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Semiconductor diode laser device adjuvanting intradermal vaccine

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ABSTRACT

A brief exposure of skin to a low-power, non-tissue damaging laser light has been demonstrated to augment immune responses to intradermal vaccination. Both preclinical and clinical studies show that this approach is simple, effective, safe and well tolerated compared to standard chemical or biological adjuvants. Until now, these laser exposures have been performed using a diode-pumped solid-state laser (DPSSL) devices, which are expensive and require labor-intensive maintenance and special training. Development of an inexpensive, easy-to-use and small device would form an important step in translating this technology toward clinical application.

Here we report that we have established a handheld, near-infrared (NIR) laser device using semiconductor diodes emitting either 1061, 1258, or 1301 nm light that costs less than $4000, and that this device replicates the adjuvant effect of a DPSSL system in a mouse model of influenza vaccination. Our results also indicate that a broader range of NIR laser wavelengths possess the ability to enhance vaccine immune responses, allowing engineering options for the device design.

This small, low-cost device establishes the feasibility of using a laser adjuvant approach for mass-vaccination programs in a clinical setting, opens the door for broader testing of this technology with a variety of vaccines and forms the foundation for development of devices ready for use in the clinic.

1. Introduction

In recognition of the clear advantages of skin-based vaccination [1–3], a growing range of technologies are now in use or at an exploratory stage of their development for intradermal delivery of vaccine antigen [4,5]. For the maximum efficacy, the intradermal vaccination strategy would further benefit from use of adjuvants to reduce antigen dose or the number of vaccinations used in order to generate protective immunity [5–8]. However, development of adjuvants for intradermal vaccines is especially challenging, as most conventional adjuvants are likely to be inappropriate for use in the skin due to issues related to viscosity, formulation with antigen, or the potential to induce persistent local inflammation [9,10]. There is clearly a need for new adjuvants that are compatible with intradermal vaccination.

A physical parameter such as laser light may serve as an alternative to conventional chemical or biological adjuvants [11,12]. Brief exposures of a small area of the skin to non-tissue damaging high-power, nanosecond pulses of visible light lasers before intradermal vaccination has been shown to increase immune responses to a clinically relevant vaccine in mice and humans [10,12–14]. However, these initial approaches are constrained by a key barrier; production of such a pulsed laser requires large devices that cannot easily be reduced to small and economical form factors. We have recently demonstrated the ability of short duration treatment with

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low-power, non-pulsed, 1064 nm lasers to augment immune responses to intradermal vaccine in a lethal challenge murine influenza model [15]. In contrast to the complicated technical requirements for visible pulsed lasers, elimination of the need for high-power pulses on continuous wave (CW) 1064 nm near-infrared (NIR) laser makes it feasible to produce relatively small and inexpensive laser devices.

In spite of the obvious advantages to utilizing such small CW NIR laser devices, appropriate devices are not currently being produced for medical applications. This device development gap forms an obstacle to further translation of the laser vaccine adjuvant approach into human studies. Here we report that we successfully developed a handheld NIR laser device for adjuvanting vaccines that can be made at a substantially lower cost than current commercially-available systems. Showing that the same laser adjuvanting effects can be produced by lasers based on inexpensive diodes as are produced by more expensive diode-pumped solid-state lasers (DPSSLs) addresses one of the practical limitations of using NIR lasers in a clinical setting.

2. Materials and methods

2.1. Design and assembly of a handheld NIR laser device

SemiNex Corporation (Peabody, MA) incorporated a Gallium arsenide (GaAs) 1061 nm semiconductor laser diode from Axcel Photonics (Marlborough, MA) or Indium phosphide (InP) 1258 semiconductor laser diode from Innolume (Dortmund, Germany) or 1301 nm semiconductor laser diode from SemiNex into the SemiNex Laser Engine platform, which had previously developed for consumer market applications at a different wavelength [16]. The details are described in the Supplementary Materials and Methods.

2.2. Measurement of the spot size of a handheld NIR laser device

To measure the spot size of the device, an infrared (IR) camera (Sensors Unlimited, Princeton NJ) was used, as the emitted light is not visible to the naked eye. The IR camera consisted of an array of pixels which would translate the IR light into a color image on a computer screen. The details of the measurement are described in the Supplementary Materials and Methods.

2.3. Measurement of the wavelength profile of a handheld NIR laser device

To measure the wavelength emitted from the device, an Anritsu MS9710B Optical Spectrum Analyzer (OSA) was used. The reported emission wavelength from a laser diode is the nominal center wavelength. However, laser diodes emit within a distribution of wavelengths around the center wavelength. Average output powers were determined using a power meter for each illumination (Thorlabs).

2.4. Control software design of a handheld NIR laser device

A control board of a handheld laser device was modified to accommodate greater operational flexibility within the R&D environment so researchers could modify the laser parameters such as power level, pulse width, duty cycle, and overall energy profile within the target tissue. The control board used an industry-standard wireless Bluetooth communication protocol and USB hardware connection to connect the laser to a PC for programming via a web interface. A web-based user interface was designed for programming the handheld units and monitoring the testing progress. The software was hosted on a Microsoft 2012 Server platform running Microsoft SQL Server 2012. Data was communicated over the Internet using PHP protocol to a PC client that connected to the handheld laser device via USB.

2.5. Determination of skin thermal responses to handheld NIR laser

The maximum non-tissue damaging dosages for the handheld device were tested in mice as previously described [15] (see Supplementary Materials and Methods). Briefly, the handheld laser device was applied to the shaved and depilated backs of eight-week-old female C57BL/6J mice (stock no:000664 from Jackson Laboratory, ME) over a range of powers using three-minute exposures, with skin temperatures monitored using an infrared thermal imaging camera (FLIR Systems, North Billerica, MA) and assessment for skin damage using visible inspection and histology. All animal procedures were performed following the Public Health Service Policy on Humane Care of Laboratory Animals and approved by the Institutional Animal Care and Use Committee of Massachusetts General Hospital.

2.6. Mouse model of intradermal influenza vaccination

In order to examine the efficacy of the handheld laser as a vaccine adjuvant, we compared the handheld lasers to a previously explored DPSSL, (1064 nm neodymium-doped yttrium orthvanadate [Nd:YVO_4] laser) (RMI Laser, Lafayette CO), using an established mouse model of influenza vaccination as previously described [15]. Briefly, two days before the laser treatment, mice were depilated using a hair remover (Nair, Church & Dwight). Immediately after the completion of the laser treatment, mice were then injected intradermally with whole inactivated influenza virus A/PR/8/34 (H1N1) (1 μg in 10 μl saline, 1 spot, Charles River). Blood samples were taken 28 days after immunization and at 4 days post-challenge with an intranasal application of homologous live influenza virus (2×10^5 50% egg infectious doses per mouse, which is equivalent to $20 \times 50\%$ mouse lethal dose (MLD_{50}), in 30 μl saline 28 days after vaccination. A part of mice was monitored for body weight and survival time for 14 days post-challenge to assess protection. The details of the treatment are available in the Supplementary Materials and Methods.

2.7. ELISAs for quantitating anti-influenza antibodies

We assessed anti-influenza IgG and its subclasses and IgE antibody responses in serially diluted mouse serum samples by using ELISA, as previously described [15,17] (see Supplementary Materials and Methods).

2.8. Hemagglutination inhibition (HAI) titration

Mouse sera were analyzed for hemagglutination inhibition (HAI) titers by SRI International (see Supplementary Materials and Methods).

2.9. Quantitation of T-cell response to influenza vaccination

For assessment of T cell responses after influenza challenge, we isolated splenoctyes 4 days post live influenza challenge as previously described [15]. Each splenocyte preparation was divided into 1×10^6 cells per 96-wells in duplicate and incubated with or without 10 μg/ml of influenza A peptides NP311 (MHC Class II, $NP_{311\text{-}325}$ QVYSLIRPNENPAHK, Anaspec) or 10 μg/ml PA224 (MHC class I, $PA_{224\text{-}233}$ SSLENFRAYV) for 60 h. IFN-γ or IL-4 amounts within supernatants (pg/ml) were determined using DuoSet ELISA kits (R&D Systems) following manufacturer's instructions.

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