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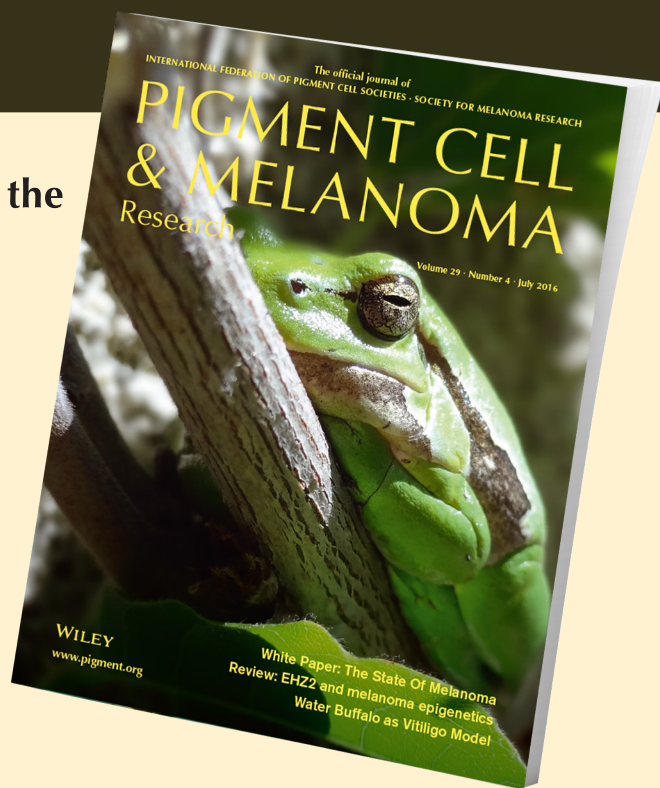

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Micro holes for delivering melanocytes into the skin: an ex vivo approach

Claire Regazzetti[1], Damien Alcor[2], Bérengère Chignon-Sicard[3] and Thierry Passeron[1,4]

1 C3M, Team 12, INSERM U1065, Nice, France **2** C3M, biophotonic platform, INSERM U1065, Nice, France **3** Department of Plastic Surgery, University Hospital Center of Nice, Nice, France **4** Department of Dermatology, University Hospital Center of Nice, Nice, France
Correspondence Thierry Passeron, e-mail: passeron@unice.fr

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Dear Editor,

Vitiligo affects 0.5% of the population worldwide. Among available treatments, surgical grafts are indicated for segmental vitiligo and stable and localized forms of non-segmental vitiligo (Ezzedine et al., 2015; Van Geel et al., 2010). The procedure for preparing the grafting bed requires a dermabrasion that can be performed surgically or by using an ablative laser. These techniques are relatively painful and require a trained physician with an adapted platform preventing many patients to benefit from cell grafting. Micro holes performed using microneedles, erbium or CO_2 fractional ablative lasers have been shown to enhance the penetration of topical agents, such as methyl-ALA used in photodynamic therapy (Bahadoran et al., 2015; Haedersdal et al., 2010). These techniques are easy to perform and induce little or no pain when the epidermis or the superficial dermis is targeted. Using such micro holes to deliver melanocytes into the skin might provide an easy and reproducible way to graft vitiligo skin. The aim of this study was to investigate the feasibility of this approach using an ex vivo human skin model and to determine the optimal technique and parameters.

To differentiate the transplanted melanocytes from those already present in the treated skin, we first infected normal human melanocytes obtained from foreskins with lentivirus carrying GFP (GFP-NHM). Fresh skins (10×10 cm) used in this study were obtained from abdominoplasty. Several pretreatments were then applied on skin samples. In the first set of experiments, we compared three techniques: 500-μM-depth microneedles, fractional ablative erbium laser with 60 J or fractional ablative CO_2 laser with 5 mJ. Note that the two last fluencies were chosen to make a 300-μM hole depth. A density of 5% was first applied. In the second set of experiments, the same procedures were performed using a density of 60%. In the third one, the microneedles of 500 μM were replaced by a 200 μM depth. This has been chosen to distinguish the efficiency of both methods, with a first depth able to reach the dermis to a depth targeting only the epidermis.

After applying these procedures, 8-mm punch biopsies were taken from each condition and placed in an ex vivo culture in semiliquid condition. GFP-NHM-injected cells were suspended in 1% hyaluronic acid, and 100 000 GFP-NHM were delivered on the surface of each skin sample in a 30-μl suspension. Half of the samples were cultured for 24 h and the other half for 4 days. Skin samples were then fixed and histological analyses were performed.

Additional methodological information is provided in supplementary data.

The results show that the use of a 5% density provided few holes with a limited number of GFP-NHM cells observed at two time points, regardless of whether the holes were made with microneedles or using lasers. The 500-μm needles made very deep holes and delivered most melanocytes into the dermis (data not shown). We, therefore, switched to a 60% density with either erbium or CO_2 lasers or 200-μm microneedles. With erbium or CO_2 lasers, skin morphology was altered and the upper layers of the epidermis were damaged (Figure 1B, C). Under these conditions, at 24 h and 4 days after treatments, we observed GFP fluorescence exclusively at the upper surface of the skin but the alteration of the skin did not allow us to clearly identify individual GFP-labeled cells (data not shown). In contrast, the application of microneedles did not affect the overall aspect of the skin. In fact, nearly all holes were closed after 24 h (Figure 1D) and we could clearly observe GFP-NHM in the epidermis. Nevertheless, the combination of skin autofluorescence and low GFP fluorescence made it impossible to determine whether there were additional cells located in the dermis (Figure S1). To better discern the GFP signal, we used FLIM (fluorescence lifetime imaging microscopy) as a contrast method. This method allows one to differentiate the GFP signal from skin autofluorescence based on the difference in fluorescence lifetime (Figure S2). The fluorescence lifetime of GFP-NHM cells integrated into the skin was determined by comparison to the control skin sample. As has been noted previously (Joosen et al., 2014), fluorescence lifetime depended on the method of sample preparation, but using the same sample preparation, the fluorescence lifetime of GFP-positive cells seen in the epidermis after transplantation was invariant (2.7 ± 0.15 ns) (Figure S2). Hence, this

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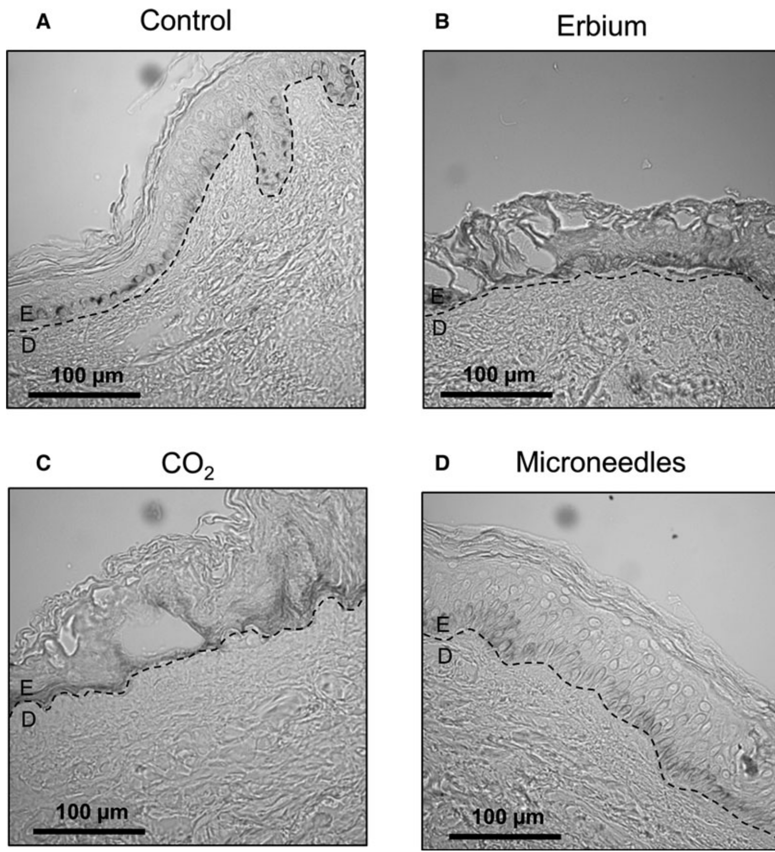

Figure 1. Skin morphology after laser treatments and microneedles processing. The skin was maintained in ex vivo culture for 24 h (A) or treated using 60% density with fractional ablative erbium laser (B), fractional ablative CO2 laser (C) or 200-μm needles (D), and maintained in ex vivo culture for 24 h. E: epidermis; D: dermis.

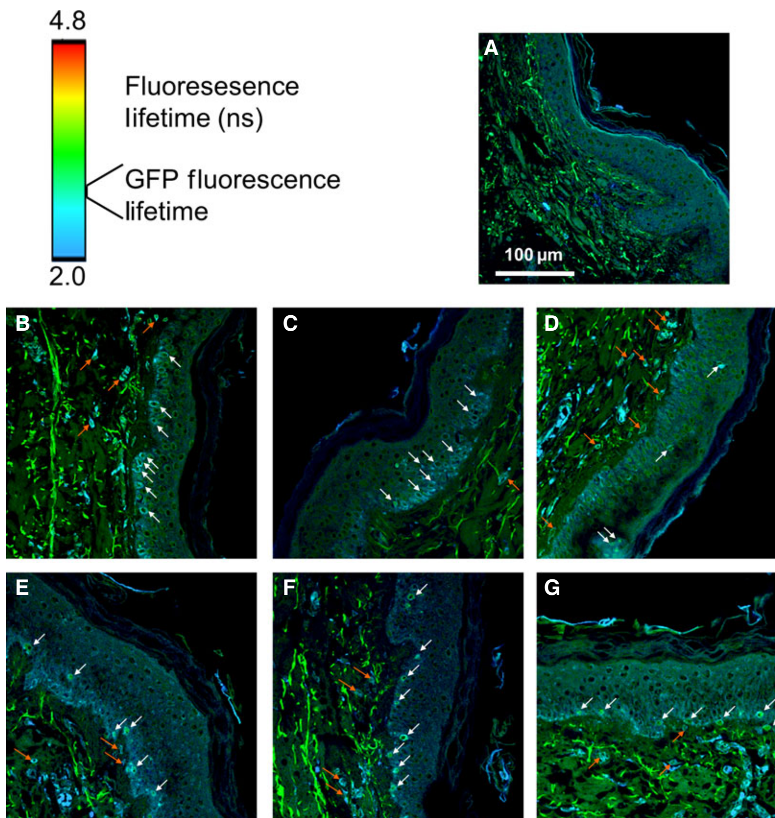

Figure 2. Use of 200-μm microneedles allows effective transfer of melanocytes into the skin. The skin was treated using 60% density with 200-μm microneedles treated (B–G) or not (A) with GFP-NHM. 24 h (B–D) and 4 days (E–G) after ex vivo culture, GFP-NHM integrated into the skin were detected using fluorescence lifetime imaging in green/blue (fluorescence lifetime = 2.7 ± 0.15 ns). Epidermal GFP-NHM are marked with white arrows and dermal GFP-NHM are marked with red arrows.

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value was used to identify the grafted cells in the skin. To confirm that integrated cells identified either by GFP fluorescence or pseudocolored in green/blue according to their fluorescence lifetime were indeed melanocytes, we also performed immunohistochemistry, using the gp100 melanocytic marker (Figure S3). After 24 h of ex vivo culture, we observed green/blue pseudocolored GFP-NHM both in the epidermis (white arrows) and in the upper dermis (red arrows) (Figure 2B–D). Four days later, GFP-NHM were still present in the skin and most of them were located on the basal layer of the epidermis (Figure 2E–G).

These results show that micro channels can be used for delivering living cells into the skin. The use of 200-μM microneedles with a high density (at least of 60%) appears as the optimal procedure to deliver sufficient numbers of cells into the epidermis. All procedures succeeded in delivering GFP-NHM into the skin. However, fractional CO_2 and erbium laser induce a marked disruption of the skin. Laser technique methods are still interesting, but appear (at least under ex vivo conditions) as less feasible for therapeutic purposes compared to microneedles. The coagulation induced by the lasers might also be an obstacle for an optimal delivery of the cells. The 500-μm microneedles also allowed delivering numerous cells into the skin but the holes were clearly too deep, resulting in predominant delivery of the cells to the dermis. Moreover, this extreme depth could be a marked caveat in vivo as bleeding could decrease the delivery of the cells. Interestingly, at 200 μm depth, cell delivery is mainly targeted to the epidermis. Previous study performed on human subjects comparing CO_2 laser and microneedles showed that neither pain nor bleeding were associated with using 200-μm microneedles for enhancing the penetration of methyl-ALA(Bahadoran et al., 2015). Moreover, the use of 200-μM microneedles does not require any specific platform and could be performed by any physician. Thus, the 200-μm microneedles appear as the best approach, allowing delivery of numerous melanocytes along the basal membrane, which represents their physiological location. Homing of melanocytes at the basal membrane is consistent with previous reports showing that differentiated melanocytes spontaneously take up residence at this place (Li et al., 2010), and so, even cells that after delivery through channels are not initially found at the basal membrane may finally migrate to this location. Nevertheless, it is important to note that our approach is different from the one used in clinical practice. In the clinic, the origin of melanocytes, their number and their purity are all different from the pure melanocyte suspension used in our study, and the presence of keratinocytes, for instance, may modify the integration and homing of melanocytes.

Taken together, our results show that melanocytes can be delivered effectively into the skin through micro holes and that they eventually reach their physiological location. It appears based on the ex vivo approach that the 200-m microneedles with a 60% density provide optimal results. A prospective comparative trial in vitiligo patients comparing this approach to dermabrasion should be conducted to evaluate its validity for the clinic.

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Conflict of interest

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References

Bahadoran, P., Le Duff, F., Pascual, T., Petit, L., Martel, P., Lacour, J.P., and Passeron, T. (2015). Comparative methods for improving transepidermal methylaminolevulinate delivery: a randomized clinical trial. JAMA Dermatol. *00*, 1–3.

Ezzedine, K., Eleftheriadou, V., Whitton, M., and Van Geel, N. (2015). Vitiligo. Lancet *386*, 74–84.

Haedersdal, M., Sakamoto, F.H., Farinelli, W.A., Doukas, A.G., Tam, J., and Anderson, R.R. (2010). Fractional CO(2) laser-assisted drug delivery. Lasers Surg. Med. *42*, 113–122.

Joosen, L., Hink, M.A., Gadella, T.W. Jr, and Goedhart, J. (2014). Effect of fixation procedures on the fluorescence lifetimes of Aequorea victoria derived fluorescent proteins. J. Microsc. *256*, 166–176.

Li, L., Fukunaga-Kalabis, M., Yu, H., Xu, X., Kong, J., Lee, J. T., and Herlyn, M. (2010). Human dermal stem cells differentiate into functional epidermal melanocytes. J. Cell Sci. *123*, 853–860.

Van Geel, N., Wallaeys, E., Goh, B.K., De Mil, M., and Lambert, J. (2010). Long-term results of noncultured epidermal cellular grafting in vitiligo, halo naevi, piebaldism and naevus depigmentosus. Br. J. Dermatol. *163*, 1186–1193.

Supporting information

Additional Supporting information may be found in the online version of this article:

Figure S1. Use of 200μm microneedles allows effective transfer of melanocytes into the skin.

Figure S2. Fluorescence lifetime of the GFP signal.

Figure S3. Detection of GFP-NHM and endogenous melanocytes.

Data S1. Supplementary data.

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