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Characterising the material properties at the interface between skin and a skin vaccination microprojection device

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ABSTRACT

The rapid emergence of micro-devices for biomedical applications over the past two decades has introduced new challenges for the materials used in the devices. Devices like microneedles and the Nanopatch, require sufficient strength to puncture skin often with sharp-slender micro-scale profiles, while maintaining mechanical integrity. For these technologies we sought to address two important questions: 1) On the scale at which the device operates, what forces are required to puncture the skin? And 2) What loads can the projections/microneedles withstand prior to failure. First, we used custom fabricated nanoindentation micro-probes to puncture skin at the micrometre scale, and show that puncture forces are ~0.25–1.75 mN for fresh mouse skin, in agreement with finite element simulations for our device. Then, we used two methods to perform strength tests of Nanopatch projections with varied aspect ratios. The first method used a nanoindenter to apply a force directly on the top or on the side of individual silicon projections (110 μm in length, 10 μm base radius), to measure the force of fracture. Our second method used an Instron to fracture full rows of projections and characterise a range of projection designs (with the method verified against previous nanoindentation experiments). Finally, we used Cryo-Scanning Electron Microscopy to visualise projections *in situ* in the skin to confirm the behaviour we quantified, qualitatively.

Statement of Significance

Micro-device development has proliferated in the past decade, including devices that interact with tissues for biomedical outcomes. The field of microneedles for vaccine delivery to skin has opened new material challenges both in understanding tissue material properties and device material. In this work we characterise both the biomaterial properties of skin and the material properties of our microprojection vaccine delivery device. This study directly measures the micro-scale puncture properties of skin, whilst demonstrating clearly how these relate to device design. This will be of strong interest to those in the field of biomedical microdevices. This includes work in the field of wearable and semi-implantable devices, which will require clear understanding of tissue behaviour and material characterisation.

1. Introduction

Micro-devices for biomedical use have in recent years received a lot of research attention, with many products advancing towards commercialisation and clinical trials [1]. One area with particular promise is devices that are used for vaccination – in particular microneedles or variants thereof. To date, these have been shown to have the capacity to perform enhanced efficacy vaccinations (in animals) due to their ability to precisely deliver vaccine adjacent to high densities of immune cells in the skin or mucosal tissue, requiring as little as 1/100th of the dose of a traditional vaccination

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route (intramuscular injection) [2,3]. The effective development of these poses some fresh and important material characterisation challenges. Specifically, it is important to know what force profile is required to position these devices within the skin to the desired penetration depth; and correspondingly, it is crucial to know what dimensional stability and mechanical integrity limits these microstructures possess.

Effective skin puncture is achieved with a highly localised stress; reported to be ~35 MPa [4] with quasi-static application. However, the skin is a complex bioviscoelastic material, with known strain-rate [5,6] and scale-effects [4,7,8] upon the mechanical properties. And so it follows that when microstructured devices are dynamically applied to the skin that they will be subjected to even greater stresses.

A further compounding factor is that the topography of skin makes it unlikely for all tips to enter the skin perpendicularly, introducing additional lateral forces on the microneedle structures. Finally, as the structures reach further into the skin they encounter a different range of skin layer material properties [8] and ultimately (in the dermis) a tight mesh of collagen that must be spread apart to puncture further. This means that there is not simply a single penetration event, but rather a progression of complex puncture events that include off-axis resultant forces.

Previous studies that have aimed to quantify the forces required to puncture skin generally make use of their single microneedles or arrays thereof [9]. However, the sharpness of the needles makes it hard to understand the puncture mechanism and to isolate the stress of failure [10]. For example, Park et al. [11] found that 1.4–3.5 N was required to insert microneedles of 700 μm to 1.5 mm in length. However, Davis et al. [9]. found that their microneedles of a similar scale required only 0.08–3.04 N for insertion. Other work that has applied patches to skin, including ours [5], has shown that a dynamic application is required for substantial puncture where an array of structures is used. Discerning skin's puncture force becomes difficult in these situations. Additionally, we have shown that skin's material behaviour is highly viscoelastic and scale-dependent [7], which means that studies with a single microneedle design/size cannot necessarily simply be translated for other (more practical) designs.

The challenge that is presented for microdevice design is to ensure that the strength of penetrating structures is sufficient for their use. But if the device is designed with excessive mechanical integrity (ie larger dimensions) then it may end up with a size that hinders its ability to penetrate skin – a problem exacerbated in high density array configurations such as with our Nanopatch (e.g. 20,000/cm^2 for a prototype for mouse use) [12]. Generally, the material testing that has been performed has been on large structures (~1 mm in length) which allow higher forces to be used and therefore simpler equipment setups [11,13,14]. Using these methods, a range of materials have been tested for microneedle designs that can withstand forces of 0.04–6.44 N [13,15]. As would be expected, for an equivalent geometry, a dissolving microneedle will have a substantially lower strength than one with a metallic or inorganic structure (silicon, glass).

Although our Nanopatch vaccination device has been used in a number of successful animal studies, we have not fully assessed either: a) its required strength to puncture skin; or b) the size of projection that is required to ensure sufficient strength for use. However, both are of critical importance for clinical translation and production scale-up, i.e. aiming for penetration in thicker human skin using an inexpensive manufacturing material. Within this paper we address both of these pieces of information, to provide a complimentary assessment of the device characteristics. First, we utilise nanoindentation using custom manufactured probes to identify the puncture forces that the tip of our projections (small microneedle structures) must reach in order to penetrate skin. This is particularly important on a scale that is unique from previous work in this area. We complement this with modelling of projection skin puncture to correlate the dynamic application of a Nanopatch device to skin with low speed nanoindentation testing. Following this, the second part of this paper is a set of mechanical tests on a range of Nanopatch projection geometries to assess their strength. We then compare these two aspects of our device and demonstrate using CryoSEM the capacity for these devices to puncture skin without fracturing.

2. Materials and methods

2.1. Manufacture of Nanopatches

Nanopatches were manufactured from silicon wafers using Deep Reactive Ion-Etching (DRIE) as published previously [16], at the Australian National Fabrication Facility – Queensland (ANFF-Q). To create projections of different sizes, the number of etching/-passivation loops was changed. Attempts were made to maintain a consistent height between patch types but this was not always possible. The projections were assessed for width, height and surface finish to ensure a range of suitable geometries were obtained. Observation and measurements were performed in a JEOL NeoScope, also at ANFF-Q, with any additional measurements being performed in ImageJ (NIH, Maryland, USA). During experiments with patches at least 5 patches were used for each experimental condition.

2.2. Manufacture and measurement of nanoindentation probes

For the nanoindentation experiments, two types of probe were required. The first type was that to be used for testing the puncture properties of skin. This required a defined frontal contact area and had to be similar in size to the tips of Nanopatch projections, to make any results scale-relevant. The method for manufacture of this probe is discussed in Crichton et al. [7]. Briefly, Omniprobes were purchased and Focussed Ion Beam (FIB) milling was used to re-shape the tip with either a 1 μm or 2 μm diameter (the same range as projection tips). The second type of probe was a 40 μm diameter probe that was also used in Crichton et al. [7], but this time an electropolished tungsten filament was used to create a blunter tip and then FIB was subsequently used to create a large, flat probe. This probe was used to indent directly onto projections using nanoindentation, with sufficient strength to avoid any material failure.

2.3. Experimentation for skin puncture force measurements

To measure the force/stress to puncture skin, the two small probes (1 μm and 2 μm diameter) were mounted in a custom nanoindenter probe mount, supplied by Hysitron (MN, USA). The Nanoindenter used was a Hysitron TI-900 Triboindenter and the probes were used with a Hi-Load transducer attachment. Indentations were performed at 100 μm/s, the maximum rate of the system. The chamber of the testing system was maintained dry with the presence of drybeads, and the machine was housed in an air-conditioned laboratory to maintain a constant humidity (25–30% RH) and temperature (25 °C) during testing.

Forces of failure were measured by the nanoindenter and it was desirable to be able to understand the local failure stress of the skin. To do this, the maximum stress, σ_{max}, was calculated using Hertzian contact theory [17], with Eq. (1).

$$\sigma_{max} = (1 - 2\nu)\frac{E_r}{\pi a} \quad (1)$$

where the value of Poisson's ratio (ν) was taken to be 0.45 based on our previous studies, the reduced modulus E_r was used for the cor-

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