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Microvascular effects of microneedles application

Johannes Hackethal[1,2], Fredrik Iredahl[3,4], Joakim Henricson[5,6], Chris D. Anderson[6,7], Erik Tesselaar[9]

1 Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, Vienna, Austria

2 Austrian Cluster for Tissue Regeneration, Vienna, Austria

3 Department of Health, Medicine and Caring Sciences, Linköping University, Division of Community Medicine, Linköping, Sweden

4 Department of Primary health care, Region Östergötland, Linköping, Sweden

5 Department of Emergency Medicine, Local Health Care Services in Central Östergötland, Region Östergötland, Linköping, Sweden

6 Department of Biomedical and Clinical Sciences, Division of Cell Biology, Linköping University, Linköping, Sweden

7 Department of Dermatology and Venerology, Heart and Medicine Centre, Region Östergötland, Linköping, Sweden

8 Department of Radiation Physics and Department of Clinical and Experimental Medicine, Linköping University, Linköping, Sweden.

Corresponding author:

Johannes Hackethal, PhD

Adress: Donaueschingenstrasse 13, 1200 Vienna, Austria

Mail: Johannes.Hackethal@trauma.lbg.ac.at

Telephone: +43 (0) 5 93 93-41248

This work was performed at the Department of Hand and Plastic surgery, Burns and Intensive care and Department of Clinical and Experimental Medicine, Linköping University, 58185 Linköping, Sweden.

Running head: Effects of microneedles on skin microcirculation

Abstract

Background

The efficiency of transdermal drug delivery may be increased by pretreating the skin with microneedles, but distinct effects of microneedles and the microneedle-enhanced delivery of vasoactive drugs on the skin microvasculature are still not well investigated.

Materials and Methods

In eight healthy human subjects, we measured the microvascular response to microneedle-induced microtraumas in the skin microvasculature using polarized light spectroscopy imaging (Tissue Viability imaging, TiVi). The microvascular response was assessed for up to 48 hours for three microneedle sizes (300 µm, 500 µm, and 750 µm) and for different pressures and application times.

Results

In our results, microneedle application increased the local red blood cell (RBC) concentration for up to 24 hours dependent on the needle lengths, applied time and force.

Conclusion

Optimization of microneedles size, pressure and application time should be taken into account when future protocols for drug delivery as well as experimental provocations.

Keywords: microneedles, TiVi imaging, skin provocation

Introduction

The human skin forms an important biological barrier that protects against external bacterial access and everyday mechanical damage, meanwhile preventing internal loss of water and other molecules. In the 1970's, the microneedle technique was introduced to enhance transdermal drug delivery (TDD). When pressed onto the human skin, microneedles penetrate the epidermis and parts of the dermis and form microchannels. During the past decades, TDD has become a popular technique to apply drugs over the skin barrier,[1,2] because it is an easy-to-perform method to deliver drugs dermally - neither passing the highly enzymatically active gastrointestinal tract nor undergoing the first pass effect in the liver.[3–5] TDD is frequently used for vaccination studies or diagnosis because conventional stainless-steel needles may be associated with fear and discomfort, especially among children.[6] In addition, the needed drug doses are significantly lower compared to oral medication and conventional needles, making TDD applications cheaper.[7]

In general, microneedles can be divided into three main categories: solid, degradable and hollow. Solid microneedles can be metal or polymer made. Stainless steel microneedles have sufficient mechanical strength, are easy to form and FDA approved. Degradable microneedles consist of polymers, are inexpensive, biocompatible and in case of breakage they will be eliminated by the body.[3,8]

Currently used microneedles typically vary in length between 50-900 µm.[3,8] However, for effective piercing of the epidermis, the minimally required needle length is generally considered to be at least 300 µm.[9] An increase in needle numbers does not increase pain where as an increase in needle length above 900 µm may significantly increase pain, because the needles may stimulate the local sensory nerves.[10,11] Immune and micro trauma responses in the skin induce both transepidermal water loss and erythema,[9] which correlates to the inflammatory response of the damaged microtissue and the applied drugs.[12]

By forming micropores through the epidermis, a therapeutic window can be produced, as the local microtraumas start to induce a transdermal response (e.g. by keratinocytes, 95% of all cells in the epidermis). These cells activate a cascade of inflammatory response including cytokines (mainly Il-1α, but also IL-6, IL-8, granulocyte-macrophage colony stimulatory factor and TNF-α). A reactive vasodilatation, which increases the red blood cell (RBC) concentration, enables a cell infiltration towards the epidermis to initiate the final restoration of the skin barrier function.[9,12]

A lot of useful applications of microneedles have already been demonstrated, including the use of microneedles as delivery systems for vaccines,[6,7] as skin allergy test devices,[13,14] for stimulating

angiogenesis by electrical signals and growth factors, for applications of immunobiologicals,[4] biopharmaceuticals,[15] drugs,[5] and for diagnosis purposes,[16] or as cosmetic products.[3] 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 objectively measure microvascular effects of microneedles in the skin by different needle lengths, durations and weights using polarized light spectroscopy imaging.

Material and Methods

Subjects

Eight healthy volunteers (4 male), 21-37 years (mean ± SD, 24.9 ± 6.1 years) and Body Mass Index (BMI) 19-26.3 (mean ± SD, 22 ± 2.8) were recruited. The subjects had no known skin or vascular disease or use of medications (except oral contraceptives) and gave their informed consent to participate. Blood pressures were measured before the experiments (mean ± SD, systolic 115 ± 8.5; diastolic 71 ± 6.8). The participants were asked to refrain from drinking caffeine or tea, use nicotine products and eating for two hours prior to the experiments. All experiments were approved by the regional ethics review board at Linköping University, Sweden.

Study protocol

All measurements were performed in a temperature-controlled room at 21 ± 1°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, Uppsala, Sweden) before the experiments. Stainless steel microneedles arrays were used in all experiments (2 rows à 8 microneedles, 300 µm, 500 µm and 750 µm lengths (Dermaroller GmbH, Wolfenbuettel, Germany).

Microneedles with a length of 300 µm, 500 µm and 750 µm were gently applied on the forearms using a weight of 100 g or 500 g for 10 or 60 seconds. TiVi images were acquired every 10 seconds in the first 5 minutes and every 60 seconds for the next 55 minutes. Additional images were acquired after 6, 12, 24 and 48 hours.

Equipment

The microvascular response was quantified by Tissue Viability imaging (TiVi).[17] 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.[18] 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, Linköping, Sweden).

Data analysis

Data is presented as mean ± SD or as mean ± 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). The microneedle time series tests were further analyzed using 2-way ANOVA with multiple comparisons. $P < 0.05$ 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”).

Results

During the first 60 min after application of microneedles to the skin, a significant increase in C_{RBC} was observed for all needle lengths, application weights and durations (Figure 1 - A). This increase in C_{RBC} was visible as local erythema, at the sites where the needles penetrated the epidermis. Mean C_{RBC} increased with the length of the needles, independent of duration or weight (Table 1). Significant differences in C_{RBC} were observed between 300 µm and 750 µm, but no other groups. Microneedle application of 500 g and 10 s duration increased C_{RBC} more than the needle application using 100 g weight and 60 s duration. Microneedle application of 500 g and 60 s showed the highest absolute C_{RBC} increase, still visible after 60 minutes, and therefore, a time series up to 48 hours was performed (Figure 1 – B and C). A significant increase in C_{RBC} after 24 h was observed with the longest needles (750 µm)

applied with 500 g for 60 s (p=0.047). Shorter needles and less weight and duration resulted in a faster return to the baseline of the C_{RBC} (Table 2). After 48 hours, no significant increase in C_{RBC} was observed in any of the experiments.C

Discussion

The main finding of this study is that the application of microneedles to skin of the forearm results in immediate vasodilatation and this vasodilatory response depends on the needle length, application weight and duration. We observed a substantial variation in maximum response between subjects. This variability in microvascular response may be caused by individual variations in vascular reactivity and circadian variations due to different measuring time points (between 7 a.m. and 9 p.m).[17]

All participants described the microneedle applications as painless, which is consistent with previous findings.[9,4]

This experiment showed that longer needles increased the C_{RBC} response, which was also described by other studies,[11,19] due to the fact that more microvascular tissue is damaged, leading to an elevated immunological responses by keratinocytes,[9] Langerhans cells and an accumulation of red blood cells.[17]

This study also showed that an increase of applied mass on the skin can elevate the concentration of RBC more than an increase of application time: 500 g weight for only 10 s application time showed higher C_{RBC} responses when compared to 100 g weight for 60 s application time. Similar results were also shown by Noh et al., where microneedle applications showed the same extent of skin redness after application on the forearm, as measured using reflectance spectrophotometry, regardless if applied for 2 or 240 min. [20]

In the 48-hour tests, 300 µm needle lengths provoked an increase in C_{RBC} up to 6 hours when applied for 60 s and 500 g, but when using lower mass or duration, erythema was not detectable any more after 6 hours. A significantly increased C_{RBC} concentration was observed up to 24 hours for the longest needles applied with 500 g for 60 minutes.

Bal et al.,[9] Haq et al.[21] and Han et al.[12] showed that the formation of erythema recovered after 48 h latest for microneedle sizes shorter than 300 µm, but they used different microneedle shapes. Since needle shape is known to be an important parameter in barrier disruption[9], the results of these studies cannot easily be compared to the findings in this study. Also, in contrast with our study, the previous

studies used laser Doppler flowmetry or a reflectance spectrometry to detect skin responses, which may also have different detection properties than TiVi imaging.

Conclusion

The results of the current study show that the microvascular skin reactions caused by microneedle application can be measured reliably and practically using polarized light spectroscopy (TiVi). The findings confirm previous findings that the extent of the microvascular response is dependent on both the needle length and the weight at which the needle arrays are applied.

From a clinical point of view, microneedles of 750 µm length, although painless during application, cause an elevated red blood cell concentration for up to 24 hours.

Conflict of interest

The authors declare on conflicts of interest.

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Figure 1: Microneedle-induced effects in skin vasculature. **(A)** 1-hour time series: The RBC concentration (shown as C_{RBC}) was assessed using TIVI (Tissue Viability images) for 60 minutes, after applying different needle lengths (300 µm, 500 µm, 750 µm) and different weights and durations (mean ± SEM, n=8). **(B)** 48-hour time series**. (C)** Representative images of the 48-hour time series.

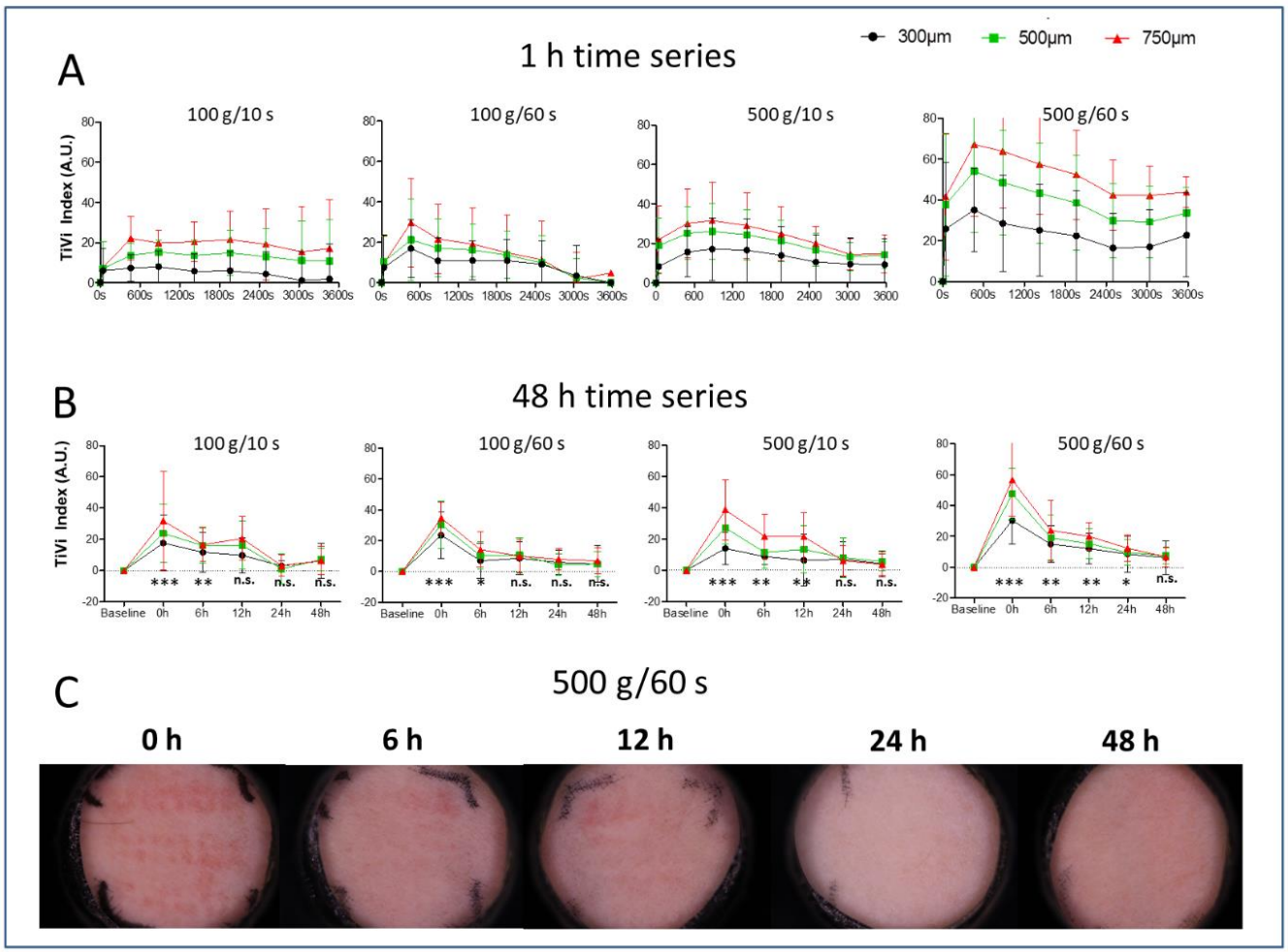

Table 1: Maximum C_{RBC} for all different needle lengths and combinations of weight and duration (n=8, mean ± SD).

Maximum responses for different needle lengths and combinations of weight and duration (n=8)			
times	750 µm	500 µm	300 µm
100g/10s	28.2 ± 14.0	21.7 ± 9.0	13.1 ± 8.2**
100g/60s	28.7 ± 17.3	24.3 ± 13.8	20.7 ± 14.5
500g/10s	38.4 ± 18.7	35.6 ± 13.5	25.6 ± 19.9*
500g/60s	81.8 ± 29.0	70.9 ± 30.3	50.7 ± 31.1***

Significant difference in C_{RBC} increase compared to 750 µm, * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Table 2: Significance of increase in C_{RBC} for different durations and weights compared to baseline during the first 48 hours after microneedle application.

Column stats analysis (2way ANOVA with multiple comparisons vs. Baseline [0], p=0.05)						
Time[h]	length	max	6h	12h	24h	48h
100g/10s	750 µm	<0.001	n.s	<0.001	n.s	n.s
	500 µm	<0.001	0.005	0.004	n.s	n.s
	300 µm	0.001	n.s	n.s	n.s	n.s
100g/60s	750 µm	<0.001	0.015	n.s	n.s	n.s
	500 µm	<0.001	n.s	n.s	n.s	n.s
	300 µm	<0.001	n.s	n.s	n.s	n.s
500g/10s	750 µm	<0.001	<0.001	<0.001	n.s	n.s
	500 µm	<0.001	n.s	0.03	n.s	n.s
	300 µm	0.02	n.s	n.s	n.s	n.s
500g/60s	750 µm	<0.001	<0.001	<0.001	0.047	n.s
	500 µm	<0.001	<0.001	0.008	n.s	n.s
	300 µm	<0.001	0.009	n.s	n.s	n.s