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Early circulating biomarker detection using a wearable microprojection array skin patch

Jacob W. Coffey [a], Simon R. Corrie [a], Mark A.F. Kendall [a, b, *]

[a] Delivery of Drugs and Genes Group (D2G2), Australian Institute of Bioengineering and Nanotechnology, The University of Queensland, Brisbane, QLD 4072, Australia
[b] The University of Queensland Diamantina Institute, The University of Queensland, Brisbane, QLD 4072, Australia

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ABSTRACT

Microprojection array (MPA) skin patches selectively capture circulating biomarkers from the dermal layers of the skin, avoiding the need to extract, handle or process blood. Here we investigate the effect of improving biomarker capture *in vivo* on MPA detection of a model biomarker (antigen-specific-IgG raised in response to Fluvax vaccine) in a murine model. First, we investigate targeting MPA penetration to biomarker rich regions of the skin by varying MPA penetration depth. We observed a 4-fold increase in biomarker capture from predominantly epidermal to deep dermal penetration (27 ± 9 μm–153 ± 30 μm penetration range). We then study the kinetics of biomarker capture by varying the contact time with skin from rapid application (less than 20 min) to long term application (up to 24 h) with a wearable MPA patch. We observed MPAs reproducibly captured detectable amounts of our model biomarker after 10 min application and a greater than 6-fold increase in capture was observed up to 6 h application. Combining the effect of penetration depth and application time we obtained comparable early detection (after vaccination) of our model biomarker as a standard enzyme-linked immunosorbent assay (ELISA). We expect that integration of these devices with existing detection technologies has potential advantages in rapid diagnostic tests, particularly in cases where laboratory-based sample collection and processing is not available.

1. Introduction

Alternatives to the needle and syringe will simplify blood collection and processing required for diagnostic assays, and enable rapid minimally invasive diagnosis to be performed at the point-of-care or in the field. Blood is currently the most important clinical sample for diagnostic assays [1,2]. Traditionally whole blood is collected with a needle and syringe by an intravenous blood draw which then requires downstream processing to perform the actual diagnostic assay [3]. This is both time and resource inefficient, requiring the handling and transport of contaminated blood to centralised laboratories for processing. These limits are amplified in low resource settings where centralised laboratories and trained laboratory staff are not widely available [3,4]. Moreover, needles are invasive, providing a disincentive for testing and are therefore not well suited to repeated sampling [5,6]. Rapid diagnostic tests, which typically use a lancet to collect a small volume of blood for analysis on a lateral flow strip, avoid centralised processing, yet still cause pain and require removal of potentially infectious blood.

Microneedles (MN) and microprojection arrays (MPAs) have the potential to offer a solution to these sampling challenges. MN's and MPA's are arrays of hollow or solid projections, typically 50–1000 μm in length (the field of microneedles has been reviewed elsewhere [7]). Broadly, MN or MPA devices have previously been demonstrated to penetrate the tough outer layers of the skin [8–10] and deliver a variety of payloads (*e.g.* drugs [11] and vaccines, including conventional [12,13], DNA plasmid [14], and live-virus prime-boost candidate vaccines [15]) directly within the skin for a range of therapeutic benefits. In the field of diagnostics, hollow microneedles have been proposed to remove interstitial fluid (ISF) from skin for measurement of small analytes (*e.g.* glucose, lactate, and dissolved oxygen [16–20]). However, the use of any form of MN or MPA to capture specific disease biomarkers *in vivo*, for rapid minimally invasive disease diagnosis has yet to receive significant attention. Conceptually, selective capture of biomarkers to a solid surface *in vivo* reduces the

* Corresponding author. Delivery of Drugs and Genes Group (D2G2), Australian Institute of Bioengineering and Nanotechnology, The University of Queensland, Brisbane, QLD 4072, Australia.
E-mail address: m.kendall@uq.edu.au (M.A.F. Kendall).

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downstream effort to transport and process bulk biological fluids (*e.g.* blood) for diagnostic assays.

We propose that skin presents a unique pathway to selectively sample circulating disease markers in a minimally invasive manner. The outer most skin layer, the epidermis (approximately 50–100 μm in human skin [21]), consists predominantly of densely packed keratinocyte cells [22]. The epidermis is often sub-divided into the stratum corneum (SC; outer most sub-layer consisting of dead cells which prevent unregulated fluid loss with the environment), and the underlying viable epidermis (VE; which ensures homeostatic and reactive renewal of the SC). Beneath this is the dermis, which contains a dense organised network of blood vessels including capillaries, arterioles, venules [23,24]. The surrounding dermal tissue is predominantly composed of a cross-linked matrix of structural proteins (including collagen) surrounded by interstitial fluid (ISF) – with comparatively fewer cells (including fibroblasts and dendritic cells) – and other structures such as hair follicles, sweat glands and sebaceous glands [22]. The permeable walls of dermal capillaries facilitate exchange of some plasma proteins and other solutes from capillary blood with dermal ISF, through a combination of diffusion, convective flow, and endocytosis [25,26] – which varies depending on the size and physical properties of the solute [27,28] This 'plasma turnover' is accompanied by complementary drainage (to remove waste products) by the lymphatic system [29]. The skin therefore potentially contains a range of circulating biomarkers that are present in blood plasma, either confined to the microcirculation or present in interstitial tissue fluid through transport across capillary walls [2,27].

We recently demonstrated this diagnostic potential with an MPA skin patch that selectively captured antigen-specific-IgG and dengue NS1 protein from skin [30–32]. A schematic comparison of MPA biomarker capture with traditional blood collection using a needle is represented in Fig. 1. In these works selective protein capture reduced sample processing and altogether avoided the handling of body fluids. We expect our MPAs to be near pain free (or indeed pain free): as the projections only penetrate the outer skin layers and arrays of longer microneedles have been demonstrated to cause minimal pain in human patients [10,33]. Collectively, we have shown that MPAs successfully capture circulating markers from murine skin, however, the distribution and kinetics of circulation of biomarkers within the skin is not presently well understood.

It is not known how the mechanical engagement of MPA projections with the skin – which can be tuned to range in penetration (from the epidermis right down to the deep dermis) and application time (be held on the skin for seconds or hours) – influence the ability of the MPA projections to selectively extract biomarkers. The skin is a multilayer heterogeneous material and we hypothesise that the dermis contains the majority of circulating biomarkers in skin, and increasing the dermal penetration will result in high biomarker capture. Furthermore, MPA skin patches have the potential to continue to sample biomarkers 'from the source' (*i.e.* the skin and its capillaries) during application, without being limited by sample volume. We hypothesise that longer application will allow biomarkers to accumulate to the MPA surface *in vivo*, resulting in higher capture for the same concentration of marker in skin/serum. Increasing biomarker capture may lead to more sensitive early detection of biomarkers, which can lead to improved patient outcomes and infectious disease management for many disease scenarios [34,35].

With this in mind, in this study we systematically investigate the link between the mechanical engagement of MPAs to live skin and their ability to access biomarkers. We then evaluate the effect of improved biomarker capture on MPA diagnostic sensitivity (proportion of individuals with biomarker for which a positive test result is obtained). First, we determine the effect of MPA penetration depth on capture of a model circulating biomarker (antigen-specific-IgG) from skin. Next, we investigate the kinetics of biomarker capture by varying the contact time with skin from rapid application (1–20 min) to long term application (up to 24 h) with a wearable MPA patch. Our overall hypothesis is that increasing biomarker capture *in vivo* will improve MPA diagnostic sensitivity, enabling detection of less abundant markers early during their production which we test *in vivo* using our model.

2. Materials and methods

2.1. Fabrication and characterisation of microprojection arrays

MPAs were fabricated using a process of Deep Reactive Ion Etching (DRIE) described by Jenkins et al. [36] at the Rutherford Appleton Laboratory, Oxford, UK. Briefly, a mono-crystalline <100> silicon wafer was etched by a DRIE process followed by sputter coating firstly, with an intermediate layer of chrome (approximately 10 nm) and secondly, with a layer of gold (approximately 100 nm). The wafer was then diced into MPAs with a base area of 4 × 4 mm. MPAs contained a square array of projections with a density of 20,408 cm^{-2}. The size and geometry of projections was varied in the production process while a constant distance between the centres of adjacent projections (70 μm) was maintained between different MPAs. A JEOL-500 scanning electron microscope was used to characterize the MPAs.

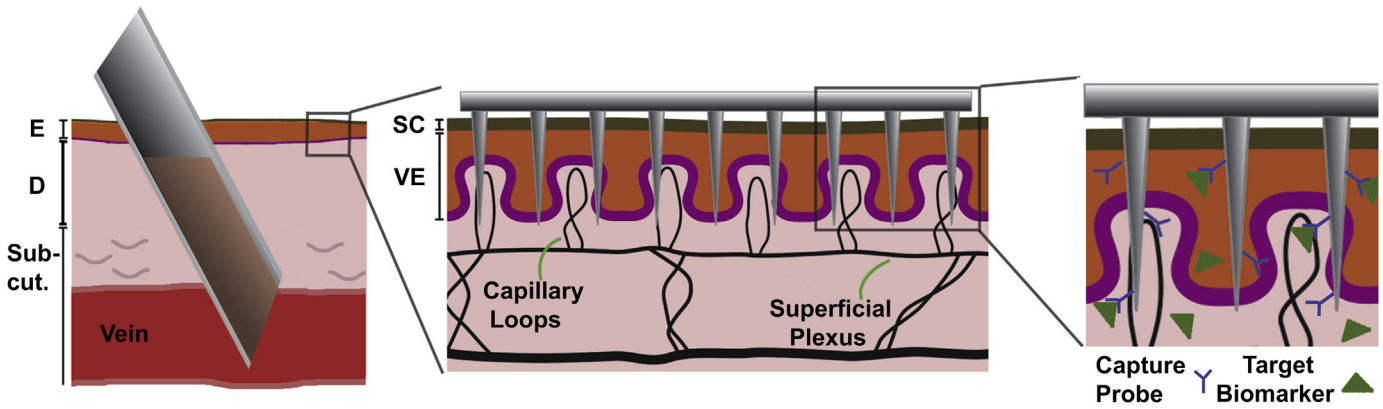

Fig. 1. Simplified schematic comparing a conventional blood draw with a needle and syringe to selective biomarker capture using an MPA skin patch (dimensions are indicative only). Left Panel: Needles must penetrate through the skin to access more deeply situated veins and withdraw unprocessed blood, Middle Panel: MPAs are engineered to penetrate through the outer skin layers only, enabling contact with biomarker containing skin fluid in the dermis (ISF or blood from capillary vessels), Right Panel: MPAs are functionalised to selectively bind only target markers from skin fluid with molecular specificity (no fluid is removed from skin).