ORIGINAL REPORT

Autologous Patch Healing vs Secondary Intent Healing after Mohs Micrographic Surgery: A Randomized Controlled Trial

Marie K. HJORTH[1], Ewa A. BURIAN[1], Tonny KARLSMARK[1], Mette MOGENSEN[1,2], Martin GLUD[1], Anna Ahm HARAGER[1], Anthony ROSSI[3] and Katrine E. KARMISHOLT[1]

[1]Department of Dermatology and Venereology Bispebjerg and Frederiksberg Hospital, Copenhagen University Hospitals, Denmark, [2]Department of Clinical Medicine, University of Copenhagen, Denmark, and [3]Department of Dermatology, Memorial Sloan Kettering Cancer Center, New York, USA

Secondary intent healing is a viable option for wound closure after facial tumour removal by Mohs micrographic surgery. Secondary intent healing involves prolonged healing time and carries risk of infection and complications related to scarring. Healing with an autologous patch made from the patient's own blood may be beneficial. This study on 22 patients evaluates the effect of applying an autologous patch to the wound after Mohs micrographic surgery. A randomized controlled assessor-blinded trial was carried out. Patients had Mohs micrographic surgery on day 0 and clinical evaluation on day 12, day 19, and after 6 months. Transepidermal water loss was measured on day 12 and day 19. Reflectance confocal microscopy was applied exploratively. At 6 months' follow-up the Patient and Observer Scar Assessment Scale was evaluated. Primary outcome was fully epithelialized wounds on day 19 and secondary outcome was 50% epithelialized wounds on day 12. One wound from each group was fully epithelialized and wound area reduction was higher in the patch group although not significant. Transepidermal water loss decreased to a larger extent, indicating that the patch creates a moist environment. Wound healing with an autologous patch is equivalent to secondary intent healing but may prompt benefits in certain wound healing factors. Patch healing appears safe with high patient satisfaction.

Key words: basal cell carcinoma; Mohs surgery; wound healing.

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Corr: Marie K. Hjorth, Department of Dermatology and Venereology Bispebjerg and Frederiksberg Hospital, Copenhagen University Hospitals, Denmark. E-mail: marie.kirstine.hjorth@regionh.dk

SIGNIFICANCE

This randomized controlled study investigates wound healing after facial tumour removal by Mohs micrographic surgery. The intervention group healed with a patch made from their own blood and the control group healed with secondary intent. The wounds were evaluated after surgery on day 12, 19, and after 6 months. We investigated whether there were adverse events, measured transepidermal water loss and wound size area, and evaluated clinical photos. The wounds were evaluated blinded by the Patient and Observer Scar Assessment Scale. We found no difference regarding epithelialization in the 2 groups. No clinical infection or recurrence of cancer was detected indicating that the patch method appears safe. Although not significant, wound area reduction was higher in the patch group as was change in transepidermal water loss indicating that the patch creates a moist environment. Each wound healing method was associated with high patient satisfaction.

The face is a common site for development of basal cell carcinomas (BCC) (1, 2). BCCs of the face can be removed surgically by fixed margin excision or with Mohs micrographic surgery (MMS). Either treatment creates a wound, which will become a scar. Scars can affect patients negatively through symptoms such as pain, contraction, and aesthetic concerns, which can all lead to reduced quality of life, especially when the scar is located on the face (3, 4). Available reconstructive methods after surgical removal of skin cancer by MMS are various and depend on factors such as defect size, location, and the surrounding skin structure. Healing by secondary intent is a viable option for wound closure in smaller defects and especially in concave regions of the face such as the alar crease, temporal regions, ala nasi, ear helix, and lateral radix nasi (5). However, secondary intent healing may involve prolonged healing and carries an increased risk of infection and complications related to problematic scarring including contraction and reduced aesthetic outcome. We theorize that in scar formation, when healing by secondary intent, the myofibrils are pulling the wound edges before granulation tissue is formed sufficiently, causing contraction from the wound edges. This can be problematic, for example on ala nasi, with a risk of retraction pulling the alae rim upwards risking asymmetry of the nostrils and furthermore affecting nasal breathing. A method to speed up the formation of granulation tissue may therefore be beneficial, resulting in improved wound healing. An option explored for wound healing is the use of autologous extracted growth factors from the patient's own blood (6). Blood withdrawn from a patient is centrifuged to separate out the buffy coat consisting of white blood cells and platelets. The buffy coat is obtained with a concentration of platelets that is higher than in circulating blood (7, 8). Platelets are promotors

of healing and release cytokines and growth factors such as platelet-derived growth factor (PDGF), vascular endothelial growth factor, basic fibroblast growth factor, and transforming growth factor β1 among others in the wound bed, which support the healing process of inflammation, tissue repair by formation of collagen matrix, and angiogenesis (9, 10). Autologous extracted white blood cells may lower the risk of infection during wound healing. A method to visualize wound healing is reflective confocal microscopy (RCM). RCM enables bedside high-resolution imaging of the skin at the cellular level, providing valuable information on skin morphology and architecture to a depth of 200 µm, and has been successfully applied to monitor wound healing after laser procedures (11–14).

Closing of a surgical wound with an autologous patch (3C Patch®; Reapplix Inc, Southlake, TX, USA) is a technique approved to accelerate wound healing including surgical wounds (15). The patch is placed in the wound bed as a biologic graft (16). The improved wound healing effect has been investigated in chronic wounds (15). The aim of the present study is to evaluate the postoperative wound healing effect of applying an autologous patch to the surgical wound after MMS.

MATERIALS AND METHODS

Study design

A prospective, randomized, controlled assessor-blinded trial was carried out comparing wound healing with an autologous patch vs secondary intent healing after removal of skin cancer with MMS. The study was approved by the Danish Research Ethics Committee (H-21052933) and registered at ClinicalTrials.gov (NCT05170035) before study initiation. All study participants provided written informed consent.

Patients

Twenty-two consecutive patients undergoing MMS by European Society for Micrographic Surgery (ESMS) certified Mohs surgeons for biopsy verified superficial or nodular basal cell carcinoma in the head-and-neck area in a location suitable for secondary intent healing and thus also suitable for healing with an autologous patch were recruited from the Department of Dermatology, Bispebjerg Hospital, Copenhagen, Denmark. Inclusion criteria were BCC on ear helices, temporal region, medial canthus, alar crease, and ala nasi and a minimum age of 18 years. If an included patient after MMS had a skin defect considered unsuitable for participation, e.g., wounds > 2 cm in diameter, the patient was considered as screening failure. Exclusion criteria were subjects with major systemic disease not stabilized and pregnancy or breastfeeding.

Randomization and blinding

Patients with a surgical defect eligible for participation were randomized to heal with either an autologous patch (intervention) or by secondary intent (control). Randomization was conducted with consecutively numbered, closed, non-opaque envelopes containing a computer-generated allocation. First-author MH was not blinded and carried out transepidermal water loss (TEWL) measurements and *post hoc* analysis of wound size measurements.

Study procedures

All patients were seen for MMS on day 0 (D0, baseline) and for clinical evaluation of the wound on day 12 (D12 ± 1), day 19 (D19 ± 1), and after 6 months. Sutures were removed on D12 from patients treated with a patch. Transepidermal water loss from the wound site was detected with a skin barrier function measurement device (GPSkin Barrier® by GPOWER Inc, Seoul, South Korea) on D12 and D19 as a supplement to measurement of epithelialization. Three measurements were carried out and the mean value was noted. Clinical photos were taken. RCM was performed on 4 patients on D19 as a proof of concept. Six months after baseline all patients were seen for clinical photos and scar evaluation using the validated Patient and Observer Scar Assessment Scale (POSAS, version 3.0; https://www.posas.nl/) by a blinded physician. Wound size area (mm^2) was measured digitally by MH as a *post hoc* analysis after last study visit on clinical photos from D12 and D19 measuring length and width using a digital ruler. The clinical photos included a ruler beside the wounds.

Intervention: autologous patch

On D0 immediately after surgery patients randomized to the intervention group had 18 mL of venous blood drawn from their arm. The blood was centrifuged for 15–20 min (class IIa 3C Patch®; Reapplix Inc) to separate the buffy coat consisting of platelets, fibrin, and leucocytes subsequently pressed to form a compact moist patch (Fig. S1). The patch was applied to the surgical defect and fixed with Ethilon 6-0 sutures.

Reflectance confocal microscopy

To supplement the clinical findings, patients with a wound area and location receptive to the RCM scanning probe were asked to volunteer in an additional protocol where the wound was scanned by RCM. VivaScope Multilaser 1500® (VivaScope GmbH, Munich, Germany) was used for *in vivo* confocal imaging of the patch in order to show a possible integration of the patch into the wound. The autologous patch has not been visualized integrated in the skin previously and was used descriptively and as an explorative outcome.

Wound dressing

All wounds in both groups received standard wound care dressing post-surgery D0 with gauze and 3M Micropore™ medical tape (3M/Solventum, Maplewood, MN, USA), which was changed on day 3–4 post-surgery. All included patients received Terramycin®–Polymyxin B ointment from D0–D19, which is also standard care at the department where the study was carried out. Four patients received oral antibiotics (dicloxacillin) for 5–7 days postoperatively, prescribed by the Mohs surgeon as standard of care according to an assessment of the wound regarding localization, number of rounds of Mohs surgery, and time period with an open wound. Patients were recommended to cover the scar daily for 3 months with 3M Micropore™ medical tape and to avoid sun light the first year post-surgery.

Sample size calculation

A sample size of 22 patients provided 95% power, SD 3, with expected improved healing by 25% by healing with an autologous patch. The sample size was estimated according to a similar study on acute wounds using platelet rich plasma vs secondary intent healing (17).

Outcome measures

Primary outcome measure was blinded assessment of wound healing on D19 by a trained physician (EB) and an experienced dermatologist (TK) on clinical photos (**Fig. 1**). Wounds were evaluated as *fully epithelialized yes/no*.

Secondary outcome measures were the following: detection of adverse events during wound healing especially clinical infection; hypergranulation tissue and recurrence of cancer; blinded assessment of wound healing on D12 by EB and TK on clinical photos; TEWL measurement on D12 and D19; and POSAS. Wounds were evaluated as *50% of wound epithelialized yes/no*. In cases of different physician ratings, they met to discuss and reach a consensus. On-site blinded clinical evaluation of POSAS at 6 months' follow-up was evaluated by EB. According to POSAS, scar components were evaluated separately regarding 7 parameters, which are evaluated on a 5-point scale: 1 equals normal skin and 5 corresponds to the worst imaginable scar.

Explorative outcome measures were, first, RCM for proof-of-concept monitoring differences in microscopic healing and epithelialization between the 2 groups in 4 patients on D19; second, objective blinded evaluation of the scars and patients' satisfaction after 6 months by POSAS; and lastly the question "*would you recommend the treatment to friends and family yes/no*".

Statistical analysis

A Wilcoxon rank sum test was carried out as a non-parametric test on data not normally distributed. Fisher's exact test was used for categorical variables ≤ 5 and Pearson's χ^2 test was used for categorical variables > 5. TEWL values and wound size are indicated as median. An inter-rater reliability analysis was carried outbetween EB and TK when evaluating wound epithelialization. For

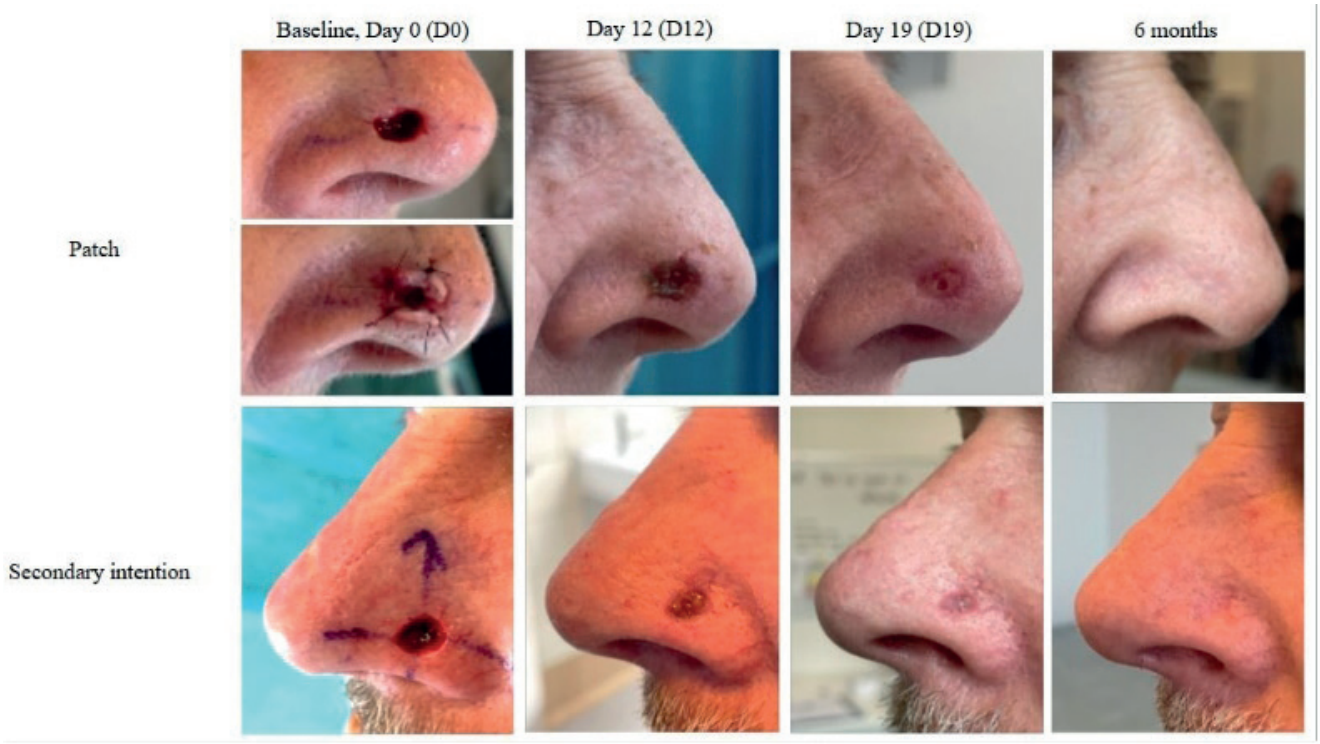

Fig. 1. Wounds healing with secondary intent or with patch from baseline to 6 months later. Photos shown with permission from patients.

this purpose Cohen's kappa was calculated. Statistical analysis was carried out by R Studio for Windows (R Foundation for Statistical Computing, Vienna, Austria). All data were double checked in all entries by MH and EB.

RESULTS

Study population

Twenty-two patients were enrolled from April 2022–May 2023 and followed up until October 2023. There were 11 patients in each group. There was no dropout or loss to follow-up.

The control and intervention groups were similar with no statistically significant differences in demographic characteristics except for sex (**Table I**). Most tumours were localized on ala nasi. Although not significant, the mean surgical defect size was larger in the patch group.

Outcomes

Primary outcome. There was no difference in full epithelialization between the intervention group and the control group on D19 with 1 patient (9.1%) healing in each group (**Table II**). The Cohen's kappa showed a moderate agreement between EB and TK with $\kappa = 0.462$.

Secondary outcome: Safety. No adverse events related to the patch treatment group or secondary intent group were registered including clinical infection, hypergranulation tissue, severe scarring, and recurrence of cancer after 6 months.

Table I. Baseline characteristics

	Patch ($n = 11$)	Secondary intent ($n = 11$)	p-value
Sex			0.030[1]
F	2 (18.2%)	8 (72.7%)	
M	9 (81.8%)	3 (27.3%)	
Age			0.904[2]
Mean (SD)	65.727 (19.432)	64.909 (10.977)	
Range	27–84	46–86	
Smoking status			1.000[1]
No	9 (81.8%)	10 (90.9%)	
Yes	2 (18.2%)	1 (9.1%)	
Anticoagulative medicine			1.000[1]
No	7 (63.6%)	8 (72.7%)	
Yes	4 (36.4%)	3 (27.3%)	
Localization			0.586[1]
Ala nasi	8 (72.7%)	8 (72.7%)	
Ear helix	2 (18.2%)	0 (0.0%)	
Medial canthus	1 (9.1%)	2 (18.2%)	
Temporal region	0 (0.0%)	1 (9.1%)	
Defect size mm^2, baseline			0.299[2]
Mean (SD)	78.364 (57.931)	56.273 (36.865)	
Range	12–198	25–144	
Subtype			1.000[3]
Nodular*	11 (100.0%)	11 (100.0%)	
Oral antibiotics after MMS			0.586[1]
No	10 (90.9%)	8 (72.7%)	
Yes	1 (9.1%)	3 (27.3%)	

One patient had a nodular tumour with infiltrative characteristics.
[1]Fisher's exact test for count data. [2]t-test. [3]χ^2 test for given probabilities.
SD: standard deviation; MMS: Mohs micrographic surgery.

Secondary outcome: Healing. In 1 patient in the control group 50% epithelialization was achieved on D12 ($p = 1.000$). The Cohen's kappa showed that there was a low agreement between EB and TK when evaluating wound epithelialization with $\kappa = -0.063$. The intervention group had the greatest delta percentage reduction in wound size area from D0–D19 by 61.8% compared with 47% in the control group, though not significant ($p = 0.459$).

Secondary outcome: TEWL. TEWL measurements showed a larger relative change in TEWL in the intervention group between D12 and D19, however this was not significant (**Fig. 2** and Table II).

Secondary outcome: POSAS. Blinded POSAS at 6 months' follow-up showed a small difference regarding surface texture (rated *2*) in the patch group and (rated *1*) in the control group ($p = 0.034$) (**Table III**). The POSAS observer evaluation was equal between the 2 groups. POSAS patient evaluation reported 1 patient with a raised scar in the control group ($p = 0.003$). All other factors were similar between the 2 groups (Table SI).

Explorative outcome: RCM imaging of wound healing. RCM scans of the wound at D19 demonstrated excellent integration of the autologous patch into normal adjacent epidermis. A fluent gradual transition from patch to normal well-organized keratinocytes in epidermis is illustrated in **Fig. 3**, where RCM images of a patient's ear in Fig. 3B show leucocytes and debris in the patch, and in Fig. 3C small uniform dark keratinocytes surrounded by white cytoplasm, in a well-organized normal honeycomb pattern. Fig. S2 illustrates the different healing processes with RCM images from 3 patients with patches and again the gradual transition from amorph patch material to a well-organized honeycomb pattern of normal keratinocytes. In Fig. S2D the secondary intent healing appears slower with no visualization of re-epithelialization. RCM images with acceptable quality were chosen for publication (see Fig. S2).

At 6 months' follow-up all the participants would recommend the received treatment.

DISCUSSION

This randomized controlled, assessor-blinded clinical trial including 22 patients compared wound healing with an autologous patch with secondary intent healing after MMS of BCC in the face. The primary outcome showed no statistical difference in complete epithelialization on D19. Interestingly, the intervention group healed with a larger reduction in wound size area from D12–D19, although this was not significant. TEWL measures tended to be lower in the intervention group and decreased to a larger extent at D19.

Recurrence of BCC is rare after tumour removal by MMS (18). Using an autologous patch containing growth factors may raise the concern of stimulating a potential

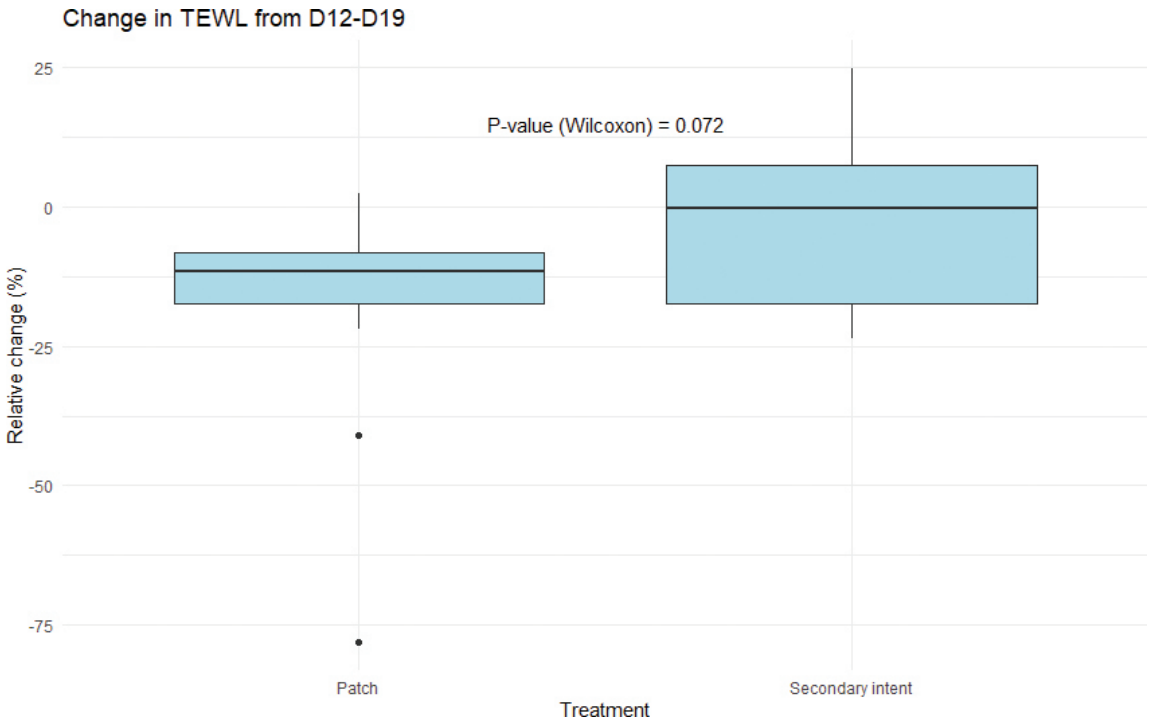

Fig. 2. Change in transepidermal water loss from D12–D19.

recurrence of cancer if any remaining cancer cells are left in the wound bed. We saw no hypergranulation tissue during wound healing nor recurrence of cancer at 6 months' follow-up. We did not identify a statistically significant difference in epithelialization between the intervention and control group regarding 50% epithelialization at D12. A similar study utilizing topical recombinant human PDGF vs placebo found that the intervention group healed 4 days more quickly (day 15 vs day 19) (17). A study by Saba et al. investigating topical PDGF vs petroleum-based gel found that PDGF treated wounds healed after 16 days vs 19.5 days in the control group. However, this study did not apply a randomized design (19). We chose to see patients on D19 before complete wound healing to evaluate a potential difference between the 2 healing methods. Despite an initial, although not significant, bigger mean size of the surgical defect in the intervention group, we found a bigger reduction in wound size from D0–D19 in the intervention group in a *post hoc* analysis.

The tendency towards a lower median TEWL and a larger relative change in TEWL in the intervention group between D12 and D19 may indicate that the patch functions as a protective barrier in the wound and suggests that the patch promotes a moist environment, which could facilitate the wound-healing process (20, 21).

Table II. Wound characteristics

	Patch ($n = 11$) Median [range] (IQR)	Secondary intent ($n = 11$) Median [range] (IQR)	*p*-value
Defects size mm² D0	64 [12–198] (41.5, 110.5)	40 [25–144] (35.5, 68)	0.450[2]
Wound size mm² D12	60 [12–130] (40, 76)	45 [10–90] (24, 56)	0.250[2]
Wound size mm² D19	31.5 [8–130] (13, 53.5)	20 [8–121] (15.5, 30)	0.549[2]
Wound size reduction % from D0–D12	20 [−300–81.3] (0.8, 37.2)	31.8 [−71.4–71.4] (−6.3, 36.8)	0.844[2]
Wound size reduction % from D12–D19	39.4 [−10–75] (0, 61.8)	37.5 [−34.4–68.8] (21.1, 45.8)	0.972[2]
Wound size reduction % from D0–D19	61.8 [−50–82] (39.5, 76.6)	47 [−33.3–80] (31.9, 70.6)	0.459[2]
Primary outcome: wound fully epithelialized			
No	10 (90.9%)	10 (90.9%)	1.000[1]
Yes	1 (9.1%)	1 (9.1%)	
Secondary outcome: wound 50% epithelialized			
No	11 (100.0%)	10 (90.9%)	1.000[1]
Yes	0	1 (9.1%)	
TEWL wound site D12	27.7 [10.3–80] (23.5, 40.3)	31 [0.7–51.7] (17.7, 38.7)	0.818[2]
TEWL wound site D19	15 [2–27.7] (10.8, 21.2)	22 [5–55] (13.3, 34.5)	0.158[2]

IQR: interquartile range; TEWL: transepidermal water loss. [1]Fisher's exact test. [2]Wilcoxon rank sum test

Table III. POSAS observer

POSAS observer	Patch Median (IQR)	Secondary intent Median (IQR)	*p*-value
Overall quality	2 (2, 2.5)	2 (1.5, 2)	0.066
Difference in pigmentation	2 (1, 2)	2 (1, 2)	0.750
Difference in vascularity	2 (2, 2)	2 (1, 2)	0.188
Difference in surface level	2 (1, 2)	2 (2, 2)	0.354
Difference in surface texture	2 (1, 2)	1 (1, 1)	0.034
Firmness of the scar	1 (1, 2)	1 (1, 2)	0.852
Adhesion of the scar to underlying tissue	1 (1, 1)	1 (1, 1)	0.544
Under how much tension is the scar	1 (1, 1)	1 (1, 1)	1
Like normal skin 1	2	3	4	5 Worst imaginable scar	

IQR: interquartile range. *p*-values are from a Wilcoxon rank sum test.

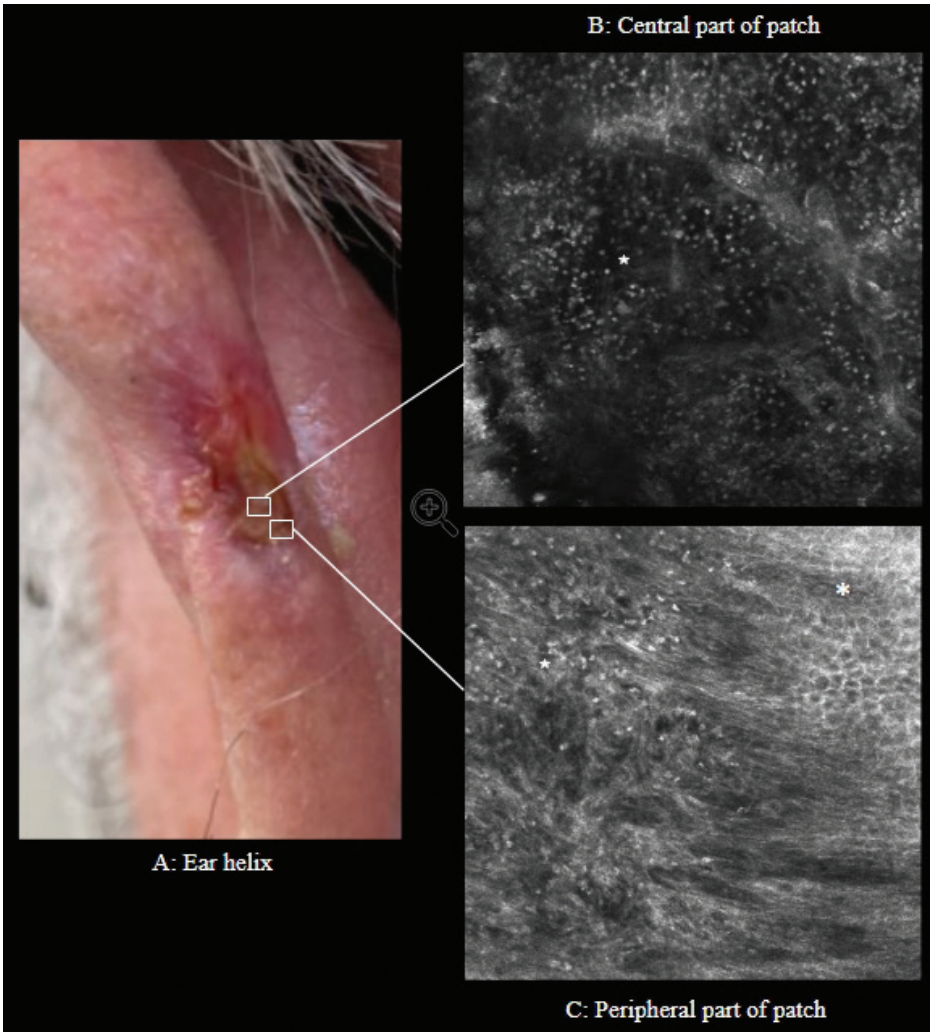

Fig. 3. Reflectance confocal microscopy post Mohs micrographic surgery with autologous patch healing ear helix D19). (A) Patient's ear. Shown with permission from patient. (B) RCM image illustrating leucocytes (star), miscellaneous cells and debris. (C) RCM image shows the gradual, fluent transition of the patch into normal, well-ordered normal keratinocytes in a honeycomb pattern (asterisk).

RCM further corroborated this hypothesis by showing the presence of a membrane, likely stemming from the patch, which may explain the numeric differences in TEWL between the 2 groups (20, 21). More frequent monitoring of the healing process until wound closure could be considered in future investigations. Our outcome measures did not demonstrate the full potential of wound healing because the patch covered the wound bed, making it difficult to visualize the new epithelium. In future studies a punch biopsy could investigate the wound bed by showing the amount of granulation tissue and epithelium underneath the patch.

The POSAS was comparable between the 2 groups at 6 months' follow-up. Overall, all study participants were satisfied with the treatment received and would recommend it to others. To our knowledge this study presents the first data on patient satisfaction in wound healing by secondary intent after MMS on a validated scar scale.

Strengths of this study included that autologous patch healing vs secondary intent wound healing after MMS was investigated in a randomized controlled manner. Furthermore, wounds were evaluated by 2 blinded investigators and onsite clinical evaluation using the validated POSAS scale at 6 months' follow-up was carried out by a blinded evaluator. Lastly, there were no dropouts.

We acknowledge that there are some limitations to our study, including unblinded measuring of wound size, which was done *post hoc* digitally on clinical photos. It is difficult to maintain full blinding during wound evaluation due to the patch being secured to the wound with sutures, which can influence scar appearance and visible remains of the patch, with a risk of revealing group allocation to the blinded assessors. The low to moderate agreement between the raters can be explained by the low number of patients and that evaluation of wound healing is a difficult criterion to objectify and dichotomize. Also, a study design where patients are seen with closer follow-ups until epithelialization could be envisioned in order to document the exact time points for complete wound healing. However, increasing study visits could

increase the dropout rate, which was absent in this study. The relatively small sample size was justified by a power calculation depending on expected healing time from a similar study (17).

In conclusion, this study shows that surgical wound healing with an autologous patch is safe and equivalent to healing by secondary intent after removal of BCC in the face by MMS. Wound healing with an autologous patch may have advantages by creating a moist environment improving TEWL. Both wound healing modalities show high satisfaction among the included patients.

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