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Comparison of Neurovascular Characteristics of Facial Skin in Patients undergoing Primary and Revision Rhytidectomy

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Abstract

Importance—Wound healing influences both the cosmetic and functional outcomes of facial surgery. Epidermal nerve fiber decrease may be predictive of impaired wound healing. Study of cutaneous innervation may afford insight into patients' wound healing potential preoperatively and aid in selection of proper procedures.

Objectives—To present the quantitative and qualitative differences of epidermal nerve fibers, neurotransmitters, vasculature and mast cells in facial skin among primary and revision rhytidectomy patients.

Design, Setting and Participants—Cutaneous specimens were collected from female patients (ages 42–66) undergoing primary (n=5) and revision rhytidectomy (n=3). Tissue was processed for confocal/epifluorescence microscopy and indirect immunofluorescent localization of several neural and tissue antigens as well as basement membrane and mast cell markers.

Interventions—Primary versus revision rhytidectomy with selection of a small area of redundant, otherwise disposed of tissue anterior to the tragus for epidermal nerve fiber study.

Main outcome Measures—Demographics including smoking status, 10 point rating scales for facial sensation, pain, and paresthesias, epifluorescence/confocal microscopy to quantify epidermal nerve fibers, neurotransmitters, vasculature and mast cells.

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Results—Primary rhytidectomy subjects had an average of 54.4 epidermal nerve fibers (ENFs) per mm (SD 31.6, range 14.2–99.2) compared to 18.6 (SD 5.8, range 13.8–25.0) in revision subjects. The primary rhytidectomy patient with lowest ENF count (14.2; reduced to the low average level of the revision samples) was the only smoker. In addition to these structural neural changes, functional neural changes in secondary rhytidectomy samples included changes in normal neural antigen prevalence (Substance P, CGRP, VIP). Capillary loops appeared less robust and were less common in dermal papilla of revision versus primary samples, and mast cells were more degranulated. There was no difference in subjective self-reported post-operative facial sensation.

Conclusions—Prior skin elevation was associated with decreased ENF density and qualitative changes in dermal nerves, capillaries and mast cells in a clinical sample of rhytidectomy patients. Smoking may also decrease ENFs, but requires further study. Future research is needed to determine whether histological findings predict wound healing and to better understand effects of surgery on regenerative capacity of epidermal nerve fibers.

Introduction

Favorable wound healing is crucial in facial plastic/reconstructive surgery and dermatologic procedures. Facelift, rhinoplasty, local flap reconstruction, split-thickness or full-thickness grafts, laser resurfacing, chemical peels, and dermabrasion are all techniques that exploit the intrinsic healing capacity of skin. Patient outcome studies have found generally high patient satisfaction after facial plastic surgery, however variability exists¹. Epidermolysis, delayed healing, infection, scarring, and flap or graft compromise after surgery are major sources of dissatisfaction for patients and surgeons alike. Altered facial sensation, nerve injury, and aberrant nerve regeneration may also significantly affect patient quality of life and daily functioning², ³.

Impaired wound healing is observed in a host of settings including deficient oxygenation/ perfusion, infection, alcohol and tobacco use, poor nutrition, radiation, diabetes mellitus, hypothyroidism and use of blood thinners, corticosteroids and other immunomodulators⁴. Patients demonstrate a wide difference in wound healing capacity, and choice of surgical procedure is based upon imperfect information. Better understanding of which patients are at risk, and how to predict wound healing could improve surgical decision making.

Wound healing occurs via a carefully orchestrated progression through hemostasis, inflammation, proliferation and remodeling. A variety of cellular components and markers are essential to this complex cascade, including platelets, neutrophils, fibroblasts, and growth factors.^{5, 6} Recent attention has also focused on the role of cutaneous nerve fibers in wound healing.^{7, 8, 9} Substance P neuropeptide neurotransmitter and calcitonin gene-related peptide (CGRP) neurotransmitter, present in some non-myelinated nerve fibers, have a role in inflammation and burn wound healing, inducing vascular permeability and vasodilation as well as mast cell degranulation.¹⁰ Likewise, neuronal processes in the skin are postulated to result in differentiation of fibroblasts to myofibroblasts via direct contact, thus allowing for collagen contraction and wound maturation¹¹. Epidermal nerve fibers also play a role in

keratinocyte growth,¹² and interact directly with the immune system by directing interleukin production¹³ and perhaps other yet to be characterized pathways.

Quantitative assessments of epidermal nerve fiber density are the most sensitive and specific measures available for diagnosing peripheral neuropathy.¹⁴ Reduced density of epidermal nerve fibers is observed in diabetes and other disease states that may negatively impact wound healing. No quantitative data on these neural changes are currently available for the role of postoperative regeneration of cutaneous nerves after facial surgical procedures.

The main objective of this pilot study project was to quantify epidermal nerve fibers and their regeneration in un-operated, normal facial skin versus previously operated facial skin in rhytidectomy patients. We hypothesized that there would be a greater number of epidermal nerve fibers in samples from patients undergoing primary facelifts versus patients undergoing revision facelifts. In addition, we sought to characterize any differences between the two groups in terms of neurotransmitters, vasculature, subepidermal nerve plexus, and mast cell status.

Methods

Participants and Samples

Cutaneous specimens were collected from female patients (ages 42–66) undergoing primary (n=5) and revision rhytidectomies (n=3). A standard facelift incision was made in a preauricular crease extending behind the tragus and into the hairline. The preauricular skin flap was elevated in a subcutaneous plane and a small sample was excised from what would otherwise be discarded skin anterior to the tragus. Information and relevant clinical history were collected and subjects completed a survey questionnaire regarding their facial sensation. The Institutional Review Board at the University of Minnesota approved this study.

Processing

Specimens were immediately placed into cold Zamboni's fixative and stored at 4°C for 24 hours, then cryoprotected with 20% sucrose in 0.1 M phosphate buffered saline (PBS) until processed. Skin samples were frozen and 60 µm thick sections were cut with a sliding cryomicrotome. After blocking for non-specific binding with antibody buffer containing 5% normal donkey serum/Triton X-100/PBS, samples were immunostained using rabbit polyclonal antibody against protein gene product 9.5 (PGP 9.5) (AbD Serotec, 1:1,000) to label nerves and mouse monoclonal antibody against type IV collagen (Chemicon International, 1:6,000) to stain the epidermal basement membrane. Immunostaining for neuropeptides–with antibodies to substance P (SP), calcitonin gene-related peptide (CGRP), and vasoactive intestinal peptide (VIP), endothelium–with anti-CD31, and mast cell–with anti-tryptase was also performed. Biotinylated *Ulex europaeus* agglutinin type I and subsequent addition of Cy 5-conjugated streptavidin, further highlighted epidermis and blood vessels.¹⁴ Following overnight antibody incubation and washes to remove the primary antibodies, fluorophore-conjugated (Cy 2, Cy 3, and Cy 5) secondary antibodies (Jackson ImmunoResearch Laboratories, Inc.) were applied.

Tissue Analysis and Statistics

Images were acquired with Carv II confocal microscope (Becton Dickinson, Franklin Lakes, NJ) as16 z-sections at 2 μ m increments using a 20× objective. ENFs were quantified in three-dimensions using NeuroLucida image analysis software (MBF Bioscience, Williston, VT). ENFs that were seen penetrating the basement membrane were counted. ENF density was expressed by the number of nerve fibers per epidermal length (mm). ¹⁶

Mean values with standard deviation and range were calculated and presented by group. Because epidermal nerve fiber counts acquired from a given patient specimen are not independent data, the study was not powered to determine statistical significance at p<.05. For assessment of pain/dysesthesia, a Likert scale from 0 to 10 was used, with 0 representing no pain and 10 representing worst possible pain. Numbness sensation was evaluated with similar scale. For assessment of epidermal nerve fibers, subepidermal neural plexus, and mast cell degranulation, categorical scales were used (see table caption).

Results

Rhytidectomy skin specimens were collected from eight female patients ages 42 to 66 years old. Five subjects underwent a primary facelift and 3 subjects underwent revision facelifts 11 to 15 years after their initial procedure (Table 1). Chart review did not reveal any history of diabetes, chemotherapy or radiation therapy, hypothyroidism or other clinical history predisposing to poor wound healing other than smoking in one case.

There were dramatic differences in the appearance and number of epidermal nerve fibers between patients who underwent a primary lift compared to revision surgery. The primary facelift group overall demonstrated an abundance of robust appearing epidermal nerve fibers (Figure 1A). In comparison, the revision facelift group had a qualitative difference in the number and appearance of epidermal nerve fibers compared to the first time rhytidectomy patients (Figure 1B).

Table 1 shows the ages and ENF values in our primary and revision rhytidectomy patients. No correlation was seen between the number of nerve fibers and age. Subject 5 (Table 1) was a 47-year-old female who underwent a primary rhytidectomy and had 25-pack year history of smoking, quitting 1 month before surgery. Her epidermal nerve fiber (ENF) count of 14.2 per mm was the only outlier in the primary rhytidectomy group and was similar in value to the lower range of the revision group. Excluding this outlier, the other four primary rhytidectomy patients had ENF counts from 40 to 99 with a mean of 64. The other four revision rhytidectomy subjects had ENF counts between 13 and 25 with a mean of 18.

Table 1 also shows qualitative and quantitative characteristics of the epidermis in our primary and revision rhytidectomy patients. There was a trend towards a more robust subepidermal neural plexus (SNP) in primary rhytidectomy samples, with mild thinning noted in two secondary rhytidectomy patients versus one primary rhytidectomy patient. In addition, the SNP in the primary group included more nerve fibers positive for Substance P and CGRP. Capillary loops appeared more robust with complex structure in the primary compared to revision samples in which the capillary loops were more irregular. Mast cells in

revision rhytidectomy samples displayed a greater amount of degranulation, a phenomenon mostly absent in the primary rhytidectomy group. VIP-positive nerve fibers, which are normally sparse, were increased in revision samples. Self-reported sensation did not differ between the two treatment groups (see Table 2), including self-reported pain and paresthesias.

Discussion

To our knowledge, this is the first study comparing healthy, unoperated skin innervation with operated, surgically disrupted skin and concomitant postoperative nerve regeneration in primary and revision rhytidectomy samples. Facial skin of primary and revision rhytidectomy patients exhibited differences in epidermal nerve fiber density, dermal nerve plexus structure, preferentially expressed neurotransmitters, and associated neurovasculature. Mast cell degranulation was also significantly affected after the initial rhytidectomy. Changes in regeneration of peripheral nerves have been previously described.¹⁷ Possible theories for this disruption in cutaneous nerves in our study include devascularization of neurovascular structures during previous surgery, degeneration of neural fibers after transection, and hindrance of nerve fiber growth during attempted regeneration after collagen deposition from scarring.

Differences in skin neuropeptide expression were also apparent between primary and revision groups. Patients in the primary rhytidectomy group generally display more normal profiles of neurotransmitter levels, with CGRP more common than Substance P and minimal VIP expression (Kennedy lab, personal communication). This profile is shifted towards increased Substance P and VIP expression in the revision rhytidectomy group. VIP in particular has been shown to be more prominent in the context of nerve regeneration. This suggests that not only is the density of epidermal nerve fibers altered, but there appears to be a fundamental shift in the function of the remaining nerve fibers with potential downstream effects on other cutaneous components including epidermal and dermal vasculature.

Whereas the primary rhytidectomy group showed no mast cell degranulation, the revision rhytidectomy group displayed minimal to widespread mast cells degranulation. Mast cells play an integral role in wound healing¹⁸. This may be an indication of disrupted immune system function with downstream effects due to release of histamines, proteoglycans, serotonin and serine proteases from mast cell granules, as well as changes in endothelial cell functionality. It is important to note that mast cell degranulation is an integral part of wound healing, and the observed changes may not necessarily represent a disruption in proper immune system function

The samples from the primary rhytidectomy subject who smoked cigarettes are of particular interest due to low epidermal nerve fiber density and decreased values in other neuronal markers. The cutaneous innervation of this individual, with significant tobacco history, resembled that observed in secondary rhytidectomy patients. Wound healing is known to be impaired in smokers resulting in higher graft and flap failure rates ^{19, 20}. This finding provides additional support for the deleterious wound healing effects of tobacco use. While the mechanism of smoking-impaired wound healing is certainly multifactorial and only

partially elucidated, the findings of decreased nerve fiber density and neuronal markers suggests neuromodulation in the skin may play a role.

It is unclear why the dramatic reduction in epidermal nerve fibers after surgery did not result in more complaints about decreased sensation in our patients. It is possible that the subjective assessment was not able to detect subtle differences in sensation threshold that more objective quantitative nerve fiber studies would be able to elucidate. The use of a nonvalidated instrument is a limitation of this study. Assessing sensation pre-operatively and post-operatively at serial time points, using subjective and objective measures would increase sensitivity and increase the likelihood of detecting clinical correlation with epidermal nerve fiber counts. We plan to include quantitative sensory testing in future studies to evaluate any functional sensory changes further and address a potentially subtle correlation to postoperative anatomic and structural epidermal nerve fiber alterations.

Several limitations of this pilot study include the relatively small sample size and the descriptive nature of our study design. Although we can hypothesize that the primary procedure caused disruption of normal cutaneous innervation, the cause of this difference cannot be determined by the current study design. Larger scale investigations are needed to determine if the anomalous findings in the former tobacco user are typical.

The current pilot study lays the groundwork for several future additional directions. First, we plan to include additional immunohistochemical markers in future studies to address a connection between cutaneous nerve disruption and mesenchymal stem cell signaling. Cutaneous nerves likely provide trophic signals for tissue regeneration after injury and mesenchymal stem cells may be a downstream recipient of such signaling. Specifically, these stem cells may serve as a proxy measure for upstream neuronal signaling during tissue repair after injury. Second, additional staining for collagen can be included in future studies to quantify the amount of scarring present in primary and revision rhytidectomy samples and address the effect of any differences in scar formation between the two groups on wound healing. Finally, we plan to expand our biopsy site selection. The biopsies used for this study were taken from the area near the incision, where one would expect the greatest mechanical disturbance of epidermal nerve fibers during rhytidectomy. It remains to be studied if vascular and neurologic characteristics differ across different epidermal sites, and future studies can include a second biopsy site for comparison.

Cutaneous nerves may help orchestrate multiple players involved in wound healing. Transection of cutaneous nerves is inevitable during surgical procedures, but baseline state may offer valuable insight into the wound healing potential of prospective surgical patients and nerve regeneration. Further study is needed to clarify this potential effect. Despite small sample size, our use of rhytidectomy skin affords insight into rhytidectomies and flap elevations. Cutaneous innervation is easily accessible to study via a small, 2–3mm punch biopsy preoperatively. Should epidermal nerve fiber density become a useful marker for the postoperative healing course, we would have a better understanding of which patients are at risk for potentially poor cosmetic outcomes and could counsel them on their treatment options accordingly.

Conclusion

Rhytidectomy is associated with decrease in ENF density and qualitative changes in the nerve fibers, capillaries, and mast cells. The data raise the possibility that smoking may decrease ENFs compared to non-smokers. Future studies are needed to determine whether clinical symptoms and wound outcomes correlate with immunohistochemical findings. The development of screening tools for predicting wound healing potential could be valuable to counseling patients before surgical treatment. This may also have implications on selection and the adjunctive use of ablative treatments, such chemical peels and laser treatments and topical treatments to aid in wound healing and/or minimize scars.

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Figure 1. Representative Confocal Microscopic Images of Sample Subjects' Cutaneous innervation and Mast Cells

A) Confocal microscopic image taken of facial skin from a 53-year-old female who underwent a primary facelift. (40.6 epidermal nerve fibers per millimeter).

Basement membrane (BM) - red, epidermal nerve fibers (ENFs) - green, capillaries (CAP) - pink.

B) Specimen from a 64-year-old female who underwent a revision facelift 11 years after her primary surgery. (13.8 epidermal nerve fibers per millimeter)

C) Specimen from a 47-year-old female with a 25 year history of smoking who underwent a primary facelift. (14.2 epidermal nerve fibers per mm²)







Figure 2. Representatitive Images of Neuropeptides in Dermis compared with Non-peptidergic Neural Fibers

A) Substance P

- B) Calcitonin Gene-Related Peptide
- C) Vaso-active Intestinal Peptide



Figure 3. Representative Mast Cell Images

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Table 1

Demographics and Confocal microscopy results

Subject	Primary vs. Revision Rhytidectomy	Age/Sex	Smoker	Number of ENF (mm)	SNP	Sebaceous Glands	SP	CGRP	VIP	Mast Cell Degranulation	Capillaries
1	Primary	66 F	oN	67.0	0	0	14	15	4	0	Normal
2	Primary	47 F	Yes	14.2	-1	-1	1	6	1	-1	Complex
3	Primary	56 F	oN	50.8	0	0	7	1	0	0	Normal
4	Primary	42 F	oN	99.2	0	0	4	15	0	0	Normal
5	Primary	53 F	oN	40.6	0	0	1	8	0	0	Normal
9	Revision	64 F	oN	13.8	-1	-2	0	3	1	7-	Irregular
7	Revision	65 F	oN	17.0	-1	0	0	0	2	-3	Irregular
8	Revision	64 F	oN	25.0	0	0	0	7	17	-1	Complex
F – female,	ENF, epidermal nerve fiber, SNP – sube _f	pidermal neu	rral plexus, S	SP – substance P, CGRP – c	alcitoniı	n gene-related peptide,	VIP-v	asoactive	intestina	al peptide	

Grading System (ENF, Sebaceous Glands): 0 (normal), -1 (mild loss), -2 (moderate loss), -3 (severe loss), -4 (no nerve), +1 (hyper)

Grading System (SNP): 0 (normal), -1 (mild thinning), -2 (moderate thinning), -3 (sparse), -4 (no nerve)

Grading System (Mast Cell Degranulation): 0 (none), -1 (minimal or <10%), -2 (moderate or <50%), -3 (diffuse or <75%)

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Table 2

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	Rig
	Chin Sensation (Standard)
e Clinical Findings	Primary vs. Revision Rhytidectomy
Subjectiv	Subject

Subject	Primary vs. Revision Rhytidectomy	Chin Sensation (Standard)	Right Facial Sensation (0 – 10)	Left Facial Sensation (0 – 10)	Right Facial Pain (0 – 10)	Left Facial Pain (0 – 10)	Right Facial Paresthesia (0 – 10)	Left Facial Paresthesia (0 – 10)
1	Primary	10	10	10	0	0	0	0
2	Primary	10	8	6	2	2	4	4
3	Primary	10	9	10	0	0	0	0
4	Primary	10	10	10	0	0	0	0
5	Primary	10	10	10	0	0	0	0
9	Revision	10	8	8	0	0	0	0
7	Revision	-	Ι	-	I	I	I	-
8	Revision	10	1	3	0	0	5	0