Fractional Transepidermal Delivery: A Histological Analysis

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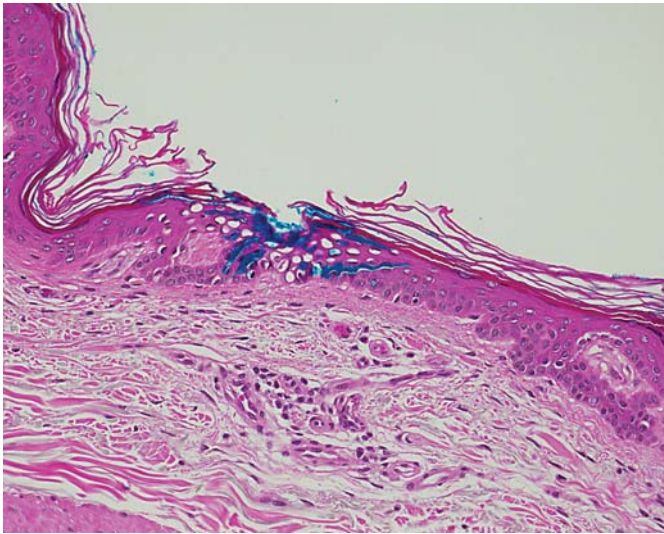
Abstract

Background: In autologous cell therapy, e.g. in melanocyte transplantation for vitiligo, a minimally invasive mode of transepidermal delivery of the isolated cells is of crucial importance to reduce potential side effects such as infections and scarring as well as to minimize the duration of sick leave. *Objectives:* To compare the characteristics of the microscopic treatment zones induced by ablative fractional CO_2 laser and by microneedle treatment in ex vivo human breast skin. *Results:* Ablative fractional CO_2 laser treatment resulted in superficial, mainly epidermal defects reaching at most the upper papillary dermis (0.1–0.3 mm), covered by a thin eschar and coated by a small zone of collagen denaturation. Tissue injury characteristics depended on spot size as well as the energy delivered. Microneedle treatment led to thin vertical skin fissures, reaching the middermis (up to 0.5 mm) and injuring dermal blood vessels, but without surrounding tissue necrosis. *Conclusions:* Both technologies are able to create small epidermal defects which allow to deliver isolated cells such as melanocytes to an epidermodermal site, with microneedle treatment having the advantage of lacking devitalized tissue and eventually enabling vascular access for the transplanted cells.

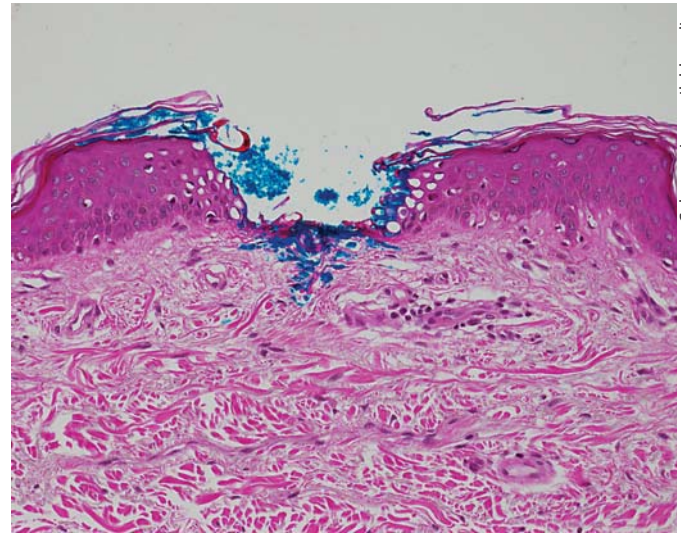Copyright © 2011 S. Karger AG, Basel

Introduction

The best method to transepidermally deliver isolated cells, i.e. in melanocyte transplantation in patients with vitiligo, is not yet defined. Usually, large-scale de-epidermization by ablative lasers or dermabrasion is used [1–3]. These methods have several disadvantages, i.e. (1) difficulties in stable application of cell solutions needing additional fibrin glue or sophisticated dressing technologies, (2) depending on the surface area treated relevant sick leave, (3) risk of infection and (4) scarring [2]. Other technologies include 'intraepidermal' injection, which is cumbersome for large areas [4]. For the transepidermal delivery of drugs various 'skin-breaching' modalities have previously been discussed in the literature [5]. To deliver single cells in physiological solutions aiming at a definitive take, the skin has to be 'opened' at least down to the epidermodermal junction, with defects wide enough to enable the passage of cells such as melanocytes, i.e. around 10–20 µm in size. Efficient delivery of the cells depends on many factors, e.g. the cell number, the medium in which the cells are suspended (viscosity, ingredients to keep the cells viable), density and distribution of the skin defects, retraction phenomena of the skin after injury as well as the time between wounding and application. In the course of a proof of concept to transplant autologous outer root sheath melanocytes [6], we compared two fractional de-epidermization

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Fig. 1. Ablative fractional CO_2 laser (spot size 120 μm, power 5 W, pulse energy 60 mJ, spot density 151 MTZ/cm^2): focal epidermal necrosis without dermal injury, no penetration of blue dye into the dermis.

Fig. 2. Ablative fractional CO_2 laser (spot size 120 μm, power 10 W, pulse energy 60 mJ, spot density 151 MTZ/cm^2): full-thickness epidermal necrosis with tissue injury down to the superficial dermis (width 200 μm, depth 100 μm), focal penetration of blue dye into the papillary dermis.

modalities in ex vivo human skin samples: ablative fractional CO_2 laser and microneedle treatment. Thereby tissue injury characteristics were analyzed histologically.

Materials and Methods

Freshly excised human skin (n = 17) from breast reduction surgery in a 22-year-old Caucasian female was fixed in formalin 4% for at least 48 h and then exposed to a fractional CO_2 laser (n = 12) or treated with microneedle application (n = 5) in order to investigate their effect on ex vivo human skin.

The eCO$_2$™ laser (Lutronic Inc., San Jose, Calif., USA) was used in different settings, i.e. varying spot size (diameter of 120 vs. 1,000 μm), pulse energy (40, 60 mJ), power (5, 10 and 20 W) and spot density [51 vs. 151 microscopic treatment zones (MTZ)/cm^2], resulting in 12 treatment modalities. The CIT8 Model Dermaroller® (Dermaroller GmbH, Wolfenbuettel, Germany) used in this study is a drum-shaped roller studded with 192 fine microneedles in 8 rows, each 1 mm in length and 0.09 mm in diameter, that was repeatedly (3 passes in 2 directions) passed over the skin. Rolling the Dermaroller over an area for 6 times results in approximately 100 holes/cm^2. Any microneedle treatment with a needle length of more than 0.25 mm breaches the dermis where nerves are found. Therefore, this treatment is painful. Hence, topical anesthesia has to be administered locally prior to the treatment when used in vivo. The same holds true for CO_2 laser application.

Within minutes after the laser treatment and just before the microneedle application, the surface of the skin was stained with methylene blue dye, enabling the color to penetrate into the resulting skin defects to facilitate localization and measurement of

dimensions of the lesions. Treated specimens were stored for 24 h in 4% buffered formalin, then routinely processed and embedded in paraffin. These specimens were then cut into vertical sections of 5 μm thickness and stained with hematoxylin and eosin. Serial sections have been performed to increase the chance to identify the treated areas. Histological slides were evaluated by a dermatopathologist (H.B.) to record characteristics of the lesions. Special attention was paid to depth and width of skin defects, epidermal changes, and further tissue damage. Stained samples were imaged using a light microscope (Nikon 80i) and a digital camera (Nikon Digital Sight DS-Ri1). The lesion dimensions were measured with a Visual Basic computer program (NIS-Elements).

Results

Treatment of the ex vivo human skin with the fractional eCO$_2$ laser (n = 12) caused a small epidermal necrosis with a diameter of 300 μm (fig. 1, 2), depending on the parameters used. At maximum there was a full-thickness epidermal defect penetrating the superficial dermis to a depth of 300 μm (fig. 3). At the border of the lesions, we always observed thin zones of eschar and adjacent tissue necrosis as a consequence of the thermal injury. Adjacent to the lesions the epidermis showed cellular damage of the keratinocytes (ballooned or necrotic cells).

Microneedle treatment of the ex vivo human skin with the Dermaroller (n = 5) caused many thin vertical epidermodermal fissures with a surface opening width of 10–
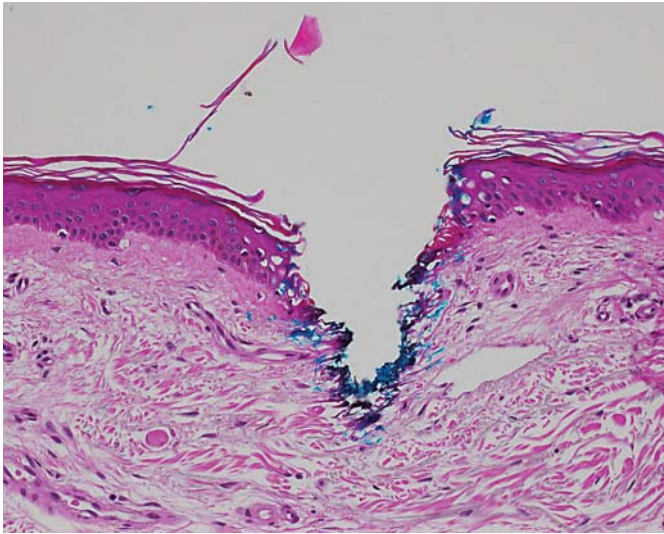
Fig. 3. Ablative fractional CO_2 laser (spot size 120 µm, power 20 W, pulse energy 60 mJ, spot density 151 MTZ/cm²): epidermal and superficial dermal tissue injury (width 200 µm, depth 300 µm), lined by a thin zone of eschar and collagen denaturation. Blue dye limited to the margins of the skin defect.
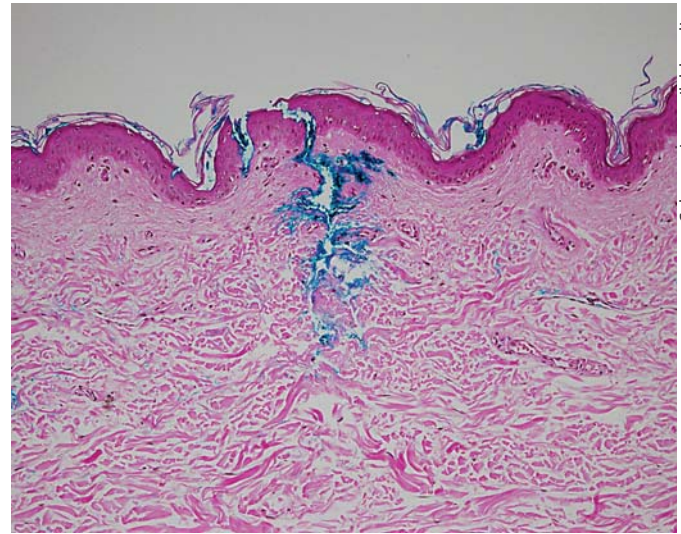
Fig. 4. Microneedling: thin vertical epidermodermal fissure with a surface opening width of 10 µm and a depth of 400 µm. No perilesional tissue necrosis, blue dye strictly perilesional.

30 µm (fig. 4) and a depth of injury into the dermis up to 500 µm. Obviously, with this technique we did not observe any tissue coagulation or eschar.

In both eCO_2 laser- and Dermaroller-treated skin, interlesional tissue was without any sign of injury at light microscopy. In specimens bearing only epidermal injury not reaching the dermis, the blue dye was only seen on the surface of the lesions. In case of full epidermal defects, the presence of blue dye within the dermis was strictly limited to the treated zones.

Discussion

Both ablative fractional laser and microneedle application technologies lead to a fractional disruption of the epidermal barrier, inducing small defects in the surface epithelium, which will allow implantation of isolated cells into the skin. By which procedure the better niche for cell survival and take is created remains speculative and will also depend on the preferential localization of the cell type applied (e.g. keratinocytes and melanocytes with epidermal localization vs. fibroblasts, vascular endothelial cells or even mesenchymal stem cells with dermal localization). However, as shown in the present study, there are relevant differences between skin defects created by a fractional CO_2 laser and microneedle treatment,

which could have an enormous impact on the success rate of transepidermal cell delivery.

Based on the theory of selective photothermolysis, ablative fractional lasers create microscopic vertical channels in the skin, known as MTZ [7, 8]. Compared to the fully ablative laser, the fractional ablative technologies damage only a fraction of the skin (3–40%) while leaving microscopic interspersed areas of untreated healthy skin. This allows a more rapid re-epithelization by an enhanced wound healing response to the thermal injury and therefore results in less severe unwanted effects. The degree of epidermal and dermal tissue coagulation depends on factors such as spot size, pulse energy and power. The dimension of each MTZ as the zone of collagen denaturation measured histologically in hematoxylin and eosin-stained tissue has been described, and an ex vivo model correlated well with the in vivo conditions [9–12]. We mainly observed necroses of the epidermis and at most of very superficial parts of the papillary dermis. The dimension of these defects will allow single cells to be delivered into the skin. The CO_2 laser is hemostatic so that there is little or no bleeding or fluid loss through the laser-ablated defects, which potentially could impede cell implantation. On the other hand, the perilesional zones of tissue necrosis present in CO_2 laser-treated skin might hamper nutritional supply as well as cell migration from the defects into the surrounding tissue.

The range of depths that can be acquired with microneedles obviously depends on the size of the needles, some of them not penetrating the epidermis [13, 14]. Microneedle treatment with the Dermaroller resulted in deeper skin defects damaging also the blood vessels of the superficial vascular complex [15]; however, there was no perilesional tissue necrosis. This might even give access to a systemic dissemination of the cells, which could be of interest for future treatments with autologous adult stem cells, e.g. derived from the outer root sheath of hair follicles [6]. Measurement of the depth of tissue damage induced by microneedle application bears some problems. We stained the surface of the skin and the needles of the Dermaroller with methylene blue dye in order to stain the created fissures of the skin. It is our hypothesis that the extent of blue stain corresponds to the grade of dermal injury by the needle as the dye is not supposed to penetrate markedly into the formalin-fixed tissue. This hypothesis is sustained by the fact that blue dye in the dermis was always limited to the margins of the skin defects caused by the needle. We made a similar observation in the tissue treated with laser, where blue dye was seen in the dermis only if there was a complete epidermal defect and where the blue color was always strictly limited to the treated zones. We want to emphasize the importance of serial sectioning of specimens in order to detect the treated zones as they are very thin and many sections do not contain any, or at least not their central part with the most extensive injury, which is essential to document maximum depth and width.

In conclusion, the niche created for the applied cells might be quite different depending on the de-epidermization technique used. In this context, the local reaction to the mode of tissue injury resulting in the release of inflammatory cytokines and growth factors among others will play an important modulatory role. In humans, this can only be further explored by comparative clinical proof-of-concept applications using a cell type that allows an unequivocal readout in an autologous setting without marking of the cells. Melanocyte transfer to treat vitiligo would be a most suitable approach, repigmentation documenting the take and preserved functional activity of the transplanted cells. Controls applying the ablative method with the transfer medium without cells will be essential in such clinical trials. The disappointing patient benefit reported for actual therapeutic options in vitiligo [16] indicates a great need to improve the modalities of melanocyte transplantation.

Disclosure Statement

The authors declare no conflict of interest.

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