British Journal of Pharmacy

https://doi.org/10.5920/bjpharm.1164

British Journal of Pharmacy

www.bjpharm.hud.ac.uk

Proceedings of the 13th APS International PharmSci 2022

A Microneedle-Microplate Platform to Detect Biomarkers in Skin

Rachael Victoria Dixon[a, b], Wing Man Lau[a, b], Keng Wooi Ng[a, b]*

[a]School of Pharmacy, Faculty of Medical Sciences, Newcastle University, UK, [b]Translational and Clinical Research Institute, Faculty of Medical Sciences, Newcastle University, UK.

ARTICLE INFO

Received: 16/06/2022
Accepted: 07/07/2022
Published: 04/11/2022

*Corresponding author.
Tel.: +99 1912082343
E-mail:
keng.ng @newcastle.ac.uk

KEYWORDS: Microneedle Array (MNA); Point-of-Care Testing (POCT); Diagnostics; ELISA.

SUMMARY

This research aimed to integrate an established signal quantification technique with immunocapture microneedle arrays (MNAs) to improve biomarker-based disease diagnostics in the skin. The miniaturized sandwich enzyme-linked immunosorbent assay (ELISA) captured endogenous porcine immunoglobulin G (IgG) direct from porcine skin. Immunocapture MNAs were incubated in o-phenylenediamine (OPD) solution in a 384-well microplate. Absorbance was measured using a microplate spectrophotometer at 450 nm. This technique allows for rapid biomarker detection with high-throughput processing. Immunocapture microneedle devices such as these can be easily adapted for targeting different biomarkers or multiplexed leaving plenty of scope for future work and assay optimisation.

INTRODUCTION

The recent COVID-19 and Ebola outbreaks have emphasized the need for sensitive and rapid disease diagnosis using point-of-care testing (POCT). Immunocapture microneedle arrays (MNAs), are an emerging technology with the potential to fill this gap in global healthcare. The MNAs comprise small (≤ 1 mm long), immuno-functionalised projections (i.e. microneedles) protruding from a solid base (Dixon *et al*, 2021). Such microneedles can be inserted effortlessly and painlessly (Kaushik *et al*, 2001; Haq *et al*, 2009) into the skin to selectively capture biomarkers from the dermal interstitial fluid (Dixon *et al*, 2020). This technology could vastly accelerate and improve disease diagnosis, but their development and adoption have been impeded by the lack of a facile, low-cost detection technique for large-scale clinical deployment. This research aimed to integrate an immunocapture MNA platform with established off-the-shelf microplate spectrophotometry to demonstrate selective biomarker detection in skin.

MATERIALS AND METHODS

Polylactic acid (PLA) was from MakerBot Industries (Brooklyn, USA). Full-thickness porcine skin recovered from animal by-products were kindly donated by NU Farms (Newcastle, UK), frozen within 3 hours of extraction and thawed immediately prior to use. Uncoated 384-well microplates were from VWR (Lutterworth, UK). The matched immunoglobulin G (IgG) antibody pair was from antibodies.com (Cambridge, UK). All other reagents were purchased from Sigma Aldrich (Dorset, UK).

Vacuum-moulded PLA MNAs were surface-functionalised as described previously (Ng *et al*, 2015). The microneedles were designed to spatially align with the wells in a 384-well microplate (Figure 1). Porcine IgG-specific capture antibodies were immobilised on the microneedle surfaces as a miniature sandwich enzyme-linked immunosorbent assay (ELISA) platform. Following blocking with 5% w/v bovine serum albumin (BSA), the MNAs were pressed into porcine skin and remained in situ

https://doi.org/10.5920/bjpharm.1164

for 1 hour to capture IgG from the dermal interstitial fluid. As experimental controls, MNAs were incubated in (a) 50 ng/mL IgG solution (positive control) or (b) 1% w/v BSA solution (negative control).

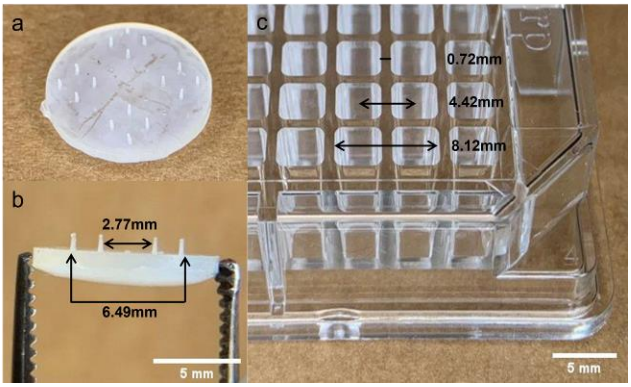

Fig. 1. *Modified immunocapture MNA designed to spatially align with the wells of a 384-well microplate. Each 2 x 2 cluster of microneedles fit into one well on the microplate.*

The MNAs were then incubated with a horseradish peroxidase (HRP)-conjugated detection antibody, washed, dried and placed on a 384-well microplate. The microneedles were inserted into wells filled with o-phenylenediamine (OPD) solution. After a 45-minute incubation, the MNAs were removed from the microplate and the developed signal was measured at 450 nm in a microplate spectrophotometer (Multiskan FC, Thermo Scientific).

RESULTS AND DISCUSSION

Using the integrated microneedle-microplate platform, endogenous IgG was detected in the excised porcine skin with a good signal-to-noise ratio (Figure 2).

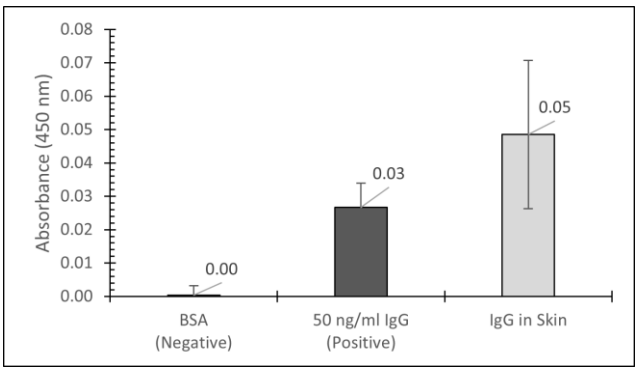

Fig. 2. *Colorimetric signal quantification of endogenous IgG from porcine skin. Error bars represent n=4*

This was corroborated by the visible colour change in the OPD solution in the microplate (not depicted). The higher absorbance in the test microneedle array compared to the positive control suggests that the IgG concentration in the skin sample was >50 ng/mL, although hydrodynamic differences in the skin matrix and the control solution may affect IgG capture and complicate the interpretation.

CONCLUSIONS

The microneedle-microplate platform successfully detected endogenous IgG in excised porcine skin. However, additional assay optimisation is required to further enhance the absorbance values, and thus the sensitivity of the assay, and confidence in its interpretation. The results demonstrate the potential of immunocapture MNAs as a simple, rapid, low-cost POCT platform when integrated with existing high-throughput analytical systems. This integrated system also allows for multiplexing as each cluster of microneedles on the same MNA could be coated with different antibodies to target different biomarkers simultaneously.

ACKNOWLEDGEMENTS

This work was supported by the Faculty of Medical Sciences, Newcastle University, UK. The authors have no conflicts of interest to declare.

REFERENCES

Dixon, R.V., Lau, W.M., Moghimi, S.M., Ng, K.W., 2020. The diagnostic potential of microneedles in infectious diseases. Prec. Nanomed., 3, 629-640.

Dixon, R.V., Skaria, E., Lau, W.M., Manning, P., Birch-Machin, M.A., Moghimi, S.M., Ng, K.W., 2021. Microneedle-based devices for point-of-care infectious disease diagnostics. Acta Pharm. Sin. B., 11, 3244-2361.

Haq, M.I., Smith, E., John, D.N., Kalavala, M., Edwards, C., Anstey, A., Morrissey, A., Birchall, J.C., 2009. Clinical administration of microneedles: skin puncture, pain and sensation. Biomed. Microdevices, 11, 35-47.

Kaushik, S., Hord, A.H, Denson, D.D., McAllister, D.V., Smitra, S., Allen, M.G., Prausnitz, M.R., 2001. Lack of pain associated with microfabricated microneedles. Anesth. Analg., 92, 502-504.

Ng, K.W., Lau, W.M., Williams, A.C., 2015. Towards pain-free diagnosis of skin diseases through multiplexed microneedles: biomarker extraction and detection using a highly sensitive blotting method. Drug. Deliv. Transl. Res., 5, 387-396.