Controlled release of bacteriophage from PLGA microparticles delivered by microneedle patches to induce innate and adaptive immune system

Rezvan Jamaledin[1], Rossella Sartorious[2], Raffaele Vecchione[1], Pier Giuseppe De Berardinis[2], Paolo Netti[1]
[1]Italian Institute of technology, [2]Institute of Protein Biochemistry, Naples, Italy

Abstract

While vaccines represent the strongest weapons against a contagious disease, several shortcomings still limit their optimal function. This can largely be attributed to the lack of a proper delivery system as well as route of administration for effective activation of the immune system. The intradermal route has the potential to be an outstanding route of vaccination due to the localization of potent antigen present cell (APC) and other immune cells in the dermis. MNPs are tiny needles, small enough (100 –1,000 μm) that perforate superficial layers of the skin to deliver therapeutic agent into the dermis in a relatively painless and invasive manner [1, 2]. MNPs are generally preferred by patients as compared to traditional hypodermic injections. The majority of works with microneedles has involved bolus delivery of drugs and vaccines using either coated or dissolvable microneedles, but introducing microparticles into microneedles in order to sustained release has received less attention. Given these objectives and challenges, we designed a multi-compartment dissolvable and degradable polymeric microneedle patches for the sustained release of fd-OVA [3]. **Experimental and methods:** Fabrication of PLGA MP. MPs were prepared by double emulsion method of water in oil in water. 100 mg PLGA was dissolved in 1ml Dichloromethane (DCM). 100µl of bacteriophages was added to PLGA solution and homogenized for 30 sec at 20,000 rpm. 7.5mg ABC in 1 ml water was added to the first emulsion and was homogenized at 20,000 for 30sec and then mixed with 10ml PVA solution. The final emulsion was added to 40ml water. MPs were washed three times to remove PVA and then were exposed to lyophilization for overnight. Fabrication of microneedle patches. Casting solution contained Polyvinylpyrrolidone(PVP) and Hyaluronic acid (HA) was poured onto the mold. After being dried for 2 hours under the chemical hood, microparticles were incorporated into the cavities. 150µl solution of PVP and glycerol was poured on a PMMA film and after drying was placed onto the mold to extract the microneedles. **Results**: **Phage-encapsulated MPs**. We prepared empty and phage-encapsulated porous microparticles poly (lactic-co-glycolic acid) (PLGA) via water-oil-water double emulsion (Fig. 1). Porous particles were generated by including ammonium bicarbonate (ABC) in the inner aqueous phase. We generated highly porous empty MPs with the average size (d= 13 µm) and direct encapsulated phage with the average size of (d= 17 µm). **Fabrication of microneedles:** PLGA, PVP and HA were selected as microneedle materials because these biodegradable polymers are biocompatible, mechanically strong and can be formulated for slow drug release for weeks to months.

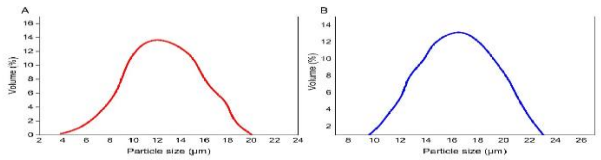


Fig. 1. Particle size distribution analysis of PLGA µPs performed by Mastersizer 2000. (A) Empty µPs, (B) phage-loaded µPs.

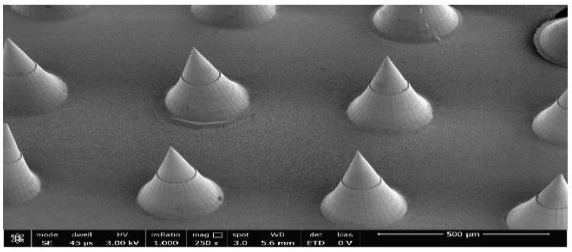


Fig 2. Scanning electron microscopy images of microneedles arrays. Base 600µm and height 300µm.

References:

[1] S.P. Sullivan, D.G. Koutsonanos, M. del Pilar Martin, J.W. Lee, V. Zarnitsyn, S.-O. Choi, N. Murthy, R.W. Compans, I. Skountzou, M.R. Prausnitz, Dissolving polymer microneedle patches for influenza vaccination, Nature medicine 16(8) (2010) 915.
[2] K. van der Maaden, W. Jiskoot, J. Bouwstra, Microneedle technologies for (trans) dermal drug and vaccine delivery, Journal of controlled release 161(2) (2012) 645-655.
[3] R. Sartorius, L. D'apice, M. Trovato, F. Cuccaro, V. Costa, M.G. De Leo, V.M. Marzullo, C. Biondo, S. D'auria, M.A. De Matteis, Antigen delivery by filamentous bacteriophage fd displaying an anti-DEC-205 single-chain variable fragment confers adjuvanticity by triggering a TLR9-mediated immune response, EMBO molecular medicine 7(7) (2015) 973-988.



Rezvan Jamaledin is a Ph.D. student in Industrial Product and Process Engineering at Federico secondo university. She has received her Master degree from Shahid Chamran University, Ahvaz, Iran. Her field is a vaccine, drug delivery by the microneedles.

Email: jamaledinrezvan@gmail.com