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BCG vaccine powder-laden and dissolvable microneedle arrays for lesion-free vaccination

Fan Chen[a,b], Qinying Yan[a,c], Yang Yu[a], Mei X. Wu[a,*]

[a] Wellman Center for Photomedicine, Massachusetts General Hospital (MGH), Department of Dermatology, Harvard Medical School (HMS), Boston, MA 02114, USA
[b] Hubei Collaborative Innovation Center for Green Transformation of Bio-Resources, Life Sciences School of Hubei University, 368 Youyi Road, Wuhan 430062, China
[c] College of Pharmaceutical Sciences, Zhejiang University of Technology, 18 Chaowang Road, Hangzhou 310032, China

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ABSTRACT

Live attenuated Bacille Calmette–Guerin (BCG) bacillus is the only licensed vaccine for tuberculosis prevention worldwide to date. It must be delivered intradermally to be effective, which causes severe skin inflammation and sometimes, permanent scars. To minimize the side effects, we developed a novel microneedle array (MNA) that could deliver live attenuated freeze-dried BCG powder into the epidermis in a painless, lesion-free, and self-applicable fashion. The MNA was fabricated with biocompatible and dissolvable hyaluronic acid with a deep cave formed in the basal portion of each microneedle, into which BCG powder could be packaged directly. Viability of BCG vaccine packaged in the caves and the mechanical strength of the powder-laden MNA did not alter significantly before and after more than two months of storage at room temperature. Following insertion of the MNA into the skin, individual microneedle shafts melted away by interstitial fluid from the epidermis and upper dermis, exposing the powder to epidermal tissues. The powder sucked interstitial fluid, dissolved slowly, and diffused into the epidermis in a day against the interstitial fluid influx. Vaccination with BCG-MNA caused no overt skin irritation, in marked contrast to intradermal vaccination that provoked severe inflammation and bruise. While causing little skin irritation, vaccination efficacy of BCG-MNAs was comparable to that of intradermal immunization whether it was evaluated by humoral or cellular immunity. This powder-laden and dissolvable MNA represents a novel technology to sufficiently deliver live attenuated vaccine powders into the skin.

1. Introduction

Tuberculosis (TB) is caused by *Mycobacterium tuberculosis* (*M.tb*) and continues to be a leading cause of mortality among bacterial diseases. Although major progress has been made towards the global reduction of TB, TB still killed 1.5 million people and 9.6 million new TB cases were estimated in 2014 alone [1]. Approximately one-third of the world's population is currently infected with *M.tb* and about 5% of these infected people may progress to active disease during their lifetime. The risk of reactivating the infection and the resultant mortality are significantly escalated in HIV-infected individuals. This, along with emergence of multi-drug resistant strains [2,3], complicates this already miserable situation and raises an urgent need for an efficacious vaccine against TB. The Bacille Calmette-Guerin (BCG) vaccine, first introduced in 1921, remains to be the only licensed vaccine for TB prevention [4]. A great deal of efforts have been devoted to the development of a new vaccine against TB in the past two decades, but no any substitute or booster over the BCG vaccine has been developed so far [5]. A recent failure of a clinical trial of a new TB vaccine named MVA85A to enhance efficacy of the BCG vaccine in South African infants reaffirms the challenges we are facing to improve BCG-induced immune protection [6]. Alternatively, TB epidemics can be better controlled by broadening BCG vaccination coverage in neonates before they are exposed to any infection [7]. Development of a pain-less, lesion-free, self-applicable, and cost-effective vaccination approach would undeniably facilitate this alternative strategy in mass neonatal vaccination in developing countries.

BCG vaccine is currently administered intradermally to newborns or neonates using a hypodermic needle. It requires skilled healthcare professionals and it is often unreliable [8–10]. If the vaccine is accidentally given subcutaneously, which occurs ~5%, the bacillus may infect local tissues and the infection can spread to the regional lymph nodes, causing either suppurative or nonsuppurative lymphadenitis [11,12]. Hence, intradermal (ID) immunization of BCG vaccine poses a real risk to both vaccinees and healthcare providers. Moreover, the skin vaccination is well known to cause pain, accidental needle stick

* Corresponding author.
E-mail address: mwu5@mgh.harvard.edu (M.X. Wu).

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injuries, serious skin inflammation, and scars. These adverse events discourage children and parents, leading to a high level of non-compliance [11,13]. Microneedle arrays (MNAs) were investigated for delivering BCG vaccines in which the vaccine was coated onto a metal MNA but its viability lost > 50% in the absence of preservatives or surfactant or 25% in the presence of trehalose and surfactant after storage for only 7 days at 25 °C [14]. Dissolving MNAs were next engineered to encapsulate and deliver bioactive molecules and vaccines [15–18], which required a mixture of the bioactive molecules with monopolymer prior to polymerization. The polymerization process often compromises the activity or immunogenicity of the bioactive materials considerably and thus dissolving MNAs are not suitable to live attenuated BCG bacilli.

Currently BCG vaccine is freeze-dried powder and sealed under vacuum in a glass ampoule. The powder has to be diluted with saline water and injected within hrs, which often incurs errors owing to inappropriate opening of the glass ampoule and mishandling of the reconstitution. To eliminate the reconstitution procedure and minimize skin irritation, we explored a micro-fractional delivery of powdered vaccines into the skin, in which an array of self-healing microchannels were generated in the epidermis and upper dermis by ablative fractional laser, followed by topical application of powdered vaccine-coated array patches [19,20]. While greatly facilitating entry of powdered vaccines into the skin, the laser-perforated skin showed little reactogenicity. However, laser was required to perforate the skin and powdered vaccines fractionally coated in the patch must be aligned precisely with the individual microchannels on the skin for a high efficiency of delivery. These drawbacks are overcome by our newly engineered dissolvable, powder-laden MNAs as presented in the current study. The novel MNA could efficiently deliver powdered BCG vaccines and elicit innate, humoral, and cellular immunity similarly as traditional ID vaccination, while causing little skin irritation. The MNA could be stored for > 60 days at room temperature without adverse effects on the penetration ability of the MNA or viability of the BCG vaccine.

2. Materials and methods

2.1. Mycobacteria and animals

Mycobacterium bovis BCG strain (Danish 1331) was purchased from American type culture collection and expanded per manufacturer's instructions (ATCC-35733). For powder preparation, the *Mycobacterium* was grown at 37 °C to a mid-log phase in BBL™ Mycoflasic Middlebrook 7H9 broth with 0.2% glycerol from the Becton, Dickinson and Company (BD). Colony forming units (CFU) were enumerated after a series of 10-fold dilutions and culturing the diluted bacilli on BBL™ Middlebrook and Cohn 7H10 agar plates supplemented with 0.5% glycerol (BD). Inbred BALB/c and C57BL/6 mice at 5 weeks of age were purchased from Charles River Laboratories and both genders were used randomly with no notable difference. The animals were housed in the specific pathogen-free animal facilities of Massachusetts General Hospital (MGH) in compliance with institutional, hospital and NIH guidelines. All studies were reviewed and approved by the MGH Institutional Animal Care and Use Committee.

2.2. Fabrication of BCG powder-laden, dissolvable MNAs

MNAs were fabricated via micromolding technologies as described with some modifications [21]. In brief, a female polydimethylsiloxane (PDMS, Dow Corning) mold was used to fabricate a 6 × 9 array of microneedles each in a height of 200 μm and a base diameter of 100 μm. A 20% sodium hyaluronate (HA) (750–1000 kDa from Lifecore) solution in distilled water was added into the female PDMS mold mounted in a 6-well plate followed by centrifugation for 5 min at 1500 rpm/min at 4 °C twice with the plate rotated horizontally at 90° following each centrifugation. The plate was centrifuged for 5 min at 3000 rpm/min twice again as above to make sure that each cavity of the female MNA mold was evenly filled with HA and no bubbles were formed in the gel. Excess HA was removed gently and flatly using a cotton swab without disturbing HA microneedles in the cavity of the negative MNA mold. HA was polymerized in a negative pressure container at room temperature for overnight, forming a cave at the upper portion of each microneedle at 25–30 μm in depth.

To prepare BCG powder, bacilli at a mid-log growth phase were collected, washed three times, re-suspended with PBS, and lyophilized at − 40 °C. The powdered BCG vaccine was ground to make them more homogeneous before adding into the base of the MNA. The plate was centrifuged for 10 min at 3000 rpm/min at 4 °C twice with the plate rotated horizontally at 90° following each centrifugation. The process was repeated two to three times until all microneedles in the array were fully laden. Additional HA solution at 15% was added to cover the MNA at ~0.5 mm in thickness, followed by centrifugation at 3000 rpm/min for 10 min twice with 90° rotation in between. The plate was dried in a negative pressure container overnight at room temperature. The resultant BCG-MNA was peeled off by a transparent adhesive tape. As a control, MNA without powder was prepared similarly as empty MNA. To visualize the powder packed within microneedles, powdered Alexa fluor 555-ovalbumin (OVA) (Molecular Probes) was added to the hollow of the microneedles in place of BCG, whereas HA solution mixed with a small amount of fluorescein isothiocyanate (FITC, Sigma) was used to fabricate the cave MNA as above. The resultant MNA was scanned by two photon confocal microscopy.

2.3. Quantification and viability of encapsulated BCG vaccine

To quantify BCG vaccine encapsulated in each microneedle in the array, we first established a standard curve by mixing sulforhodamine B (SRB) powder with BCG vaccine powder at a 1: 4 ratio by weight in dH_2O, followed by measurement of SRB fluorescent density on SpectraMax®, Molecular devices, USA. The amount of BCG vaccine powder was correlated linearly with SRB absorbance at 640 nm. The experiment was repeated for three times with 6 MNAs in each experiment. To evaluate viability of the BCG vaccine encapsulated within the MNA before and after storage at room temperature in a dark and dry desiccator, a portion of the MNA consisting of 24 microneedles was cut for each test at indicated days of storage and dissolved in 1 ml PBS, followed by a series of ten-fold dilution. The diluted and reconstituted BCG vaccine of 100 μl from each sample was inoculated onto 7H10 agar dishes and cultured for 30 days at 37 °C. Bacterial colonies of the reconstituted BCG vaccine from 24 microneedles in the agar dishes were counted manually in a sample-blind fashion. The result was expressed as an average CFU number of BCG vaccine powder per 24 microneedles.

2.4. Immunizations and skin reaction observation

BALB/c mice were anesthetized by intramuscular injection of ketamine at 100 mg/kg and xylazine at 10 mg/kg followed by hair removal on the lower dorsal skin with an animal hair clipper and depilatory cream. The mice were randomly divided into four groups with six in each and immunized by BCG vaccine with MNA or ID injection at a dose of 6 μg BCG per mouse. Control mice were mock immunized with empty MNA or PBS, respectively. Only a portion of a MNA containing 24 microneedles was used to immunize each mouse. The MNA was inserted into the skin by pressing it firmly with a thumb and removed after 15 min of the application. The inoculation sites were photographed daily for 10 days post-immunization to track skin reaction. Skin temperature was also monitored daily by an infrared thermometer (LA CROSSE) for 10 days at the sites of inoculation as well as a distant skin area of the same mouse as references. The differences in the skin temperature of the two sites in the same mouse