Combination of medical needling and non-cultured autologous skin cell transplantation (ReNovaCell) for repigmentation of hypopigmented burn scars

K.H. Buscha,1, R. Bendera,1, N. Walezko a, H. Aziza, M.A. Altintas b, M.C. Aust a,*

a Department for Plastic and Reconstructive Surgery, Johanniter Hospital, Bonn, Germany
b Department for Plastic and Reconstructive Surgery, Bergmannsheil und Kinderklinik Buer, Gelsenkirchen, Germany

Burn scars remain a serious physical and psychological problem for the affected people. Clinical studies as well as basic scientific research have shown that medical needling can significantly increase the quality of burn scars with comparatively low risk and stress for the patient with regards to skin elasticity, moisture, erythema and transepidermal water loss. However, medical needling has no influence on repigmentation of large hypopigmented scars.

The goal of this study is to evaluate whether two established methods – needling (for improvement of scar quality) and non-cultured autologous skin cell suspension (for repigmentation) – can be successfully combined.

Twenty subjects with mean age of 33 years (6–60 years) with scars from deep second and third degree burns have been treated. The average treated surface area was 94 cm² (15–250 cm²) and was focused on prominent areas such as the face, neck, chest and arm.

Percutaneous collagen induction or “medical needling” was performed using a roller covered with 3 mm long needles. The roller is vertically, horizontally and diagonally rolled over the scar, inducing microtrauma. Then, non-cultured autologous skin cell suspension (NCASCS) was produced and applied using the ReNovaCell Autologous Cell Harvesting Device (Avita Medical), according to the manufacturer’s instructions.

The patients were followed 12 months postoperatively. Pigmentation changes were measured objectively, as well as with patient and observer ratings. Patient satisfaction/preference was also obtained.

Taken together, the pigmentation ratings and objective measures indicate individual improvement in 17 of the study participants. The melanin increases seen 12 months after NCASCS treatment are statistically significant.

Medical needling in combination with NCASCS shows promise for repigmentation of burn scars.

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1. Introduction

Approximately 11 million people worldwide each year suffer from burns receiving medical treatment [1]. Due to improvements in medical practice and research, the mortality rate after burns has decreased significantly over the last decades [2]. More people with deep and extensive burns survive their injury. There is a corresponding increase of long term consequences like scarring, and patients frequently request treatment to improve scar quality, such as textural problems or pigmentation. Melanocyte death, melanogenesis disruption, weakened paracrine signaling between melanocytes and other skin cells all have been shown to affect skin pigmentation [3]. Dyspigmentation of burn scars is therefore a common consequence after partial and full thickness burns [4]. Additionally, the scar tissue itself may present a barrier for melanin transfer and melanocyte migration [5]. Physical changes like higher sensitivity for sunburns [6] and psychological impairments also affect the patients, as conspicuous scars are a frequent reminder of traumatic situations [7].

Currently, numerous methods are available to treat hypopigmented skin, such as split skin grafting [8], lasers [9] and cultured skin cell transplantation [10]. In recent years, research focused additionally on the use of non-cultured skin cell suspension (NCASCS). Although the primary objective when using NCASCS was to achieve reepithelization in acute and chronic wounds [11], repigmentation has also been observed. Hence, the method has also been used to treat hypopigmentation associated with vitiligo and burn scars [12,13]. The Autologous Cell Harvesting Device is used to create a spray suspension of viable autologous skin cells. These cells are harvested intraoperatively and applied directly, in suspension, to the prepared wound.

Wounds are prepared for application of NCASCS using dermabrasion or laser, which are both ablative methods. By nature, ablative treatments remove skin structures and cells, including the basement membrane, which are then replaced by a thinner epidermis with flatter rete ridges [14]. The associated inflammatory response stimulates fibroblasts to produce parallel oriented scar collagen instead of physiological lattice pattern collagen [15]. Additionally, the risk of dyspigmentation increases after ablative treatments due to associated damage to the melanocytes [16].

Medical needling as non-ablative treatment overcomes the shortcoming of ablative treatments by not destroying structures of the epidermis, but rather promoting the formation of physiological collagen instead of scar collagen and initiating the expression of growth factors. In recent years, it has been shown that it is possible to achieve these ideal treatment goals with percutaneous collagen induction or “medical needling” [17,18]. It has been shown that medical needling does not have the risks of hyper- or hypopigmentation. However, repigmentation of large hypopigmented scars is not achieved after medical needling [19].

Therefore, the aim of this study is to evaluate if it is possible to achieve repigmentation of large (>10 cm²) hypopigmented burn scars by combining non-ablative medical needling and NCASCS. The hypothesis is that the melanocytes of the cell suspension link through the parenchymal canals onto the basal membrane. They may be successfully transplanted after 24 h, when all needling channels are closed [18].

2. Methods

2.1. Study design

This study is a prospective randomized controlled within-subject comparison. Subject’s hypopigmented scar areas were divided into 3 subareas for which treatment was randomly allocated as (1) the combination of medical needling and NCASCS, (2) medical needling alone (positive control) and (3) no treatment (negative control). The subjects were assessed at baseline (pre-treatment) and after 3, 6, 9 and 12 months. Pigmentation was objectively measured for each of the subareas at each study visit. Scar outcomes for the area treated with the combination of medical needling and NCASCS were assessed by both the patient and an observer using the Patient and Observer Scar Assessment Scale (POSAS). POSAS outcomes of scars treated with Medical Needling only are already published [17,20]. Hence, we focused on the scars treated by medical needling and NCASCS to clarify the difference regarding repigmentation.

2.2. Subject selection

In order to be included, patients were required to have hypopigmented burn scars that had healed by secondary intention, and were at least both 10 cm² in size and 1 year in age. Exclusion criteria were pregnancy and severe underlying diseases or skin lesions like cancer or infections.

2.3. Procedure

We received approval from the local ethical committee for this study. All subjects signed an informed consent. Informed consent was obtained from parents of subjects younger than 18 years of age. The treatment, including general anesthesia, medical needling, skin sample harvesting and preparation and application of the cell suspension was performed in a surgical suite.

2.4. Medical Needling and NCASCS

Medical needling is repetitive puncturing of the scar with a roller equipped with 3 mm long needles (Figs. 1 and 2). The needling device is repeatedly rolled over the scar in three directions, longitudinally, diagonally and horizontally. According to the extent of the scar, this procedure can last 30 min or longer. It is important to use the device with constant pressure and in a straight way to prevent shear forces. The needles disrupt the old collagen structures that connect the scar with the upper dermis. The needles do not have a lumen. Hence, they temporarily displace the skin cells rather than destroy them. They penetrate the dermis 2.5–3.0 mm and lead to thousands of microwounds and intradermal bleedings. A scar is sufficiently prepared for NCASCS when multiple and confluent hematomas develop; the skin is swollen and has a livid appearance.
To produce NCASCS a 2 cm by 2 cm donor skin sample with a thickness of 0.2 mm is required, and is harvested using a dermatome. The skin sample is processed within the device which contains a processing unit composed of an incubator, a well for buffer solution, a work surface and a collecting well for the final suspension. The split-thickness skin sample is digested with the enzyme trypsin for 20 min in order to release the skin cells from the extracellular matrix. Next, the digestion is stopped by rinsing the skin sample in buffer solution. The cells are removed mechanically by scraping them from the skin sample with a scalpel. NCASCS is collected with a syringe which is then covered with a spray nozzle.

After cleaning the prepared wound with wet compresses, NCASCS is applied (Fig. 3). The area is immediately covered with Telfa™ Clear wound dressing and sterile poultices. We did not apply the cell solution prior to the needling because the bleeding caused by medical needling would have rinsed the cell suspension out of the treated area.

The superficial donor site wound is covered with wet compresses. At the end of the procedure, a small amount of remaining suspension is applied on the donor site to advance the wound healing phase.

After surgery, patients are instructed to immobilize the treated area for a minimum of 24 h, until the needling channels are closed. All patients where exposed to direct sun during the one year period.

### 2.5. Assessment

Outcomes were assessed subjectively using POSAS and objectively using the Mexameter® (Courage + Khazaka electronic GmbH) which quantify the presence of melanin.

The POSAS is a partially observer administered and partially patient administered scale and covers a range of scar characteristics. The patient score includes: pain, pruritus, color, thickness, relief, pliability and overall opinion. Similarly, the observer score includes: vascularization, pigmentation, thickness, surface roughness, pliability, surface area and overall opinion. Patients and observer each score these items on a 10 point ordinal scale, 1 reflecting “normal skin” appearance and 10 reflecting the “worst imaginable scar”. The POSAS was used to make pre- to postoperative comparisons of the areas treated with NCASCS. With regard to this study we concentrate on the “pigmentation” and “overall opinion” ratings.

![Fig. 1 – Schematic representation of medical needling [17].](image1)

![Fig. 2 – Roller device for Medical Needling [19].](image2)

![Fig. 3 – Application of the cell suspension.](image3)

![Fig. 4 – Measurement principle of the Mexameter® (image source by Courage & Khazaka).](image4)
Each scar was photographed before and after treatment using a single lens reflex camera. Each picture was taken in the same room in outpatient clinic in ambient light, without direct flash. The subjects were positioned in front of a blue wall.

The melanin measurement is based on absorption of light. The device’s probe (which is little bigger than a pencil) emits light (Fig. 4) in defined wavelengths (for melanin 660 and 880 nm), detects the reflected amount of the emitted light within 5 mm² and computes the Melanin Index, which is proportional to the melanin content of the skin. Measurements of melanin were made three times in a row within 1 cm² in each of the subjects’ 3 scar areas, along with a melanin measurement in an area of healthy skin for comparison. At the “Baseline” time point the individual measuring points were marked with a marking pencil and photographed afterwards. Hence, it was possible to measure always at the same skin location.

2.6. Statistics

Statistical analysis was performed using the software SAS version 9.3. Due to the sample size only nonparametric tests (Fisher’s exact tests for categorical variables respectively Wilcoxon signed rank tests) were used. Significance was accepted at a level of \( p < 0.05 \).

3. Results

3.1. Subjects

The demographic data of 20 subjects are listed in Table 1. Fifteen subjects were women and five were men. The average age was 33 years, with a range from 6 to 60 years. The majority of injuries were due to scalding hot fluids \((n = 10)\). Others suffered from fire accidents \((n = 7)\). One subject was the victim of an acid attack. Noteworthy is that 2 of the subjects had hypopigmented scars resulting from cosmetic interventions, namely chemical peeling and laser treatment.

3.2. Patient and Observer Scar Assessment Scale

The data of 19 subjects are considered. One subject was lost to follow-up after moving to another country. The values from each subject’s last visit (6–15 months) were used. There were no infections detected at any subject and all scars were 100% epithelialized.

3.3. Patient ratings (Fig. 5)

The subjects evaluated the color of the scars treated with needling and NCASCS preoperatively with a median of 9 (Fig. 5).
8.0 ± 2.1 SD (standard deviation) points. Postoperatively they rated 4.0 ± 2.5 SD points which is an improvement of 50% and statistically significant with \( p < 0.05 \). Additionally, the subjects rated the overall opinion of their scars preoperatively with a median of 7.0 ± 2.6 SD points. Postoperatively they evaluated 3.0 ± 2.3 points which is an improvement of 57.1%. With \( p < 0.05 \) the improvement is statistically significant.

### 3.4. Observer ratings (Fig. 6)

The observer rated the scars regarding pigmentation preoperatively with a median of 8.0 ± 1.4 SD points and postoperatively with 5.0 ± 2.1 points. This is an improvement of 37.5% which is statistically significant with \( p < 0.05 \). Regarding “Overall opinion” the observer evaluated the scars preoperatively with a median of 6.5 ± 1.7 SD points. Postoperatively

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**Fig. 6 – Observer rating for “Pigmentation” and “Overall opinion”, preoperatively and on last follow-up.**

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**Fig. 7 – Patient 1, forehead, pre- and 1 year postoperatively after medical needling and NCASCS.**
they rated 4.0 ± 1.5 points. This shows an improvement of 38.5% which is statistically significant with \( p < 0.05 \).

3.5. Photo documentation

Hereafter, exemplary outcomes are shown (Figs. 7–13).

3.6. Mexameter®

Hereafter, the results of the Mexameter® measurements are depicted. Regarding melanin measurement, we chose 1 year as the primary endpoint because of the potentially confounding effect of season and the associated exposure to sunlight. Four subjects were unable to follow up for the 1 year mexameter measurement.

Regarding “Scar Needling + NCASCS” the median for preoperative melanin index was 125.3 ± 31.9 SD points. Postoperatively it increased to 162.0 ± 48.2 points, an improvement of 29.3% (Fig. 14). This is statistically significant with \( p < 0.05 \).

The “needling only” scar was preoperatively measured with a median of melanin index of 149.5 ± 100.7 SD points and postoperatively with 137.0 ± 77.0 points (Fig. 15). Hence, the amount of melanin decreased about 8.4% which is not statistically significant (\( p > 0.05 \)).

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**Fig. 8** – Patient 1, right temple, pre- and 1 year postoperatively after medical needling and NCASCS.

**Fig. 9** – Patient 1, abdomen, pre- and 1 year postoperatively after needling and NCASCS.
The "Untreated scar" was measured and the median for the melanin index was accounted preoperatively for 186.0 ± 59.6 SD points. Postoperatively the index was measured for 169.5 ± 90.9 points (Fig. 15). The amount of melanin decreased about 8.9% which is not statistically significant ($p > 0.05$).

The median for the amount of melanin was measured in "Healthy skin" for 158.0 ± 46.7 SD points preoperatively. One year later the amount was accounted for 153.0 ± 46.9 points (Fig. 15). Hence, the melanin index was nearly consistent.

4. Discussion

There are various methods to treat burn hypopigmentation.

It has been shown that split-skin grafts produce a good pigmentation outcome [21]. A limiting factor of this procedure is that the skin of the donor site can only be expanded by a factor of 1.5 [12]. Consequently, the larger the hypopigmented area is to be treated, the larger the donor site must be,
increasing associated risks of infection, pain and wound healing problems with new scarring [22].

Cultured skin cell transplantation has been shown to achieve good but also frustrating results regarding repigmentation [23,24]. It is a two-stage procedure, where the harvested cells are cultured over a few weeks in special laboratories. In a second surgery the cells are applied to the prepared wound bed [25]. The necessities of two operations and highly qualified laboratories make this procedure expensive.

NCASCS can be considered as a modification of the cultured cell technique, involving the disaggregation step but not the expansion or amplification step. The advantages are that it is cheaper, faster and a single-stage procedure. An advantage over split-skin grafts is that it is possible to expand the donor site by the factor 1:80 [25]. Minimizing the donor site thus minimizes the risk of new scarring, infection or dyspigmentation. Additionally, it is possible to treat an area of 320 cm² with a 4 cm² skin sample. The average treated area in our study was $94.1 \pm 67.6$ cm².

NCASCS is usually combined with ablative treatments like lasers or dermabrasion as wound bed preparation.

Ablative and fractional laser treatments are not only used for skin resurfacing and tightening [26,27]. It has been shown that it is possible to achieve good results regarding repigmentation in vitiligo [9,28]. Since burn scars have a lack of skin appendages with stem cell reservoirs, they have reduced...
potential for repigmentation compared to vitiligo lesions [29]. Furthermore, despite risk minimization with fractional lasers, the risk of dyspigmentation is increased [30,31]. Complication rates for hypopigmentation are reported ranging from 12 to 14% [32]. The most common complication after dermabrasion is pigment alteration after treatment [16,33]. Additionally, darker skin types are more likely to suffer undesirable pigment changes [33].

Medical needling as wound preparation for NCASCS is very promising. It is a non ablative procedure which has been shown to result in high patient satisfaction [17]. It has been shown that after medical needling the level of transforming growth factor (TGF) – β3 remains high even beyond the initial wound healing phase [34]. TGF-β3 supports the formation of physiological lattice pattern collagen as it is found in healthy skin [35].

Furthermore, medical needling results in a thickening of the epidermis, a growth of the extracellular matrix with increased collagen and elastin and a decreased transepidermal waterloss [19,20,34].

Medical needling does not change the amount of melanocytes but the expression levels of melanocyte-stimulating hormone (MSH) and Interleukin-10 (Il-10) are modified. MSH which influences the proliferation and activity of melanocytes is significantly down regulated two weeks after treatment. Il-10 as an anti inflammatory cytokine is upregulated post operative [19]. These findings indicate that medical needling minimizes the risk of postinflammatory dyspigmentation.

The POSAS is currently considered the most suitable scar assessment scale [36]. It is reliable, complete, can be used within minutes on males or females and was made for detecting scar parameter changes over time [37]. Patients and
observers both rated scar pigmentation and overall opinion significantly better postoperatively as compared to before the treatment. The photos support these findings.

However, the POSAS is based on subjective impressions either of the patient or the observer. Therefore, it was important to reinforce these positive results with objective data.

The Mexameter<sup>10</sup> melanin index showed a significant pigmentation improvement only in the scar treated with medical needling and NCASCS. The control areas remained nearly unchanged or even showed a slight decrease in the amount of melanin.

Based on the fact that Medical Needling has no influence on the pigmentation of the treated area<sup>19</sup>, these results suggest that it is possible to transplant melanocytes of the cell suspension through the needling channels. In future, it is important and necessary to support these results with histological samples.

Three patients showed no increase of the melanin index postoperatively. They can be classified as non-responders (see Fig. 14). The reasons might be ascribed to the following circumstances: invalid or incorrect needling technique or invalid wound cleaning and washing before the application of the suspension made with NCASCS, whereby the puncture channels can be agglutinated with serous fluid. Furthermore, a lack of immobilization of the treated scar or early removal of the wound dressing can lead to shear force and thus provoke death of the transplanted cells.

5. Conclusion

Our results show a subjective and objective improvement in pigmentation and overall opinion. Both medical needling and NCASCS preserve the epidermis which results in a reduced risk of new scarring or dyspigmentation.

Considering all factors, we come to the conclusion that the combination of medical needling and NCASCS is a very promising approach to repigment large hypopigmented burn scars.

Conflict of interest

None declared.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.burns.2016.04.009.

References


