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Microvascular effects of microneedles with subsequent histamin application in the skin prick test

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Abstract

Background: Since decades, histamin applications are routinely performed in skin prick tests using a lancet. However, this technique is associated with various drawbacks.

Materials and Methods: In healthy human subjects, we investigated the effects of microneedle-enhanced histamin delivery (wheal size, erythema size) in the skin microvasculature using polarized light spectroscopy imaging (Tissue Viability imaging, TiVi). Histamin was applied on microneedle-pretreated skin or on -untreated skin, to assess the microvascular response in the local skin.

Results: In our results, histamin was delivered more rapidly into the skin after microneedle pretreatment compared to passive diffusion, visible as wheal and erythema.

Conclusion: The here presented technique might be useful for a personalized drug-testing system in the future.

KEYWORDS

histamin, microneedles, skin prick test, TiVi imaging

1 | INTRODUCTION

Histamin applications into the skin are usually performed as a positive skin test control worldwide in thousands of healthy and diseased persons. Histamin skin prick results are usually visible as wheal formation and skin redness. However, differences in this manual technique between personal causes variations in the wheal size and the interpretation of these sizes are differently performed.

Transdermal drug delivery (TDD) is frequently used for vaccination studies or diagnosis because conventional stainless steel needles may be associated with fear and discomfort, especially among children.¹ In addition, the needed drug doses are significantly lower

compared to oral medication and conventional needles, making TDD applications cheaper.²

A lot of useful applications of microneedles have already been demonstrated, including the use of microneedles as delivery systems for vaccines,^{1,2} skin allergy test devices,^{3,4} for stimulating angiogenesis by electrical signals and growth factors, for applications of immunobiologicals,⁵ biopharmaceuticals,⁶ drug,⁷ for diagnosis purposes,⁸ or as cosmetic products.⁹ However, the direct and prolonged effects of microneedle application on the local microcirculation have not been studied extensively.

The aim of this study was to assess microvascular effects of microneedles in the skin to assess the microvascular effects of histamin delivered by microneedle-produced pores.

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2 | MATERIAL AND METHODS

2.1 | Subjects

Seven healthy volunteers without known skin or vascular disease or use of medication (except oral contraceptives) were recruited to the study and gave their informed consent to participate. The participants were asked to refrain from drinking caffeine or tea, use nicotine products, and eating for two hours prior to the experiments.

2.2 | Study protocol

All measurements were performed in a temperature-controlled room at $21 \pm 1^\circ\text{C}$. All participants were in a semi-supine position in a bed for at least 10 minutes before the measurements started. All measurements were done on the forearms which were disinfected with a skin cleaner (Klorhexidin 5 mg/mL, Fresenius Kabi AB) before the experiments. Stainless steel microneedles arrays were used in all experiments (2 rows $\times$ 8 microneedles, 750 μm lengths (Dermaroller GmbH).

2.3 | Equipment

The microvascular response was quantified by Tissue Viability imaging (TiVi).¹⁰ Briefly, TiVi is based on a digital camera and two filters (polarizers). Outgoing light from the camera flash passes the first filter producing linear-polarized light. A part of this light will penetrate the skin and become randomly scattered (depolarized). Part of the backscattered, randomly polarized light will pass the second filter placed in front of the camera lens. RBCs absorb light in a range of 500 - 600 nm (the green wavelength region) to a much higher extent than light in a range of 600 - 700 nm (the red wavelength region), in comparison to the surrounding tissue, which absorbs red and green light at approximately the same amount.¹¹ These differences in absorption can be determined and calculated into a measure of RBC concentration (C_{RBC}). The polarized digital TiVi camera was placed 20-30 cm above the skin. Ambient light was turned off during all measurements. The images were reduced in size and cropped into the area of interest using image processing software (ImageJ, US National Institutes of Health, Maryland, USA) and converted into numerical data using the software provided by the TiVi system (TiVi 700 1.1 WheelsBridge AB).

2.3.1 | Assessment of histamin uptake upon microneedle treatment

Seven subjects (5 male), 23-40 years (mean $\pm$ SD, 25.4 ± 6.4 years), and BMI 19-26.2 (mean $\pm$ SD, 22.8 ± 2.4) were recruited. Blood pressures were measured before the experiments (mean $\pm$ SD, systolic 118 ± 11.1 ; diastolic 73 ± 8.9).

750 μm microneedles were gently applied on the forearms for 60 seconds with a weight of 100 g. Histamin (Soluprick positive control, Alk, Horsholm, Denmark) was dropped immediately after needle application upon the microneedle-treated skin and on untreated skin and contained in by plastic rings mounted to the skin by double adhesive tape. The drug was removed after 10 minutes followed by a 5-min skin recovery period. C_{RBC} was measured every 10 s during the whole experiment using the TiVi camera. Mean C_{RBC} was calculated inside the rings and at different distances from the center of the rings. The sizes of the wheals and the sizes of the skin redness were assessed using the LUCIA G software version 4.80 (Optoteam GmbH).

2.4 | Data analysis

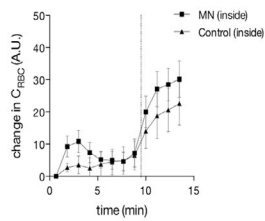
Data are presented as mean $\pm$ SD or as mean $\pm$ SEM, if not stated otherwise. Statistical analysis was further performed using a 2-tailed (no priori assumption), paired Student's t test (comparing means from the same group of subjects, before and after treatment). $P < .05$ was considered as statistically significant. All statistical analysis was calculated using GraphPad Prism version 6.00 for Windows (GraphPad Software, San Diego California USA, "www.graphpad.com").

3 | RESULTS

3.1 | Assessment of histamin uptake upon microneedle treatment

When histamin was applied on the skin areas that were pre-treated with microneedles, an immediate increase in C_{RBC} was observed, suggesting a more rapid drug uptake in the skin vasculature compared to untreated skin. A maximum increase in C_{RBC} was observed 3 minutes after histamin application, followed by a decrease (wheal formation). After removing the solution after 10 minutes, a further increase in C_{RBC} was observed, and a plateau in C_{RBC} response was reached after 15 minutes. Outside the containing ring, a similar increase in C_{RBC} was observed, which progressed during the following 15 minutes without reaching an apparent plateau. The C_{RBC} response was strongest at 1 cm outside the containing ring and decreased with increasing distance from the histamin application site. Visually, an immediate increase in skin redness (erythema) could be observed, which was visible up to 3 cm circumferential to the holding ring (Figure 1A). The sizes of the wheal formation were calculated as $48 \pm 4 \text{ mm}^2$. The sizes of the skin reaction (indicated as erythema) were calculated as $360 \pm 140 \text{ mm}^2$. On intact skin, no wheal formation was observed (Figure 1B). A montage of the images showing the development of the response over time is depicted in Figure 1C. A supplemental video of a montage of the histamin application is provided (supplemental Video S1).

(A) histamin application



(B) after 15 minutes



(C) time series montage

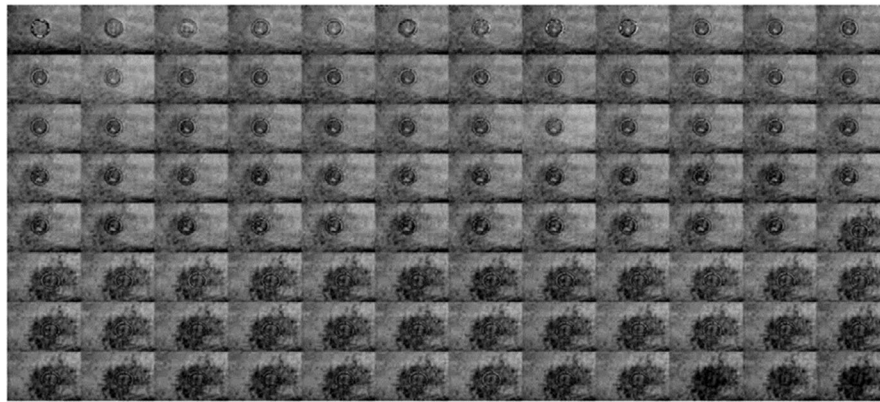


FIGURE 1 Effects of microneedle-enhanced histamin delivery in the skin microvasculature: (A) Microvascular response in the skin to topical application of histamin, after pretreatment of the skin with a microneedle array and on untreated control site (control). The left pane shows the change in C_{RBC} inside the ring containing the histamin solution. The right pane shows the change in C_{RBC} at different distances outside the containing ring. A wheal formation and an increased skin redness can be observed upon microneedle treatment, but not on intact skin; mean $\pm$ SEM $n = 7$). (B) image after 15 minutes. (C) A time series of images acquired during 16 minutes (10 s intervals) after application of histamin on microneedles-pretreated skin. After 1 minute, a redness can be clearly distinguished, later developing into a wheal formation [Colour figure can be viewed at wileyonlinelibrary.com]

4 | DISCUSSION

4.1 | Assessment of histamin uptake upon microneedle treatment

All participants described the microneedle applications as painless, which is consistent with previous findings.^{5,12} All participants described the histamin application on microneedle-pretreated skin as itchy; however, this effect faded away after a few minutes post-application, as reported in literature.¹³ No complaints were reported from all participants.

The results showed that microneedles form micropores into the epidermis which enable a rapid delivery of substances to the skin microvasculature by passive diffusion. Histamin was able to spread out in the epidermis and initiate vascular responses like (a) dilatation of capillaries, (b) a wheal formation at the site of skin rupture, and (c) a widespread dilatation of the surrounding arterioles by local reflex action, visible as red flare (erythema). Similar results were shown by Haridass et al after microneedle and histamin application in a pilot clinical case study in six participants.¹³ In this study, 250 μ m microneedles (Nanopatch[®]) were used, which affects the immune response from different cell types and in different skin layers. On the one hand, rather consistent local wheal formation sizes at the site where the microchannels were formed ($\pm 9.7\%$) and on the other hand, rather different sprouting red flare sizes ranging from 224 mm^2 up to 592 mm^2 ($\pm 33.4\%$) were detected between individuals. Although these findings are well studied, quantifying these effects in the skin is still not standardized.¹⁴

Interpreting and comparing current skin prick tests are difficult due to various application methods used by different operators.¹⁴⁻¹⁶

Currently, the wheal size is measured manually in most cases, by for example, measuring the maximal and minimal diameter of the wheal, which does not represent the exact size of the wheal.¹⁵ Other skin prick protocols measure the longest extent of the wheal and the extent 90° to the first measurement. Hence, both procedures record an estimated size of the wheal, not the exact size. TiVi methods can record the exact size and the shape of the wheal and the surrounding erythema. This addition of information makes comparisons between individuals, but also between different time points more adequate to numerical data only.

Moreover, TiVi is known to distinguish between specific wavelengths which make this procedure more sensitive and also more selective, compared to subjective manual measurements. Third, the trauma to the skin to apply allergens in a skin test is done manually by different lancet types. Therefore, standardized microneedle application could provide a new standardized procedure for allergy testing in the skin.

5 | CONCLUSION

5.1 | Assessment of histamin uptake upon microneedle treatment

These experiments disclosed that microneedles are able to form micro channels in the skin tissue and enable microvascular responses due to passive diffusion.

From a clinical point of view, this technique has shown to be painless, safe, and easy to apply. Therefore, it may become the technological basis for a smart personalized drug-testing method in the future.

CONFLICT OF INTEREST

The authors declare on conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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