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Microprojection arrays to immunise at mucosal surfaces

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ABSTRACT

The buccal mucosa (inner cheek) is an attractive site for delivery of immunotherapeutics, due to its ease of access and rich antigen presenting cell (APC) distribution. However, to date, most delivery methods to the buccal mucosa have only been topical—with the challenges of: 1) an environment where significant biomolecule degradation may occur; 2) inability to reach the APCs that are located deep in the epithelium and lamina propria; and 3) salivary flow and mucous secretion that may result in removal of the therapeutic agent before absorption has taken place. To overcome these challenges and achieve consistent, repeatable targeted delivery of immunotherapeutics to within the buccal mucosa (not merely on to the surface), we utilised microprojection arrays (Nanopatches—110 μm length projections, 3364 projections, 16 mm^2 surface area) with a purpose built clip applicator. The mechanical application of Nanopatches bearing a dry-coated vaccine (commercial influenza vaccine, as a test case immunotherapeutic) released the vaccine to a depth of 47.8 ± 14.8 μm (mean ± SD, $n = 4$), in the mouse buccal mucosa (measured using fluorescent delivered dyes and CryoSEM). This location is in the direct vicinity of APCs, facilitating antigenic uptake. Resultant systemic immune responses were similar to systemic immunization methods, and superior to comparative orally immunised mice. This confirms the Nanopatch administered vaccine was delivered ***into*** the buccal mucosa and not ingested. This study demonstrates a minimally-invasive delivery device with rapid (2 min of application time), accurate and consistent release of immunotherapeutics in to the buccal mucosa—that conceptually can be extended in to human use for broad and practical utility.

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1. Introduction

An improved reach of effective vaccines to more people globally is a priority. With most of today's vaccines being administered by the needle and syringe (in liquid form), one significant way of improving vaccines is by moving to alternative (needle-free) delivery methods which vaccinate at sites rich in immune cells (e.g., the skin or mucosal sites). This contrasts with the current conventional immunisation strategy of intra-muscular injection which delivers to a tissue low in antigen presenting cells (APCs), critical cells required for the initiation of adaptive immune responses. Novel delivery site and method advantages include: avoiding needle-phobia [1]; eliminating unsafe needle practices [2]; improving vaccine stability (indeed potentially removing the 'cold chain' with vaccines stored in dry form) [3]; dose reduction and improved immune responses [4,5].

In order to realise these potential benefits, the initial site of infection and the nature of the pathogen against which a protective immune response is desired must be taken into consideration. For many pathogens, this initial site is at a mucosal surface that is exposed to the environment and requires the rapid secretion of mucosal immunoglobulins to prevent the dissemination of infection. The ability of an antigen to induce mucosal as well as systemic immune responses depends on the cells targeted at the initial site of immunisation (reviewed in [6,7]). However, systemic routes of immunisation alone may not generate immune responses that are capable of trafficking specific antibodies to these mucosal sites. In a recent study by O'Meara et al. [8], a chlamydial vaccine antigen in combination with adjuvant delivered intra-nasally protected mice from infection but not from disease pathology, whereas the same antigen-adjuvant combination delivered trans-cutaneously protected mice from disease pathology yet had no effect on reducing the burden of infection. This study highlights the need for continued

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development of mucosal and systemic vaccine delivery systems for the generation of optimal immune responses.

Additional benefits of new mucosal vaccine delivery systems (or indeed, epithelial vaccines) include protection of the antigen from physical elimination or degradation by enzymes, direct deposition and release of the antigen to, or near APCs within the mucosa and stimulation of the immune system to generate rapid adaptive and long lived protective immunity. Recently, the feasibility of delivering vaccines to the oral mucosa using microneedles has been demonstrated, with Ma et al. [9] showing that vaccine delivery into the lip or dorsal tongue surface of rabbits was able to induce both mucosal and systemic immune responses. A mucosal site that has been largely overlooked in developing new mucosal vaccine delivery technologies is the buccal mucosa; the inside lining of the cheek that forms part of the oral mucosa. If consistently and repeatedly targeted, the buccal mucosa offers a site rich in APCs [10] that is also a convenient, accessible part of the mucosa. So far, mechanical targeting methods have deposited many drugs—but few vaccines—*on* to the buccal mucosa surface [11,12].

As a device, there may be significant mechanical benefits to delivering to the buccal mucosa—several studies have identified significant complexity in breaching skin by mechanical means, with consistency of targeting [13–17]. Whilst the buccal mucosa layers are significantly thicker than skin's epithelia, its benefits include:

1. A much simpler surface layer to breach, due to the lack of a very tough keratinised layer. Although some animals including mice have a thin mucosal keratinised layer, it is still thinner than the corresponding skin surface.
2. The buccal mucosa surface is highly and consistently hydrated. In contrast, the skin surface can have a highly variable hydration—leading to variable skin mechanical properties [18,19]. Goktas et al. [20] found mucosal ultimate tensile stress to be ~1 MPa; an order of magnitude lower than skin.
3. It lacks the hairs, sun damage, scars and cosmetic effects that cause the skin to be a highly variable surface.

However, there are other potential sources of buccal variability including, for example in humans, the buccal mucosa is covered with a salivary layer (0.1–0.07 mm thick [21] with a ~0.5 ml/min flow rate [22]) that has the potential to dilute or wash away vaccines placed on the surface.

In this paper, we present a new minimally-invasive mechanical method that directly delivers drugs *into* the buccal mucosa (using vaccines as a test-case), by breaching the surface layer—as a practical way to achieve rapid, consistent and repeatable drug delivery. We utilised the Nanopatch, a small array of densely packed (>20,000 cm^{-2}) micro-projections that has previously been shown to be effective in delivering vaccines to APCs in skin (there are many investigations targeting vaccines to skin; with [4,5,23–30] just being a subset). We conceived, developed and investigated the application of the Nanopatch to the mucosa. Dynamic application of the Nanopatch to the buccal mucosa using a simple clip applicator provides a practical means for rapid, controlled and consistent mucosal delivery of immunotherapeutics. We selected the mouse model and an influenza vaccine as the test-case immunotherapeutic vaccine. We show here that our approach delivers vaccine antigen to the interstitial space within the epithelium where APCs reside, and results in immune responses comparable to needle-based intramuscular injection, Nanopatch skin delivery and superior to oral vaccine delivery.

2. Materials and methods

2.1. Animals

Adult female C57Bl/6 mice and MacGreen transgenic mice aged 6–12 weeks were used in this study according to the University of Queensland animal ethics regulations (AIBN/020/10) and with the approval of the Office of Gene Technology and Recombination (Australia). MacGreen mice carry the enhanced green fluorescence protein (eGFP) under the cfs1r promoter. In these animals all macrophage and monocyte derived cells express eGFP, including dendritic cells and Langerhans cells.

2.2. Clip applicator design

Application of Nanopatches to the buccal mucosa was performed using a clip applicator methodology—shown schematically in Fig. 1. The clip utilised was a hinged clip with a spring that resulted in a closing force of 1.9 Newtons (+/−8%). The diameter of the clip end was ~5 mm to ensure that it would fit within the small target area of a mouse mouth. The patch was attached to one arm of the clip applicator using double sided tape.

2.3. Nanopatches and coating

Nanopatches were manufactured by Deep Reactive Iron Etching as previously described [25] in the Rutherford Appleton Laboratory (Oxford, UK). The Nanopatches are etched from silicon and sputter-coated with a thin layer of gold. Each Nanopatch is 16 mm^2 (4 mm × 4 mm) and contains 3364 projections of 110 μm length and spaced at 70 μm intervals. Nanopatches were cleaned in a solution of glycerol and water (1:1 ratio), flushed with an excess of water and allowed to dry prior to coating. Formulations for coating contained 1% methylcellulose (Sigma-Aldrich, Castle Hill, NSW, Australia), active agent (0.2 μm, yellow–green fluorescent FluoSpheres® (0.4%), DiD (20 μM) (both Invitrogen Australia Pty Ltd, Mulgrave, VIC, Australia) or the purified, inactivated detergent-disrupted split virion influenza vaccine, Fluvax 2010, that contains 30 μg/ml hemagglutinin of each of A/California/7/2009, A/Wisconsin/15/2009 (A/Perth/16/2009 like) and B/Brisbane/60/2008 (CSL Ltd, Parkville, VIC, Australia)) and PBS if required. 8 μl of coating solution was pipetted onto each Nanopatch and dried using a nitrogen gas jet coating procedure as previously described [25].

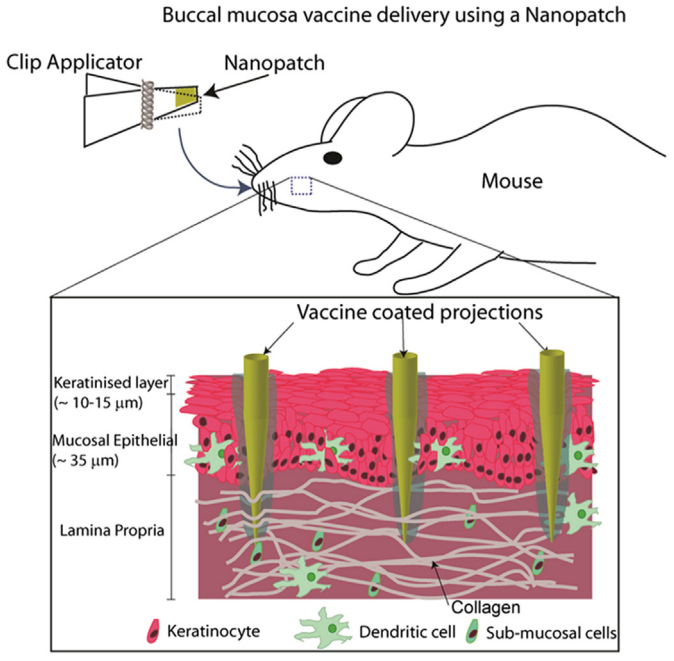

Fig. 1. The concept of applying the Nanopatch to the buccal mucosa of a mouse using a clip applicator to target APCs in the epithelium and lamina propria of the mucosa. This method uniquely places vaccine within the mucosa using a pain-free method.

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