, Republic of Korea

ARTICLE INFO

Article history:
Received 9 September 2013
Accepted 15 January 2014
Available online 23 January 2014

Keywords:
DNA vaccine
Gene delivery
Microneedle array
pH-responsive materials
Layer-by-layer assembly

ABSTRACT

Microneedle (MN)-based DNA vaccines have many advantages over conventional vaccines administered by hypodermic needles. However, an efficient strategy for delivering DNA vaccines to intradermal cells has not yet been established. Here, we report a new approach for delivering polyplex-based DNA vaccines using MN arrays coated with a pH-responsive polyelectrolyte multilayer assembly (PMA). This approach enabled rapid release of polyplex upon application to the skin. In addition to the polyplex-releasing MNs, we attempted to further maximize the vaccination by developing a polymeric carrier that targeted resident antigen presenting cells (APCs) rich in the intradermal area, as well as a DNA vaccine encoding a secretable fusion protein containing amyloid beta monomer (Aβ1–42), an antigenic determinant. The resulting vaccination system was able to successfully induce a robust humoral immune response compared to conventional subcutaneous injection with hypodermal needles. In addition, antigen challenge after immunization elicited an immediate and strong recall immune response due to immunogenic memory. These results suggest the potential utility of MN-based polyplex delivery systems for enhanced DNA vaccination.

1. Introduction

DNA vaccines have been considered as an attractive alternative to conventional vaccines due to easy and inexpensive production, superior stability in ambient temperature, and the ability to generate potent cellular and humoral immune responses [1,2]. DNA vaccines are also considered safer and are tolerated better compared with live or attenuated vaccines; however, their use in clinical settings remains limited because of their poor immunogenicity, especially in non-human primates and humans [2–4]. DNA vaccinations rely mainly on cellular delivery of plasmid DNA to induce expression of an encoded antigen, and thus lack of a proper delivery system for DNA vaccines is considered to be one of the most critical reasons for their low level of immunity [5]. Electroporation and gene gun-based ballistic delivery methods have been used to obtain improved immunization results; however, these methods often cause pain and discomfort to the patient, as well as require special expertise for handling equipment and complicated treatment procedures. Thus, electroporation and gene gun-based ballistic delivery methods are not suitable for prophylactic purposes, especially in certain regions where the necessary facilities and health care personnel are not available [6,7]. Non-viral methods of gene delivery, including cationic polymers and lipids that can form polyplexes with DNA vaccines are another promising approach, since DNA polyplexes are efficiently protected from enzymatic degradation and exhibit increased cell transfection efficiency [8,9]. Likewise, cationic polymer carriers containing mannose moieties have been reported to mediate targeted DNA delivery to professional antigen presenting cells (APCs), including macrophages and dendritic cells [10,11]. However, cationic polymer-based DNA vaccines still require a trained healthcare professional to perform the injection with a hyperdermic syringe.

Recent development of microneedles (MNs) has enabled painless cutaneous delivery of various bioactive molecules to epidermal and dermal regions replete with resident professional APCs [12,13]. Results of MN-based delivery of inactivated viruses, polypeptide antigens, and plasmid DNA encoding antigenic determinant units have been shown to be either as potent or generate even higher immunogenicity than intramuscular injections, even at a low dose [13–16]. As they can be self-administered, MNs also increase patient compliance, thereby eliminating patient anxiety and injuries caused by hyperdermic needles [17]. Therefore, MNs are a promising candidate platform for delivery of DNA vaccines. MNs coated with naked DNA vaccines can induce significant antibody- and cell-mediated immune responses, thereby providing protective immunity [18]. However, a repeated dip-coating procedure in highly concentrated and viscous solutions of plasmid DNA is required due to the lack of interaction between plasmid DNA

* Corresponding author.
E-mail address: jhjeong@skku.edu (J.H. Jeong).

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http://dx.doi.org/10.1016/j.jconrel.2014.01.016

and the surface of metal MNs [18,19]. Likewise, it may be difficult to obtain a film coating of uniform thickness on MN surfaces, which may be directly related to the DNA loading amount. Due to the lack of interaction with the MN surface, coated DNA films may be prone to separation from the MN when it is stored for an extended period of time depending on storage condition. Recently, building layer-by-layer (LbL) deposition of polyelectrolytes was identified as an efficient method for the preparation of DNA-coated MNs [17,20]. In this approach, the film thickness and DNA loading amount can be precisely controlled by alternating deposition of DNA and cationic polymer films.

In this study, we designed an efficient cutaneous gene delivery system to achieve enhanced DNA vaccination as well improved patient compliance. We fabricated MNs based on a pH-responsive polyelectrolyte multilayer assembly (PMA), which enabled rapid release of polyplex DNA vaccines upon application to the skin. We utilized a polydopamine (pDA)-based surface coating method to generate a base film, which provided a uniform cationic charge density on the surface of MNs. The versatile coating method was inspired by the amino acid composition of adhesive proteins of marine mussel threads, which can be used as a facile coating for a variety of material surfaces including polymers and metals [21,22]. Next, a pH-responsive PMA composed of alternating heparin and albumin films was built up on the base film. Albumin was employed to facilitate pH-dependent disintegration of PMA due to its charge inversion characteristics according to hydrogen concentration in the surrounding medium. Specifically, albumin has a pI value of 4.9 and is positively charged at pH values less than its pI value but become negatively charged at physiological pH. Heparin–albumin PMA acts as a stable matrix to support DNA polyplexes, which are localized in the outer most layer of PMA, and enable immediate release of polyplexes by rapid disintegration of PMA films in the skin upon injection of MNs. Importantly, released polyplexes can mediate enhanced cellular transfer of DNA vaccines. Specifically, use of polyplex- or cationic magnetic particles as a carrier for DNA vaccines has been shown to produce higher rates of transfection and better immunization results than vaccination with naked DNA, suggesting the importance of carrier-mediated gene delivery in DNA vaccination [23,24]. MNs coated with a releasable polyelelctrolyte multilayer, from which polyplexes were formed spontaneously through hydrolysis of cationic polymer layers, were recently used to achieve significantly enhanced DNA vaccination in non-human primates [25]. In addition, owing to the instant release of polyplexes, patients can conveniently use MN-based vaccination devices without the need for healthcare professionals. Along with pH-responsive polyplex-releasing MNs, APC-targetable cationic gene carrier and DNA vaccines encoding secretable antigens can be used to maximize antigen presentation by dermal resident APCs. Here, we evaluated the therapeutic potential of a MN-based cutaneous DNA vaccination system for Alzheimer's disease.

2. Materials and methods

2.1. Materials

Polycarbonate MN arrays (1 × 1 cm) were purchased from Miti systems (Daejeon, Korea). Dopamine hydrochloride, sodium heparin (17–19 kDa), bovine serum albumin (BSA), α-D-mannopyranosylphenyl isothiocyanate, D-(+)-mannose, D-(+)-trehalose, fluorescein isothiocyanate (FITC), dicyclohexylcarbodiimide (DCC), N-hydroxysuccinimide (NHS), deoxycholic acid (DA), branched polyethylenimine (bPEI 1.8 kDa and 25 kDa) and methylthiazolyldiphenyl-tetrazolium bromide (MTT) were purchased from Sigma-Aldrich (St. Louis, MO). Cell culture reagents, including Dulbecco's modified Eagle's medium (DMEM), phosphate-buffered saline (PBS), and trypsin-EDTA, and ethidium monoazide (EMA) were purchased from Invitrogen (Carlsbad, CA). Plasmid DNA (pTarget-Ig-Aβ-Fc) containing an open reading frame of $A\beta_{1-42}$ peptide, a leader sequence of mouse Igκ, and the Fc domain of human immunoglobulin, in a 5′ and 3′ order, respectively, was constructed as previously described [26]. All other organic solvents were of analytical grade and used without further purification.

2.2. Preparation of multilayered film-coated MN arrays

To build a cationic base layer, MN arrays were coated with polydopamine (pDA) by immersing the array in a dopamine coating solution (2 mg/ml, 0.1 M Tris buffer, pH 8.0). After overnight coating at 40 °C on an orbital shaker, the array was rinsed with deionized water and dried under reduced pressure. Multi-layered films (heparin/albumin) were built on the pDA-coated MN array by alternating the dipping of the array into heparin (5 mg/ml, 0.1 M citric acid buffer, pH 4.0) and albumin (5 mg/ml, 0.1 M citric acid buffer, pH 4.0) solutions for 5 min each. The array was then rinsed twice with washing buffer (0.1 M citric acid buffer, pH 4.0) before changing the dipping solution. This cycle was repeated until 16 bilayers were deposited, and was terminated with an outermost layer of heparin. The resulting array was then dipped in a solution containing DNA polyplexes, washed twice with washing buffer, and dried under reduced pressure. To prepare the polyplex coating solution, plasmid DNA (pTarget-Ig-Aβ-Fc) and cationic polymers (Man–DA3, see Supplementary data for synthetic procedure) were separately diluted in citric acid buffer (0.1 M, pH 4.0) with or without 15% D-(+)-trehalose (w/v), gently mixed, and incubated for 15 min at room temperature.

2.3. Scanning electron microscopy

The surface morphology of MN arrays was observed by SEM. For SEM analysis, the array was placed on a carbon-coated support. The sample was then coated with gold in a Precision Etching Coating System (Gantan 682 PECS, Gantan, Pleasanton, CA) and analyzed using a scanning electron microscope (JSM-7600F, JEOL, Tokyo, Japan).

2.4. X-Ray photoelectron spectroscopy (XPS)

XPS (Sigma Probe, Thermo VG Scientific) analysis, which detects the surface elemental compositions of functionalized materials, was performed after each surface functionalization step. Unmodified polycarbonate substrates were received from Kumjeon Inc. (Seoul, Korea), and the deposition procedure was performed as previously described.

2.5. Release of polyplexes from MN arrays

To observe the pH-dependent release of polyplexes, MN arrays were placed in PBS (pH 7.4) or citric acid (pH 4.1) and incubated on an orbital shaker at 37 °C. Sampling was performed at predetermined time points and replaced with an equivalent volume of fresh release medium. Plasmid DNA was separated from polyplexes by adding an excess amount of heparin (final concentration = 10 mg/ml) and extracted by the addition of chloroform. Samples were then vortexed and centrifuged at 15,000 rpm for 15 min. The resulting supernatant was saved, concentrated by ethanol precipitation, and dissolved in deionized water. The amount of DNA was quantified by PicoGreen assay (Quant-iTTM, Invitrogen, Carlsbad, CA).

2.6. Animal experiments

In vivo experiments were performed to observe the capability of multilayered film-coated MN arrays to induce antigen expression and subsequent immune response after delivery of DNA polyplexes. All animal studies were approved by the SKKU School of Pharmacy Institutional Animal Care and Use Committee. Female BALB/c mice (7 weeks old, Jungang Lab Animal Inc., Seoul, Korea) were used for all animal studies. Mice were anesthetized by intraperitoneal injection of Zoletil (10 mg/kg, Virbac Animal Health, Carros, France) and the hair on the back of the mouse was removed by applying depilatory cream,