COMBINATION OF MEDICAL NEEDLING AND NON-CULTURED AUTOLOGOUS SKIN CELL TRANSPLANTATION (RENOVACELL) FOR REPIGMENTATION OF HYPOPIGMENTED BURN SCARS IN CHILDREN AND YOUNG PEOPLE

ASSOCIATION DE L'AIGUILLETAGE MÉDICAL ET DE LA TRANSPLANTATION DE CELLULES ÉPIDERMIQUES AUTOLOGUES NON CULTIVÉES (RENOVACELL) POUR LA RECOLORATION DES CICATRICES HYPO PIGMENTÉES CHEZ LES ENFANTS ET LES ADOLESCENTS

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SUMMARY. Burn scars remain a serious physical and psychological problem for the affected. 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 is to evaluate whether both established methods – Needling (improvement of scar quality) and ReNovaCell (repigmentation) – can be combined. So far, eight patients with mean age of 20 years (6-28 years) with deep second and third degree burn scars have been treated. The average treated tissue surface was 76cm² (15-250cm²) and was focused on areas like face, neck, chest and arm. Medical Needling is performed using a roller covered with 3mm long needles. The roller is vertically, horizontally and diagonally rolled over the scar, inducing microtrauma. Then, non-cultured autologous skin cell suspension (ReNovaCell) is applied, according to the known protocol. The patients were followed 12 months postoperatively. Pigmentation changes were measured objectively, and with patient and observer ratings. Patient satisfaction/preference was also obtained. We present the final study results. Taken together, pigmentation ratings and objective measures indicate improvement in six of the study participants. Melanin increase seen 12 months after ReNovaCell treatment in the study group as a whole is notable. Medical Needling in combination with ReNovaCell shows promise for repigmentation of burn scars.

Keywords: Medical Needling, ReNovaCell, repigmentation, non-cultured autologous skin cell transplantation

RÉSUMÉ. *Les séquelles de brûlures demeurent un problème physique et psychologique pour les victimes. Les études cliniques, ainsi que les recherches scientifiques ont montré que l'Aiguilletage médical peut améliorer de façon significative la qualité des cicatrices de brûlures avec un risque faible et un retentissement psychologique mineur chez les patients et ceci vis-à-vis de l'élasticité cutanée, l'hydratation, l'érythème et la déperdition hydrique trans épidermique. Cependant l'Aiguilletage médical n'a pas d'influence sur la repigmentation des vastes cicatrices hypo pigmentées. Le but est d'apprécier la possible association des deux méthodes: Aiguilletage (amélioration de la cicatrice) et ReNovaCell (re pigmentation). Ainsi 8 patients avec une moyenne d'age de 20 ans (6-28 ans) présentant des cicatrices de brûlures du 2e degré profond et 3e degré ont été traités. La moyenne de surface traitée était de 76cms carrés (15-20cms carrés) et les zones choisies furent la face, le cou, le thorax et les bras. L'Aiguilletage médical était réalisé avec un rouleau couvert d'aiguilles de 3mm de long. Le rouleau est manié verticalement, horizontalement et en diagonale sur la cicatrice provoquant un microtraumatisme. Puis, les cellules cutanées autologues non cultivées en suspension (ReNovaCell) sont appliquées suivant le protocole connu. Les patients furent suivis pendant 12 mois après le traitement. Les changements de pigmentation étaient mesurés de façon objective par le patient et évalués suivant une grille. La satisfaction du patient et son avis étaient alors notés. Nous présentons les résultats de la fin de notre étude. Prenant en compte les taux de repigmentation et les mesures objectives, l'amélioration fut constatée chez 6 de nos patients. L'augmentation de la mélanine fut observée 12 mois après le traitement par ReNovaCell dans l'ensemble du groupe de façon notable. L'association « Aiguilletage médical + ReNovaCell » est riche de promesse pour la repigmentation des cicatrices de brûlures.*

Mots-clés*: Aiguilletage médical, ReNovaCell, repigmentation, transplantation de cellules épidermiques autologues non cultivées*

Introduction

Worldwide, millions of people suffer from burns and their related disabilities.[1] The paediatric age group especially constitutes a high proportion of hospital admissions, up 40% in comparison to adults.[2] Over the last few decades, mortality rate

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for burn injuries has decreased significantly due to an improvement in medical practice and research.[3,4] More people with deep and extensive burns survive their injury, leading to an increased incidence of long-term consequences like scarring or contractures. Since childhood and adolescence are very important periods for developing social, motor and cognitive functioning,[5] these consequences can have an enormous impact on the child's developmental, functional and aesthetic status.[6] Hence, patients and families frequently request treatments to release contractures and improve scar quality, texture and pigmentation.

Up to 25% of children with burn injuries suffer from hypopigmentation after healing.[7] Mechanisms underlying dyspigmentation can be death of melanocytes or a disruption of melanogenesis. Furthermore, a weak influence on the paracrine networks between melanocytes and other skin cells like keratinocytes and fibroblasts, which have been demonstrated to influence pigmentation,[8–10] can lead to dyschromia. Moreover, scar tissue may provide a barrier for melanin transfer and melanocyte migration.[11,12]

There are various therapeutic approaches to treating hypopigmented skin, such as skin grafting,[13,14] laser treatment[15] and cultured skin cell transplantation.[16] In recent years, research has also focused on the use of non-cultured autologous skin cell suspension (NCASCS). This method is frequently used to repigment hypopigmented lesions of vitiligo and post-burn scars. The ReNovaCell Autologous Cell Harvesting Device (Avita Medical Europe Ltd, Melbourn, UK) is used to create a spray suspension of viable autologous skin cells, prepared intraoperatively and directly applied to the prepared wound bed.

NCASCS is usually combined with ablative treatments such as dermabrasion or laser ablation to prepare the scar. These interventions remove skin structures and cells, including the basement membrane, which results in a thinner epidermis with flatter rete ridges.[17,18,19] The subsequent inflammatory response stimulates fibroblasts to produce parallel-oriented scar collagen rather than physiological lattice-pattern collagen of healthy skin.[20,17] Additionally, the risk of dyspigmentation increases after these ablative treatments.[21,22]

For wound preparation, percutaneous collagen induction or "Medical Needling" overcomes the deficits of ablative treatments by not harming the epidermis and underlying structures but rather promoting the expression of growth factors and the formation of physiological collagen, and by decreasing the risk of hyper- or hypopigmentation.[23–30] However repigmentation of large hypopigmented scars is not achieved after Medical Needling.[30]

The aim of our study is to evaluate whether it is possible to repigment large (>10 cm²) hypopigmented burn scars in a young population (under 30 years of age) with a combination of non-ablative Medical Needling and NCASCS. The hypothesis is that the melanocytes of the NCASCS go through the needling puncture channels and that they are successfully transplanted after 24 hours, when all needling channels are closed.

Methods

Study design

This study is a prospective randomized controlled within-subject comparison. The hypopigmented scars of the subjects 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 at 3, 6, 9 and 12 months post treatment. 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 observer using the Patient and Observer Scar Assessment Scale (POSAS).[31] POSAS outcomes of scars treated with Medical Needling only have already been published.[23,25] Hence, we focused our use of the POSAS on the scars treated by Medical Needling and NCASCS to evaluate improvement in pigmentation and overall appearance.

Subject Selection

Patients were required to have hypopigmented burn scars that had healed by secondary intent, and were at least 10 cm² in size and one year post-accident.

Exclusion criteria were pregnancy and severe underlying diseases or skin lesions like cancer or infections.

Procedure

In Germany, both Medical Needling and NCASCS are approved and licensed therapies that are used on a daily basis. All pre- and post-operative examinations were performed in vivo. Hence, ethical approval for invasive examinations was not needed. The whole procedure including general anaesthesia, Medical Needling, skin sample harvesting and preparation and application of NCASCS was performed in an operating theatre. All patients signed an informed consent form. Informed consent was obtained from the parents of patients younger than 18 years of age.

Medical Needling and NCASCS

Medical Needling is performed by rolling a device covered with 3mm-long needles over the scar (*Figs. 1 and 2*).

The device must be rolled repeatedly over the scar in three directions: vertically, diagonally and horizontally. To prevent wounds caused by shear forces it is important to keep the roller in a straight line. The needles penetrate 2.5 to 3.0 mm into the dermis, disrupt the scar collagen which connects the dermis to the upper layers and leads to thousands of micro wounds and

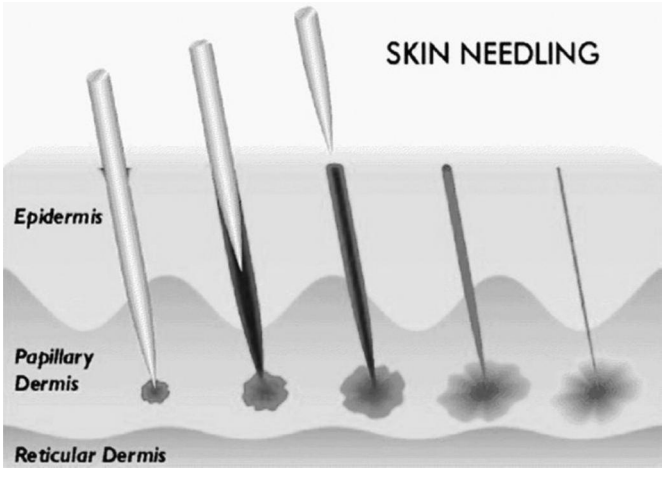

Fig. 1 - Schematic representation of Medical Needling.[23]

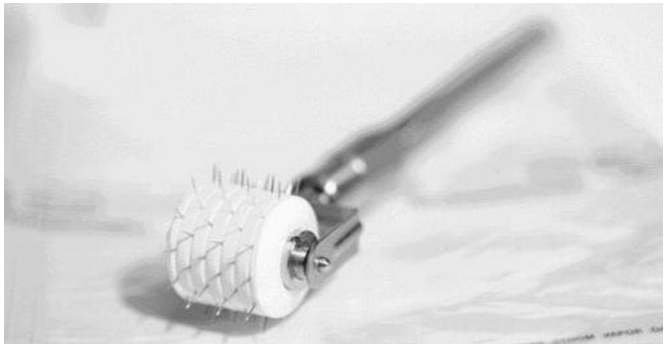

Fig. 2 - Roller device for Medical Needling.[30]

intradermal micro-bleeding. Since the needles do not have a lumen, they repress the skin cells rather than destroy them. This procedure can last 30 minutes or longer, according to the extent of the treated scar. The scar is sufficiently prepared for NCASCS when multiple and confluent haematoma develop, and the skin is swollen and has a more livid appearance.

To prepare NCASCS, a 2 cm by 2 cm split skin sample is harvested from an uninvolved area (e.g. inner upper thigh or hairline). The harvested skin sample is processed within the device that is composed of an incubator well, a well for buffer solution, a work surface and a collection well for the final suspension. To release the skin cells from their extracellular matrix network, they are processed for 20 minutes with the enzyme trypsin. After that, the skin sample is placed on the work surface. By adding 4 ml of the buffer solution (compound sodium lactate), the digestion is stopped and the skin is ready for the mechanical process of disaggregation. The cells are removed by scraping them from the skin using a scalpel. The solution on the work surface, which contains buffer and cells, is then transferred into a collection well through a sieve to create a clear solution that contains skin cells but not skin appendages or other parts of the initial skin sample. The final solution is then collected using a syringe, which is then capped with a diffusor nozzle.

After cleaning the prepared wound bed with wet compresses, the NCASCS is applied by spraying it onto the wound (*Fig. 3)*. Hereafter, the wound is dressed with TelfaClear.® The rest of the NCASCS is applied to the donor site to advance the wound-healing phase.

After surgery, the patients are instructed to keep the treated area immobile for at least 24 hours, until the needling channels are closed.

Assessment

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

The POSAS is self-administered and includes two different scales. The patient scale considers the scar parameters: pain, pruritus, colour, thickness, relief, pliability and overall opinion. The blinded observer scale considers the following parameters: vascularization, pigmentation, thickness, surface roughness, pliability, surface area and overall opinion. Each parameter is scored on a 10-point ordinal scale from 1 (reflects normal skin) to 10 (reflects the worst imaginable difference to normal skin). The POSAS was used to make pre- to post-operative comparisons of the areas treated with NCASCS. With regard to this study we concentrate on the "pigmentation" and "overall opinion" items.

Each scar was photographed before and after the treatment. To get comparable photos every follow up was performed in the same room in the outpatient clinic in ambient light, without direct flash. The subjects were positioned in front of a blue wall.

The Mexameter® (COURAGE+KHAZAKA electronic GmbH, Cologne, Germany) quantifies the amount of melanin in the skin based on the tissue's narrow wavelength light absorption (*Fig. 4*). The probe emits light in defined wavelengths (for melanin 660 and 880 nm) and detects the reflected amount of the emitted light. As the quantity of emitted light is defined, the quantity of light absorbed by the skin can be calculated. The computed "Melanin Index" is proportional to the melanin content in the skin.

Measurements of melanin were made in each of the subjects' three scar areas, along with a melanin measurement in an area of healthy skin for comparison.

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 $p < 0.05$.

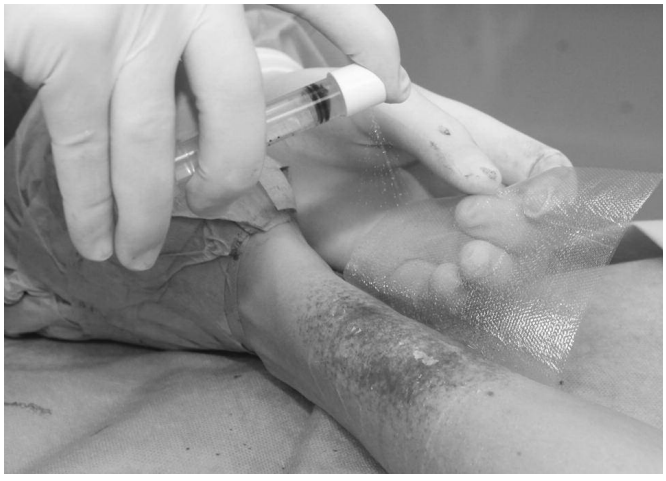

Fig. 3 - Application of NCASCS.

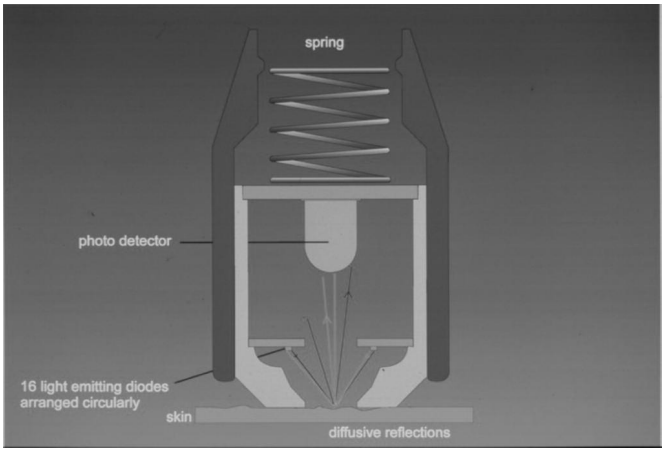

Fig. 4 - Measurement principle of the Mexameter® (Image source Courage & Khazaka).

Results

Subjects

The demographic data of eight subjects are listed in *Table I*. Six patients were female and two were male. The average age was 19.1 ± 8.7 years with a range from 6 to 28 years.

Table I - Demographic data

N	8
Gender (F/M)	6/2
Age (years)	19.9 ± 8.6
BMI	20.0 ± 4.3
Caucasian	8.0
TBSA (%)	25.0 ± 21.5
Area treated with NCASCS (cm^2)	76.0 ± 66.3

The majority of injuries were due to scalding with hot water (n=4). Others suffered from fire accidents (n=3). Noteworthy is that 1 subject was injured due to the incorrect use of a cosmetic intervention, namely chemical peeling.

Patient and Observer Scar Assessment Scale

The data of all subjects are considered. The values from each subject's last visit (8 – 13 months) were used. There were no infections detected in any subject and all scars were 100% epithelialized.

Patient Scale

Regarding "color," the subjects rated their scars treated with Medical Needling and NCASCS preoperatively with a median of 7.0 ± 2.4 SD (standard deviation) and postoperatively with 3.5 ± 2.5 points. This shows an improvement of 50%, which is statistically significant with $p < 0.05$. Furthermore, the subjects scored the overall opinion of their scars preoperatively with a median of 7.0 ± 2.1 SD points. Post-operative evaluation was 3.0 ± 1.5 points, which results in an improvement of 57.1%. This is statistically significant with $p < 0.05$ (*Fig. 5*).

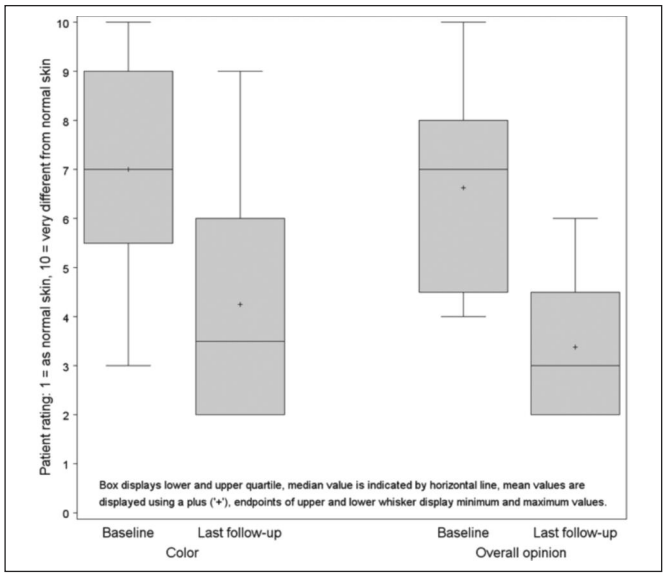

Fig. 5 - Patient rating for "color" and "overall opinion", preoperatively and at last follow-up.

Observer Scale

Regarding pigmentation, the blinded observer evaluated the scars pre-operatively with a median of 8.5 ± 1.9 SD points and post-operatively with 4.0 ± 2.6 points. This results in an improvement of 52.9%, which is statistically significant with $p < 0.05$. Additionally, regarding overall opinion the physicians scored preoperatively with a median of 7.0 ± 1.9 SD and post-operatively with 4.0 ± 1.9 points. This results in an improvement of 42.9%, which is statistically significant with $p < 0.05$ (*Fig. 6*).

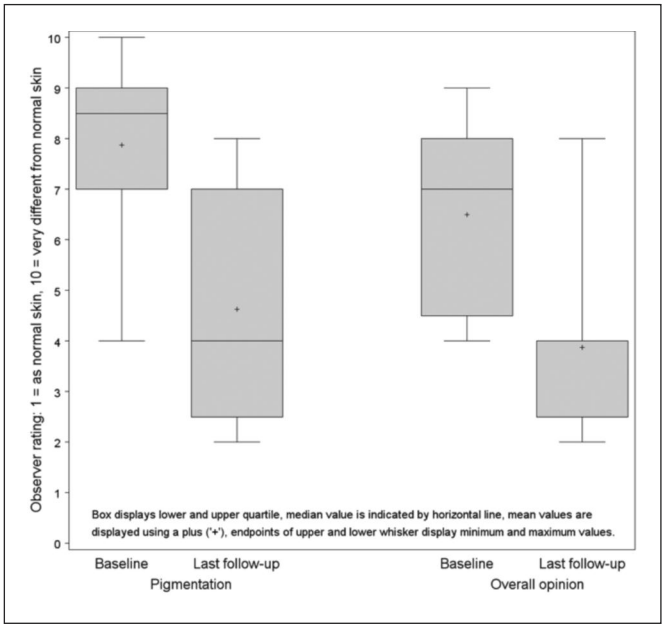

Fig. 6 - Observer rating for "pigmentation" and "overall opinion", preoperatively and at last follow-up.

Photo documentation

Hereafter, exemplary outcomes are shown (*Figs. 7, 8 and 9*).

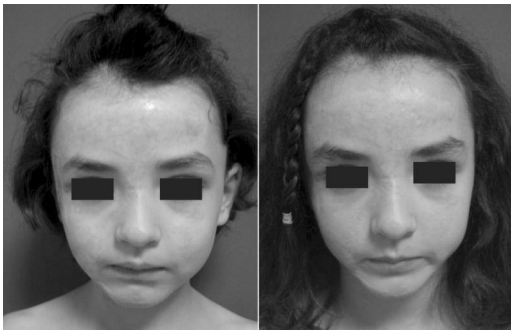

Fig. 7 - Patient 1, forehead, pre- and 1 year postoperatively after Medical Needling and NCASCS. Areas treated: forehead, temples, cheeks.

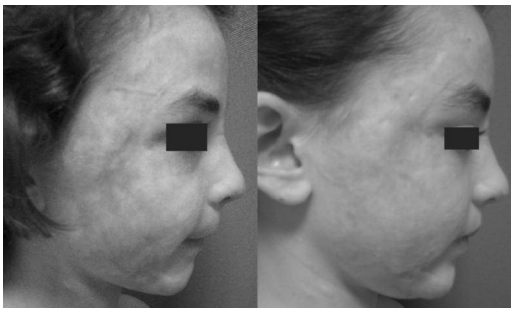

Fig. 8 - Patient 1, right temple, pre- and 1 year postoperatively after Medical Needling and NCASCS. Areas treated: forehead, cheeks and temples.

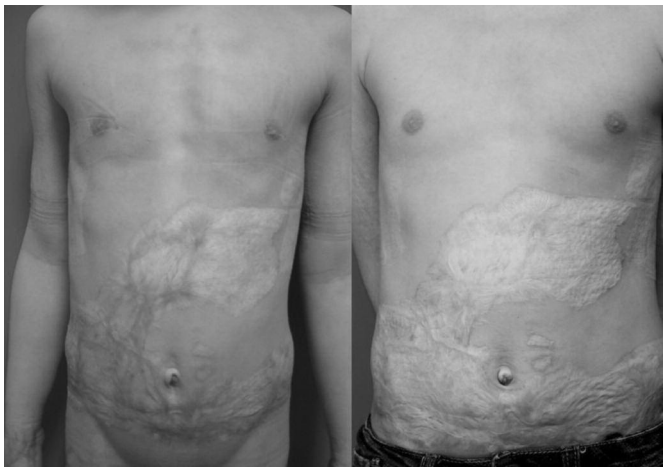

Fig. 9 - Patient 1, abdomen, pre- and 1 year postoperatively after Needling and NCASCS. Areas treated: abdomen, above the umbilicus.

Mexameter®

In the following 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. One subject was unable to follow up for the 1-year measurement.

Regarding "Scar Needling + NCASCS" the median for the melanin index amount accounted preoperatively for 106.0 ± 28.1 SD (standard deviation) points. It came postoperatively to 138.0 ± 58.0 points, which shows an improvement of 30.2%. This result is trending toward a significant difference with p =0.07, but due to the small sample size, statistical significance was not shown (*Fig. 10*).

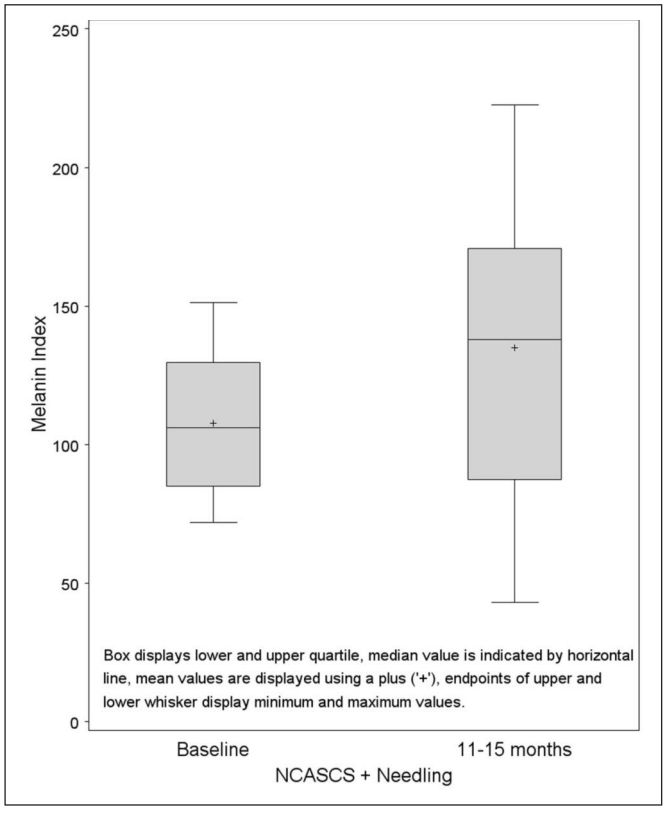

Fig. 10 - Mexameter Melanin Index for the scars treated with "Needling and NCASCS", preoperatively and 1 year later.

The "Needling only" scar was preoperatively measured with a median for the melanin index of 134.5 ± 33.7 SD points and postoperatively with 128.0 ± 32.1. Hence, the amount of melanin remained more or less unchanged.

The "Untreated scar" was measured and the median for the melanin index preoperatively was 157.0 ± 52.5 SD points. Postoperatively the index was 134.0 ± 39.5 points. The amount of melanin decreased about 14.6% which is not statistically significant with $p > 0.05$.

The amount of melanin was measured in "Healthy skin" with a median of 138.5 ± 30.4 SD points preoperatively. One year later the median was 148.0 ± 34.0 points. Hence, the melanin index was more or less consistent (*Fig. 11*).

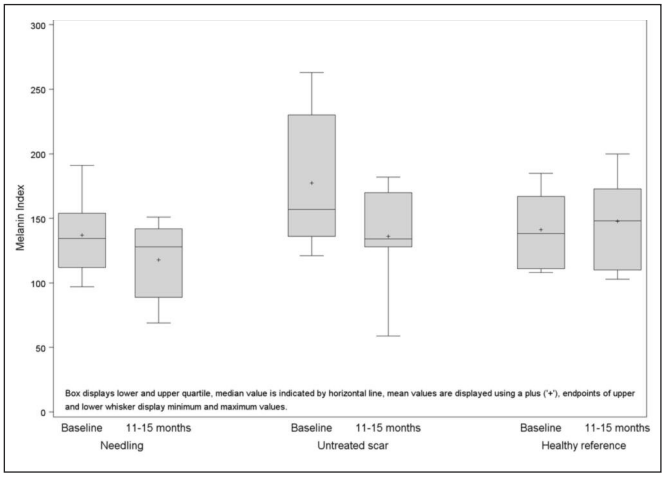

Fig. 11 - Mexameter Melanin Index for the scars treated with "Needling", the "Untreated scars" and the "Healthy reference", preoperatively and 1 year later.

Discussion

There are various therapeutic approaches available to restore pigmentation in hypopigmented skin. Since sequelae of burn injuries can have an enormous impact on a child's life and development, the goal should be to find the optimal strategy to handle these sequelae.

In reconstructive surgery, split-skin grafts are frequently used and have been shown to result in good repigmentation.[32,33] Since a split-skin sample can only be reasonably expanded by the factor 1.5, larger hypopigmented lesions require larger donor sites.[34] Consequently, the risk of infection, pain, wound healing problems and new scarring arises.[35]

In recent years, cultured skin cell transplantation has been used not only to re-epithelialize chronic wounds,[36] but also to repigment leukoderma with both good and frustrating results.[37,38] This technique requires a state-of-the-art laboratory. It is a two-stage procedure, where the harvested cells are applied a few weeks after culturing. This process makes it expensive and specially trained staff are required.

NCASCS can be considered as an advancement of the culture method. It is a one-stage procedure, where the harvested cells are directly applied to the wound. Hence it is cheaper, faster and no specially trained personnel are required. An advantage compared to split-skin grafts is that the donor site can be expanded by the factor 1:80.[39,40] Thus, the risk of new scarring, infection or dyspigmentation is minimized at the donor

site. 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 76 ± 66.3 cm².

For wound preparation, NCASCS is usually combined with ablative treatments such as laser or dermabrasion. Ablative and fractional laser therapies are frequently used for skin resurfacing and tightening.[41,42,43] Additionally, it has been demonstrated that it is possible to improve pigmentation in vitiligo lesions.[15,44] A basic therapeutic approach in vitiligo treatment is to achieve repigmentation with melanocyte stem cells from hair follicles. Since burn scars have a lack of skin appendages, they have a reduced potential for repigmentation compared to vitiligo lesions.[42]

Despite the minimized risk of fractional laser treatments, laser treatment always comes with the risk of post interventional dyspigmentation.[45,46,47,48] Complication rates with laser for hypopigmentation have been described, ranging from 12 to 14%.[49,50] The most common complication after dermabrasion is post-treatment pigmentary alteration.[22,51] Additionally, darker skin types are more likely to suffer undesirable pigmentary changes.[52,51] Thus, ablative treatments must be done with caution.

Medical Needling seems to be a promising wound preparation for NCASCS. It is a non-ablative treatment which results in high patient satisfaction, thickening of the epidermis, growth of the extracellular matrix with increased collagen and elastin, and decreased transepidermal water loss.[23,25,30,53] Additionally, levels of growth factors like Transforming Growth Factor (TGF)-ß3 remain high even beyond the initial wound healing phase.[53] TGF-ß3 supports the formation of physiological lattice-pattern collagen as it is found in healthy skin.[54]

Medical Needling does not change the amount of melanocytes but does alter the levels of Melanin-stimulating Hormone (MSH) and Interleukin-10 (Il-10). MSH influences the expression and activity of melanocytes and is significantly down-regulated 2 weeks postoperatively. Il-10, which is an anti-inflammatory cytokine, is upregulated post intervention.[30] These findings indicate that Medical Needling minimizes the risk of post inflammatory dyspigmentation.

The POSAS offers a suitable, reliable and complete scar evaluation tool.[31] It can be used in minutes and allows us to detect scar parameter changes over time.[55] Patients as well as a blinded observer rated the scars treated with Medical Needling and NCASCS for both repigmentation and overall opinion and ratings were significantly better than before the treatment.

The standardized photo documentation supports these results. Even though it is very important to evaluate the subjective opinion of the observers and especially of the patients, it was important to underline these positive results with objective data. These data were collected with the Mexameter®.

Most of the measurement points, namely scar treated with only Medical Needling, untreated scar and healthy reference, showed stable melanin levels after one year. The Mexameter® showed not only a constant melanin content in the healthy and untreated skin but an increase in melanin in the scars treated by Medical Needling and NCASCS. However, in contrast to our previously published work with Medical Needling and NCASCS on adults and children collectively, these positive results were not statistically significant.

This might be due to the following circumstances. The management of paediatric burn wounds and scars is often more challenging because of multiple factors, including immature vascularity as well as an up to 30% thinner epidermis and dermis of the child's skin, a lack of immobilization and thus a worsening of the surface area and depth over time.[56,57]

Immobilization is an important factor when it comes to melanocyte transfer through the needling puncture channels. Shear forces caused by early mobilization of the treated area or an early removal of the dressing can lead to death of the transferred cells and thus to a poorer outcome. Other circumstances that can have a negative impact on cell transfer are an invalid needling technique prior to the NCASCS application or insufficient cleaning of the wound, whereby the needling channels can be blocked by serous fluid. Another explanation is the variability, which overcomes results in a small sample size.

Conclusion

Our results demonstrate that it is possible to repigment hypopigmented burn scars by combining Medical Needling with NCASCS. The combination preserves the skin, which results in a minimized risk of new scarring and dyspigmentation.

Considering all factors, a combination of Medical Needling and NCASCS is a very promising approach to the repigmentation of large hypopigmented burn scars.

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