Smoking and other influencing factors on skin and scar quality in surgery

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Smoking and other influencing factors on skin and scar quality in surgery

An Elisabeth Karel Deliaert

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Voor Pieter en Lola

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Chapter 1

***General introduction and outline
of the thesis***

General introduction

Adequate wound healing is of great importance for survival of all living organisms. Especially in prehistoric times, uneventful and fast healing was crucial in order to prevent infection and excessive water loss, which was needed for survival. A fundamental problem in human healing resides in the fact that it is inevitably associated with scar formation. Scarring might inhibit normal function due to a limitation of motion because contractures occur. Rudimentary organisms, such as salamanders, are capable of regenerating complex tissue via regenerating rather than scarring.[1] This phenomenon of regeneration is also seen in fetal healing however this capacity is lost after birth.

In adult wound healing a certain equilibrium is necessary to ensure a healthy and functional scar. After re-epithelialization is finished, a “stop” signal is needed to end the repair process, resulting in a mature scar. Otherwise it continues unabated and causes overabundant scar tissue resulting in hypertrophic or keloidal scar tissue.

The following part of this introduction will begin with the general principles of wound and scar healing. Thereafter, the influence of the role of inflammation will be highlighted. Further, objective assessment of scar erythema is discussed. Finally, some therapies and more specifically the role of smoking and antiseptic approach in wound and scar healing will be briefly discussed.

Wound healing

Wound healing of the adult skin is a dynamic process that depends on several factors. It consists of three overlapping stages: inflammation, proliferation and remodelling.

The first stage (Figure 1) is initiated immediately after tissue damage and begins with vasoconstriction and induction of the clotting cascade. When haemostasis is achieved and a fibrin matrix is formed, degranulating platelets release histamine, which results in vasodilation. Histamine also induces blood vessels to become more porous, which results in leakage of proteins and immune cells into the extravascular space. The polymorphonuclear neutrophils are the first immune cells at the wound bed and they facilitate phagocytosis during the first two days after wounding. Their life span is relatively short and the neutrophils will be replaced by monocytes that eventually differentiate into macrophages. Macrophages play an important role in the transition between the inflammatory stage and the subsequent proliferative phase of wound healing. They first achieve phagocytosis of apoptotic neutrophils and clear debris; subsequently they have a considerable function in the secretion of growth factors such as platelet-derived growth factor (PDGF) and transforming growth factor (TGF- β) and cause fibroblast migration and activation.[2]

Figure 1. Inflammatory stage in wound healing.

The second stage of wound healing is characterised by new tissue formation: the proliferation of new cells (Figure 2). This process is estimated to begin within 2 to 48 hours and remains active until day 10 after wounding. The fibrin formed extracellular matrix is being replaced by granulation tissue that is formed by fibroblasts and endothelial cells. Granulation tissue on the other hand facilitates re-epithelialisation that occurs through migration and proliferation of keratinocytes over the injured dermis. After the inner layer, formed by endothelial cells in granulation tissue, has been completed, new blood vessels are formed. This process is known as angiogenesis. Important factors for angiogenesis such as Vascular Endothelial Growth Factor A (VEGF-A) and Fibroblast Growth Factor 2 (FGF-2) are being released.[3] Because of the collagen production, fibroblasts play a crucial role in this stage of wound healing. A part of the fibroblasts differentiate into myofibroblasts and they are responsible for the contraction of the wound. Collagen, at the beginning in particular type III, functions as a predominating tensile substance and will be replaced by the stronger type I in the final phase of wound healing. This stage of wound healing is terminated when granulation ceases, resulting in a decrease in the number of (myo)fibroblasts.

Figure 2. Proliferation of new cells.

When re-epithelialisation is finished, reorganization is initiated. This last stage (Figure 3) is dominated by apoptosis and a more acellular surface is created. Collagen type III is being replaced by the more tensile type I and reorganization of collagen fibres along the tensile lines will be installed. This process results in new tissue that however never regains the properties of uninjured skin.

Figure 3. Reorganization of cells.

Scar healing

A scar has been evaluated through different stages of maturation before ending in a mature scar that is flat and pale. This process takes about one year but can exceed over 2-3 years or even longer in case of pathologic scarring.

Pathologic forms of scarring

Hypertrophic scars and keloids (Figure 4), the two types of pathological scarring, are mainly distinguished on the basis of their clinical appearance. They are most common in the second decade of life and no preference of gender is seen. [4] Hypertrophic scars (Figure 4) are red, raised scars and the border remains typically within the confines of the original lesion.[5] This is in contrast with

keloids, which grow expansively beyond the wound borders and these scars do not spontaneously regress.[5-7] A preference for dark pigmented skin is seen in this last type of scarring.[8]

Figure 4. Hypertrophic scar after a median sternotomy (left) versus a keloidal scar after excision of a naevus (right)

Hypertrophic scars develop generally 4 to 12 weeks after re-epithelialization and occur in 39-68% of the surgical patients and in 32-72% of the burn patients.[9-11] The development of hypertrophic scarring after burn injury affects appearance, range of motion, pain sensation, sensitivity and compromises the protective function of the skin resulting in a reduced quality of life.[12, 13] Hypertrophic scars develop preferably in locations with excessive tensile forces such as sternum, back, neck, and at flexor surfaces of joints after injury or surgery. Keloids, in contrast, may develop years after minimal injury and have a preference to develop on locations with minimal tension such as earlobes, cheeks and upper arm.[14] These two types of scarring are both fibroproliferative disorders that have an abundance of dermal collagen in common, but in addition to their difference in appearance, they also have biological and molecular differences. Hypertrophic scars consist mainly of type III collagen that are organized in a wavy pattern parallel to the epidermal

surface. On the contrary, keloids have larger and more irregularly shaped collagen type I and III fibrils that lie in loose sheets, which are randomly oriented to the epithelial surface. A failure in appropriate down-regulation is suggested in both types because of the presence of multiple fibroblasts proteins and fibronectin. [14] Hypertrophic scars and keloids are two entities. Therefore, this thesis focuses only on hypertrophic scarring. In the following paragraph the pathophysiologic process of hypertrophic scarring will be discussed.

Important influencing factors in the pathophysiology of hypertrophic scar formation

Wound healing requires an accurate healing balance of different interacting factors before it results in a normotrophic scar. Impaired wound healing can result in chronic wounds or in case of enhanced healing it may result in hypertrophic scarring or keloids. Hypertrophic scarring is thought to be a consequence of prolonged inflammation, excessive proliferation and a disturbed remodeling with a decreased apoptosis. An enhanced inflammatory phase seems to be responsible for the derailment of wound and scar healing.[15] This is the reason why large burns and infected wounds, wherein healing time proceeds two to three weeks (or longer), have an increased risk of hypertrophic scar formation.[16] Fibroblasts and macrophages play a crucial role in the derailment of the inflammatory phase, resulting in aberrant wound and scar healing. Fibroblasts are responsible for the production of the new extracellular matrix (ECM) and have an important role in growth and proliferation of cells. They produce important profibrotic cytokines, which induce collagen production, such as Platelet Derived Growth Factor (PDGF), Transforming Growth Factor- β (TGF- β) and Insulin-like Growth Factor (IGF). These cytokines again have profibrotic properties and overstimulation can result in hypertrophic scarring.

Fibroblasts are naturally present in the dermal cellular layer of the skin. Macrophages are on the other hand only in a limited number present in the

skin. They have their origin in bone marrow or are derived from monocytes in circulating blood. The latter population of macrophages, which have their origin from the circulating blood, is only present after wounding and its number peaks during the inflammatory stage of wounding.[17] Macrophages can differentiate at the wound site into two phenotypes: M1 and M2; M1 macrophages are classically activated by bacterial products like lipopolysaccharide (LPS) and Interferon (IFN)- γ and M2 macrophages are activated by IL-4 and IL-13. [17] The first subgroup is important in the early inflammatory stage to phagocytise debris and to kill invading pathogens because of their release of inflammatory mediators inducing Tumour Necrosis Factor (TNF)- α , Nitric Oxide (NO) and Interleukin-6. The second group, the M2-macrophages, induces an inhibition of the inflammatory reactions and plays an important role in wound healing and angiogenesis because it is a prominent source of TGF- β .

The TGF- β family, which includes TGF- β 1-3, has a specific role in scar formation. Tissue Growth Factor- β 1 predominates in adult wound healing and is a profibrotic growth factor. Fetal wound healing, characterized by scarless healing, shows more TGF- β 3 than scarring wounds.[18] This relation is also seen in wound healing in mice; the first day after wounding TGF- β 1 expression is high in the wound area, thereafter its expression decreases and at day three TGF- β 3 dominates. A cross regulation between TGF- β 1 and TGF- β 3 is observed, resulting in an alteration between the two isoforms of the TGF- β family; when TGF- β 1 is highly expressed TGF- β 3 is low and vice versa. A high ratio of TGF- β 3 to TGF- β 1 results in clinically improved scarring. No effect was seen in wound healing rate.[19] This may also explain prolonged healing in wounds in glucocorticoid treatment. Glucocorticoid use is known to provoke delayed wound healing resulting in chronic wounds. In general, delayed wound healing is an eliciting factor for the development of hypertrophic scarring. However, hypertrophic scarring is rare in corticosteroid use. In this population reduced expression of TGF- β 1 is associated with a high ratio TGF- β 3 to TGF- β 1.[20] To date, this superfamily of growth factors is still under

research. A human recombinant TGF- β 3 is developed which shows a reduction in scar formation after full thickness skin incisions.[21]

Besides the alteration in macrophages, fibroblasts and growth factors, there is also excess in the formation of new microvessels in hypertrophic scarring. These microvessels are, compared to normal skin and normal scars, present in an increased number and are longer and more dilated.[22] They grow upward in the scar and show occlusion at the distal end of the lumina due to an excess of endothelial cells probably because of disturbed apoptosis.[23] These microvessels degenerate during time, resulting at the end of scar healing process, in a pale scar. Thereby, the regression of redness is an important parameter that is used during the follow-up of scar therapy. Clinically, an association is observed between the persistence of erythema and hypertrophic scar formation.[13]

Scar assessment

Objective measurements and assessments provide important quantitative data that can be used to determine the clinical progression of scars and to assess the efficiency of therapeutic interventions on scar healing. They are preferred over subjective rating scales since they lack the bias of subjective interpretation. [24] Frequent assessed objective parameters during scar follow-up are color, thickness, pliability (elasticity). Color assessment is an important parameter during the follow-up of scars. During scar healing erythema decreases after several months.[25] Redness or erythema (reflecting the healing activity or inflammatory response) is considered as the most important feature in the evolution of a scar. A tristimulus reflectance colorimetry, like the Minolta Chromamater (Konica Minolta, Japan), represents color in the same way the human eye does. The level of light is reflected through three broad wavelength filters and the color is described by three values $L^*a^*b^*$. [26] The a^* value is mostly used and represents the redness of the scar: the higher the value the more reddish the color of the scar.[27] A decrease in redness with the colorimeter shows a tendency towards

less hypertrophic scarring which makes the colorimeter an excellent parameter in the follow-up during scar healing.

Therapies

Scar management begins with prevention of abnormal wound and scar healing. In case of surgical wounds, the incisions must be placed parallel to Langer's lines because this results in the least amount of tension. In addition, meticulous suturing and prevention of wound infections is crucial. In case of burn wounds early surgical therapy must be performed if wound closure is estimated to be more than 2 to 3 weeks because this is the turning point when hypertrophic scarring arises. Local therapy is advised once excessive scarring is identified or prophylactic in case of wide spread scars such as burn scars with a prolonged healing. Many treatment modalities have been developed. However, few have been supported by randomized, controlled, prospective studies. The International Advisory Panel on Scar Management advises to start initial treatment with silicone gel sheeting, steroid injection and localized pressure therapy.[28, 29] Hydrating creams or silicone sheets reduce pruritus and have been shown to be beneficial for the appearance of the scars. These tools limit transepidermal water loss in function to restore the stratum corneum barrier that is damaged after injury. [30, 31]

The influence of smoking on skin and wound healing

Tobacco smoking has its origin in the Peruvian and Ecuadorian Andes. Christopher Columbus observed in 1492 that the Arawak Indians were smoking tobacco leafs. [32]. Nicotine, one of the constituents of tobacco smoke, is named after the French ambassador Jean Nicot, who found that the leaf had healing powers. After he treated several patients that were cured, he introduced the tobacco plant in France in the 16th century. First it was used as a topical treatment but soon it was taken as a tobacco-snuff. Nowadays smoking is associated with mainly negative effects resulting in addiction and an increased morbidity. Smoking is still the leading

cause of preventable death in the world. In addition, smoking is also known for its effect on the skin resulting in precocious aging. Typical aging features such as wrinkling are known to occur earlier in life in smokers compared to non-smokers. [33] The acceleration of aging due to smoking is correlated with the number of cigarettes smoked per day and the total years of smoking.[33] These features could be due to an altered biosynthesis and degradation of dermal connective tissue proteins. Precocious aging is characterized by an increased down regulation of collagen type I and III.[32] Despite the effect on skin, smoking was also shown to exert anti-inflammatory effects on different cell types. Ulcerative colitis and recurrent aphthous stomatitis benefit from smoking.[34-36] The progression of the disease aggravates after smoking cessation and it ameliorates after resumption of smoking.[37] Nicotine has also been successfully used in the treatment of pyoderma gangrenosum, Behçet's syndrome and erythema nodosum.[38-41] Smoking seems to result in a balance that on one hand results in a negative effect, or otherwise it can mute the exaggerated inflammatory response. This last effect could be beneficial in the development of hypertrophic scarring.

The use of antiseptic agents in wound healing

Inflammation, in particular the inhibition of excessive inflammation, is the keyword for proper wound and scar healing. Antiseptics inhibit or destroy the growth and development of microorganisms in or on tissues. In chronic wounds, antiseptics are used to reduce colonization and thus bring a more rapid closure of the wound. In addition, the perioperative use of impregnated materials could become a tool of choice to inhibit inflammation, which eventually create better scars with less inflammation.

Aim of the thesis

- To evaluate whether age at surgery differs between smokers and non-smokers undergoing a standard cosmetic procedure (blepharoplasty).
- To compare smoking habits with respect to a standard cosmetic procedure (blepharoplasty) in the general Dutch population.
- To evaluate the effect of nicotine during the inflammatory phase of cutaneous wound healing in mice
- To investigate the effect of triclosan-coated suture material on wound healing.
- To compare the reproducibility of the Laser-Doppler Imaging and colorimetry in the assessment of scarring.
- To investigate the effect of smoking on scar healing in women undergoing a reduction mammoplasty.
- To evaluate the effect of smoking on scar healing after cardiac surgery with a median sternotomy.

Outline of the thesis

Smoking is known for its carcinogenic effect and its negative effect on wound healing. In addition, smokers are more likely to develop typical skin aging features earlier in life. **Chapter 2** compares smoking habits between a group undergoing a standard cosmetic procedure (blepharoplasty) and the general population and to determine whether smokers undertake this procedure at an earlier age.

Inflammation plays a crucial role during cutaneous wound healing and it influences the quality of the resulting scar. Nicotine, a constituent of tobacco smoke, has been shown to exert anti-inflammatory effects on different cell types and to be beneficial in disorders where inflammation-related mechanisms are involved.[41] **Chapter 3** investigates the effect of nicotine in an experimental mouse model of cutaneous injury and healing responses during the inflammatory phase of repair.

In **Chapter 4**, a randomised prospective pilot study was performed to investigate the effect of triclosan-coated suture material on wound healing in women undergoing breast reduction.

Objective assessment of scar formation after skin injury is crucial to set up a valuable scar treatment. Several tools are used to deliver quantitative data about the scar in order to determine the clinical progression of scars. The measurement of the redness of a scar is considered to be the most important feature in the assessment of hypertrophic scars after burns. Colorimetric assessment is the standard in follow-up of scarring.[13] Therefore, we performed a comparative pilot study to investigate the reproducibility of the laser-doppler imaging and colorimetry in the assessment of scarring in **Chapter 5**.

Smoking is known to result in more wound related postoperative complications, probably by disturbing the normal inflammatory physiology.[42] A prolonged inflammation is also seen in burn wounds, resulting in hypertrophic scarring. [13, 43] However, smokers rarely present with hypertrophic scars in our experience. We therefore performed a prospective patient-controlled study to compare postoperative scar activity in smoking and non-smoking women undergoing a reduction mammoplasty in **Chapter 6**.

Hypertrophic scarring occurs frequent in areas where tension dominates.[9] The thorax is a preferential location for the development of hypertrophic scarring.[44] **Chapter 7** evaluates the redness as parameter of hypertrophy and the incidence of scar hypertrophy among smokers, ex-smokers and non-smokers after a median sternotomy.

Chapter 8 provides a summary, general discussion and challenges for future research.

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Chapter 2

***Smoking in relation to age in aesthetic
facial surgery***

A.E.K. Deliaert

E. van den Kerckhove

S. Tuinder

S. Fieuws

J.H. Savor

M.A. Caberg-Meesters

Abstract

Background: Smoking is a major cause of premature facial aging. Skin aging in general, often accompanied by wrinkling and furrowing, plays a significant role in the decision to undergo aesthetic surgery. Smoking may therefore be related to the demand for cosmetic surgery. This study aimed to compare smoking habits with respect to a standard cosmetic procedure (blepharoplasty) in the general population and to evaluate whether the age at surgery differs between smokers and non-smokers.

Methods: A questionnaire was sent to 517 patients with valid reports describing dermatochalasis of the upper eyelid who subsequently underwent an upper-eyelid correction in 2004. Smoking habits, socioeconomic status, and medical history were evaluated. The patients were classified as smokers, ex-smokers with at least 1 year of smoking cessation, and never-smokers.

Results: Of the 353 questionnaires (68.3 %) returned, 345 were eligible for statistical analysis. The smoking habits did not differ between the blepharoplasty group and the general population. However, the smokers underwent surgery an average of 3.7 years earlier than the ex-smokers ($p = 0.0007$) and 3.5 years earlier than the never-smokers ($p = 0.006$). No significant difference was observed between the ex-smokers and the never-smokers.

Conclusions: This is the first study to describe an association between smoking habits and an earlier need for upper-eyelid correction among smokers. The mechanism of skin restoration could result in a regenerative mechanism among ex-smokers, but further research is needed to support this hypothesis.

Introduction

Facial aging is characterized by excessive skin, furrows, and wrinkling. Cigarette smoke and chronic sun exposure are thought to be factors contributing to premature skin aging.[1-4] In addition to its deleterious effect on the skin, smoking is particularly known for its carcinogenic effect and the development of cardiovascular and chronic obstructive pulmonary diseases. Moreover, it results in a disturbed microcirculation and subsequent delayed wound healing.[5] Face-lifting is one of the procedures preferably not performed for smokers because of the disturbed vascularization.[6, 7] On the other hand, it is likely that smokers will develop typical skin-aging features earlier in life and may therefore feel the need for cosmetic surgery at an earlier age. However, a relation between smoking and cosmetic surgery has never been evaluated. Therefore, this study aimed to compare smoking habits between a group undergoing a standard cosmetic procedure (blepharoplasty) and the general population and to determine whether smokers undertake this procedure at an earlier age.

Material and methods

The study was performed between January and December of 2004 at the University Medical Center of Maastricht and at the VieCuri Medical Center Venlo, both situated in the south of the Netherlands. Patients were admitted to the study if a superior blepharoplasty was indicated. Until 2005, this surgical procedure was reimbursed by the patients' insurance company. To avoid socioeconomic status bias, we included only patients who underwent surgery in 2004. The indication to perform an upper-eyelid correction was determined by a plastic surgeon or a senior resident in plastic surgery. For inclusion in the study, the patients had to have valid complaints that were consistent with physical findings indicating that the complaints were amenable to surgical improvement. The complaints were excess skin of the upper eyelid, upper-eyelid folds, and periorbital wrinkles.

Moreover, the complaints had to be associated with a subjective decreased range of vision. Contraindications were Graves' disease and inflammatory disorders of the eyelid. A questionnaire was sent to all patients ($n = 517$) who underwent an upper-eyelid correction. Informed consent was included in the questionnaire, and written consent was obtained. The questionnaire was subdivided into two sections. In the first section, questions concerning gender, age, and education were asked. The highest-attained education level was defined as lower education (elementary or junior high school), middle education (middle or high school), or higher education (college or university). The second section focused on medical history and smoking status. The patients were classified as smokers, ex-smokers, or never-smokers. Smokers were asked to report the daily number of cigarettes smoked and their age when they began smoking. Ex-smokers were defined as those who had not smoked for at least 1 year. They were asked to indicate the number of years they had smoked and the year when they quit smoking. In addition, questions were asked about personal care and lifestyle. The percentages of smokers, ex-smokers, and never-smokers were related to the expected distribution in the Dutch population based on data from the Continuous Survey of Smoking Habits (DCSSH). This cross-sectional population survey of respondents 15 years of age or older is used on a weekly basis to monitor the smoking habits of the Dutch population. The DCSSH is conducted by TNS NIPO, a Dutch Institute for Public Opinion and Market Research.[8]

Statistical analysis

Kruskal–Wallis, Mann–Whitney U, and Fisher's exact tests were used to make comparisons among smokers, ex-smokers, and never-smokers. Chi-square tests were used to compare the smoking behavior in the current sample with that in the Dutch population, taking sex distribution into account. The percentages were based on subjects for whom the information was complete. All analyses were performed using the statistical package SAS (version 9.1; SAS Institute Inc., Cary, NC, USA). All p values lower than 0.05 were considered significant.

Results

Of the 353 patients (68.3 %) who returned a completed form, eight were excluded due to relocation ($n = 5$), death ($n = 1$), or refusal to participate ($n = 2$). A total of 345 questionnaires were used for statistical analysis. The mean age of all the patients at the time of operation was 54.9 ± 9.3 years. The mean body mass index (BMI) was 25.4 ± 4.6 kg/m². This study enrolled 61 male and 284 female patients (17.7 vs 82.3%). Information on smoking status was available for 323 patients (93.6%), 86 (26.6%) of whom were smokers who smoked an average of 13.8 ± 7.1 cigarettes a day. Their smoking had begun at a mean age of 20.0 ± 9.6 years. These patients included 135 ex-smokers (41.8%) who had stopped smoking an average of 20.4 ± 11.0 years before the surgical procedure. Also included were 102 never-smokers (31.6%).

Table 1. Comparison among smokers, ex-smokers, and never-smokers.

Variable	Smokers (N = 86), n (%)	Ex-smokers (N = 135), n (%)	Never smokers (N = 102), n (%)	p-value ^a
Mean age at operation (years)	52.0 $\pm$ 8.9	55.7 $\pm$ 8.0	55.5 $\pm$ 10.7	0.002*
Mean BMI (kg/m ²)	25.0 $\pm$ 5.0	25.5 $\pm$ 4.7	25.7 $\pm$ 4.2	0.15
Education				0.059
Low	36 (42.9)	36 (27.3)	40 (40)	
Middle	38 (45.2)	72 (54.5)	40 (40)	
High	10 (11.9)	24 (18.2)	20 (40)	
No information	2	3	2	
Monthly sun bed use	17 (19.8)	36 (26.9)	28 (27.5)	0.41
No info	0	1	0	

* Significant p-value

^a $p = 0.0007$ for smokers compared with ex-smokers; $p = 0.006$ for smokers compared with never-smokers; $p = 0.83$ for ex-smokers compared with never-smokers.

Table 1 presents a comparison of data relating to BMI, age, education level, and sun bed use among the smokers, ex-smokers, and never-smokers. The smokers underwent surgery 3.7 years earlier than the ex-smokers ($p = 0.0007$) and 3.5 years earlier than the never-smokers ($p = 0.006$). The ex-smokers and never-smokers

did not differ significantly. The smoking behavior of the patients in the current sample was compared with that in the Dutch population for which data relating to gender were available. The differences between the observed and expected smoking behaviors were small and not significant ($p = 0.66$) (Table 2).

Table 2. Comparison between observed smoking behavior and expected smoking behavior based on the sex distribution in the sample.

Variable		Females n (%)	Males n (%)	Total
Smokers	O	74 (86.0)	12 (14.0)	86
	E	71.2 (82.4)	15.2 (17.6)	86.4
Ex-Smokers	O	106 (78.5)	29 (21.5)	135
	E	99.2 (77.6)	28.6 (22.4)	127.8
Non-Smokers	O	89 (87.3)	13 (12.7)	102
	E	98.5 (90.5)	10.3 (9.5)	108.8
p-value	[1]	0.50	0.48	0.66
	[3]	0.34	0.23	0.66

O observed number of subjects; E expected number of subjects; [1] p-value for the comparison among the three groups; [3] p-value for the comparison of never-smokers with smokers and ex-smokers combined

Discussion

This retrospective cohort study aimed to observe whether smoking is related to cosmetic surgery. The results of the study showed that the distribution of smokers and ex-smokers in the blepharoplasty group equaled that in the Dutch population. However, the smokers were significantly younger than the ex- and never-smokers at the time of surgery. This observation appears related to the deleterious effects of cigarette smoke on facial skin. Such effects were not seen in ex-smokers. Because this study was performed at a time when all patients with dermatochalasis were reimbursed for surgical procedures, a selection bias due socioeconomic status was less likely. This is the first study to describe an earlier need for uppureyelid correction in smokers. It is known that smoking, age, and sun exposure are independent risk factors that contribute to wrinkle formation. [9] Sun exposure is one of the most studied factors leading to premature aging. However, cigarette smoke predisposes skin to aging more than sun exposure, and

the daily use of 20 cigarettes is equivalent to almost 10 years of aging.[1] This was confirmed in a study of monozygotic twins that showed a significant cumulative increase in apparent age relative to the years of smoking.[10] Besides the effect of smoking on the skin, factors contributing to wrinkle formation may be squinting of the eyes and pursing of the lips during the inhalation of cigarette smoke.[11] Findings have shown that furrow depth is greater in smokers with 35 pack-years, but the number of furrows is fewer than with never-smokers.[12] Dermatochalasis could be the result of the large furrow depth and the decreased number of furrows around the eyes. The molecular mechanism of aging seems to be accelerated by smoking. Smoking results in blockage of the cellular responses of the fibroblasts, leading to decreased collagen synthesis, an induced matrix metalloproteinases expression, and abnormal accumulation of elastic fibers and proteoglycans.[12] It is not known whether cessation of smoking results in partial restoration of the skin. In our study, we observed no difference between ex-smokers and never-smokers. The average time between cessation of smoking and surgery, however, was 20.4 years. We conclude that during this period, the effect of smoking on skin aging with respect to dermatochalasis had disappeared, probably due to the regenerative capacity of the skin. This is in line with other studies, which have shown that nicotine abstention results in restoration of the inflammatory cell oxidative burst.[13]

Conclusion

Although this study was limited because of its retrospective character, it strongly suggests a relation between smoking and the age at which cosmetic surgery is performed.

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Chapter 3

***Nicotine effect on inflammatory and
growth factor responses in murine
cutaneous wound healing***

S. Xanthoulea

A.E.K.Deliaert

A.Romano

S.S Rensen

W.A.Buurman

R.R.W.J. van der Hulst

Abstract

The aim of the current study was to investigate the effect of nicotine in an experimental mouse model of cutaneous injury and healing responses, during the inflammatory phase of repair. Nicotine injection in full-thickness excisional skin wounds minimally affected inflammatory mediators like TNF, IL-6 and IL-12 while it induced a down-regulation in the expression of growth factors like VEGF, PDGF, TGF- β 1 and TGF- β 2, and the anti-inflammatory cytokine IL-10. Analysis of wound closure rate indicated no significant differences between nicotine and saline injected controls. In-vitro studies using bone marrow derived macrophages, resident peritoneal macrophages and RAW 264.7 macrophages, indicated that nicotine down-regulates TNF production. Moreover, nicotine was shown to down-regulate VEGF, PDGF and TGF- β 1 in both bone marrow derived macrophages and RAW 264.7 cells. Using an NF- κ B luciferase reporter RAW 264.7 cell line, we show that nicotine effects are minimally dependent on NF- κ B inhibition. Moreover, nicotinic acetylcholine receptor (nAChR) subunit expression analyses indicated that while β 2 nAChR subunit is expressed in mouse macrophages, α 7 nAChR is not. In conclusion, while skin inflammatory parameters were not significantly affected by nicotine, a down-regulation of growth factor expression in both mouse skin and macrophages was observed. Reduced growth factor expression by nicotine might contribute, at least in part, to the overall detrimental effects of tobacco use in wound healing and skin diseases.

Introduction

Cutaneous wound healing after an injury is a complex and highly dynamic process that involves interaction of different players like resident cells of the skin, inflammatory leukocytes, extracellular matrix components and soluble mediators. The healing process can be divided in three consecutive and partially overlapping phases i.e. inflammatory phase, proliferative phase and remodelling phase. The repair process finally results in the formation of a mass of fibrotic tissue known as scar.[1, 2]

Numerous experimental and clinical studies have determined that inflammation plays a crucial although still not completely clear role during cutaneous wound healing and influences the quality of the resulting scar. Inflammatory cells and particularly macrophages appear to be essential for proper healing by, among other mechanisms, stimulating growth factor and anti-inflammatory cytokine production that are necessary for repair.[3] Moreover, pathological functioning of macrophages and excessive inflammation in the wound healing process can result in derailed healing, like the formation of ulcers, chronic wounds, hypertrophic scars and keloids.[4]

Nicotine, a major constituent of tobacco smoke, has been shown to exert anti-inflammatory effects on different cell types and to be beneficial in disorders where inflammation-related mechanisms are involved like in ulcerative colitis and obesity.[5] Nicotine actions are mediated through binding to cholinergic receptors termed nicotinic acetylcholine receptors (nAChR) that are expressed in many different tissues and cells in the body, including immune cells. In both human and mouse macrophages, nicotine was shown to inhibit the release of pro-inflammatory cytokines through a specific “nicotinic anti-inflammatory pathway” that involves signalling through the $\alpha 7$ nAChR and prevents activation of the NF- κ B pathway.[6-9] In human microvascular endothelial cells, nicotine was

found to inhibit TNF-induced NF- κ B activation, to suppress adhesion molecule and chemokine expression and to reduce adhesion of leukocytes to activated endothelium and consequent inflammation.[10] Moreover, nicotine was shown to have angiogenic effects and to increase endothelial cell proliferation and vascular growth in different in-vitro and in-vivo models.[11, 12]

In the skin, effects of nicotine are ambivalent and often unclear, but they generally appear more negative than positive. Although cigarette smoking has been reported to negatively affect cutaneous wound healing[13], the angiogenic action of nicotine prompted for studies for a potential beneficial role of nicotine in this process. It was reported that in either normal C57BL/6 [14] or genetically diabetic mice[15], nicotine promotes wound healing due to increased angiogenesis. Yet the effect of nicotine administration on wound inflammatory responses was not evaluated in these studies. Moreover, we have observed that scar formation, a process largely dependent on the extent of the preceding inflammatory process[16], appears to be improved in smokers, which tend to have faster and less erythematous scar healing compared to non-smokers.[17]

In the present study, we aimed to evaluate the effect of nicotine during the inflammatory phase of cutaneous wound healing responses in mice. Quantification of wound closure rate indicated that nicotine did not significantly affect the wound healing process. However, nicotine administration in wounds was found to negatively regulate the production of growth factors like VEGF, PDGF, TGF- β 1 and TGF- β 2 but to only minimally affect wound inflammatory parameters. In-vitro, in both primary mouse macrophages and in the macrophage cell line RAW 264.7, nicotine was also found to induce a down-regulation of growth factor expression and to decrease TNF production. Nicotine mediated effects were found to be independent of NF- κ B inhibition and the expression of the α 7 nAChR.

Material and methods

Animals and wound model

Ten to twelve weeks old C57BL/6 female mice (n = 3–4 mice/group) were used for experiments. To generate wounds, mice were anesthetized by isoflurane inhalation and the dorsal surface was shaved and cleaned with 70% ethanol. Full-thickness excisional skin wounds were generated on either side of the dorsal midline using a 3 mm biopsy punch (Kai medical). Mice were housed individually in special paper bedding material (7089 Harlan Teklad Diamond Soft Bedding), to avoid bedding particles interfering with the healing wounds. Two wounds were generated on the same animal and mice were divided in groups. Mice in each group received only one treatment in both wounds (i.e. saline or nicotine solutions). Solutions were injected with 3 injections of 20 µl (total 60 µl) around the wounds once daily for 3 days. Wounds were photographed daily and wound area was quantified using Image J. The percentage of wound closure was calculated using the following formula: Wound closure (%) = [(wound area on day 0 – wound area on indicated day)/wound area on day 0] x 100. At sacrifice, wounds were excised and snap frozen in liquid N₂. Experiments were performed twice. Mice were maintained under standard pathogen-free conditions and all experiments were approved by the Committee for Animal Welfare of Maastricht University. The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Reagents

Nicotine (N3876) and LPS (L2630) were from Sigma–Aldrich Chemie BV (Zwijndrecht, The Netherlands). Nicotine solutions were prepared in culture medium or PBS and were made fresh prior to each experiment. ELISA kits for murine IL-6, IL-10 and IL-12 were purchased from Invitrogen Life Technologies, Inc. (Carlsbad, CA, USA) and performed according to manufacturer's protocol. For murine TNF ELISA a hamster anti-murine TNF monoclonal (TN3) was used as capture antibody Cambridge, MA,

USA) was used as a second antibody. The ELISA had a lower detection limit of 50 pg/ml. Quantikine TGF- β 1 and PDGF-AB ELISA kits (R&D systems) and VEGF ELISA kit (Life Technologies) were performed according to manufacturer's protocol.

Primary cell isolation and cell lines

For generation of bone marrow derived macrophages (BMDM), bone marrow was isolated from femur and tibia bones of C57BL/6 mice and cultured in 15-cm bacteriologic plastic petri dishes in RPMI 1640 supplemented with 10% heat inactivated fetal bovine serum, 100 U/ml penicillin, 100 μ g/ml streptomycin, 2 mM L-glutamine, 10 mM Hepes and 15% L-929 cell conditioned medium (LCM) for 8 days. Resident peritoneal macrophages (RPM) were collected from C57BL/6 mice by flushing the peritoneal cavity with 5 ml of ice cold medium. Peritoneal cells were plated in RPMI medium supplemented with antibiotics, L-glutamine and 10% FCS and macrophages were left to adhere for 4 h. Cells were washed and the remaining macrophages were cultured overnight and stimulated the next day with nicotine and/or LPS for the indicated times. RAW264.7 cells (American Type Culture Collection, number TIB-71) were cultured in RPMI medium supplemented with antibiotics, L-glutamine and 10% FCS. 3 T3-L1 murine fibroblasts (American Type Culture Collection, number CL-173) were cultured in DMEM supplemented with antibiotics, L-glutamine and 10% FCS and SVEC4-10 small-vessel murine endothelial cells (ATCC number CRL-2181) were cultured in F12 medium supplemented with antibiotics and 10% FCS. RAW 264.7 cells stably transfected with the 3x- κ B-luc plasmid[18] were a generous gift from Dr. M. de Winther (AMC, Amsterdam).

RNA isolation and quantitative gene expression

RNA was isolated either with the RNeasy Fibrous Tissue kit (Qiagen GmbH, Hilden, Germany) for mouse skin tissue or with Trizol (Sigma-Aldrich Chemie BV) for cell monolayers. Residual DNA was digested with the RQ1 RNase-free DNase (Promega GmbH, Mannheim, Germany) and cDNA synthesis was performed using the iScriptTM cDNA synthesis kit (Bio-Rad, Hercules, CA, USA). Quantitative PCR was

performed in 10 ng of cDNA, with 1 x Absolute qPCR SYBR Green FluoresceinMix (Westburg, Leusden, The Netherlands) and 150 nM of gene specific forward and reverse primers. Cyclophilin A and β -actin were used as housekeeping genes. Primer sequences are indicated in Table 1.

Table 1. Primer sequences used in this study.

Primer name	Forward sequence	Reverse sequence
CD-68	5'-TGACCTGCTCTCTCTAAGGCTACA-3'	5'-TCACGGTTGCAAGAGAAACATG-3'
TNF	5'-CATCTTCTCAAAATTGGAGTGACAA-3'	5'-TGGGAGTAGACAAGGTACAACCC-3'
IL-6	5'-TTCAACCAAGAGGTAAGATTACATAA-3'	5'-CACTCTTCTGTGACTCCAGCTT-3'
IL-12	5'-TGAGAAGTACAGCAGGCTTCTT-3'	5'-CTTCAAAGGCTTCACTTGCAAGT-3'
IL-10	5'-GCTCTTACTGACTGGCATGAG-3'	5'-CGCAGCTCTAGGAGCATGTG-3'
VEGF	5'-GCTTTACTGCTGTACCTCCACCA-3'	5'-GGGACTTCTGCTCTCTCTCTGTC-3'
PDGF	5'-CGCCTGCAAGTGTGAGACAG-3'	5'-GAATGCTCACCCGAGCTTGA-3'
TGF- β 1	5'-GCCCTTCCTGCTCTCTCATG-3'	5'-CCGCACACAGCAGTTCCTCTC-3'
TGF- β 2	5'-GACTTAACATCTCCACCCA-3'	5'-CCATCAATACCTGAAATCTCG-3'
α 7 nAChR-1	5'-CACATTCCACCAACGCTCTT-3'	5'-AAAAGGGAAACGAGCGTACATC-3'
α 7 nAChR-2	5'-TGCTGGTATTCTTGCTGCTGC-3'	5'-GGTGCTGGGGAAGTACTGTGCTAT-3'
α 7 nAChR-3	5'-GCCTAAGTGGACGAGATCA-3'	5'-CTGGGAAGCCAATGTAGAGC-3'
β 2 nAChR	5'-GGGCAGGCACACTATTCTTC-3'	5'-TCCAATCTCCCTCACACTC-3'
cyclophilin	5'-TTCTCTCTTTCACAGAATTATCCA-3'	5'-CCGCCAGTGGCATTATGG-3'
GAPDH	5'-TTCACCACCATGGAGAAGGC-3'	5'-GGCATGGACTGTGGTCATGA-3'
β -actin	5'-GACAGGATGCAGAAGGAGATTACTG-3'	5'-CCACCGATCCACACAGACTTCTT-3'

MTT assay, luciferase activity and ELISA

For MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; Sigma–Aldrich Chemie BV) assay, 105 cells/well of BMDM, RAW 264.7, PEC or 5 Å–104 cells/well of SVEC4-10 or 104 cells/well of 3 T3-L1 cells were plated in 96-well plates overnight and stimulated the following day with nicotine and/or LPS (100 ng/ml) for 24 h. Nicotine was added to cell cultures 30 min before LPS. MTT assay was performed after 24 h by addition of MTT solution to a final concentration of 0.5 mg/ml, for 2 h. Dye was solubilized with DMSO (Sigma–Aldrich Chemie BV) and absorbance was measured at 570 nm. Data represent mean $\pm$ SEM of 4 independent experiments performed in triplicate.

For luciferase activity, RAW 264.7 cells stably transfected with the 3x- κ B-luc plasmid were plated at a density of 105 cells/well in 96 well plates and stimulated with the indicated compounds for the indicated times. Cells were lysed in lysis buffer (Promega GmbH) for 20 min and 10 μ l lysate was added to 50 μ l Luciferin (Steady-

Glo Luciferase assay system, Promega GmbH). Luciferase activity was measured with a Lumac Biocounter M1500 luminometer (Promega GmbH). Data represent mean $\pm$ SEM of 2 independent experiments performed in quadruplicate.

For ELISA, 2×10^5 T3-L1 fibroblast cells or 5×10^5 macrophages or SVEC4-10 endothelial cells were plated in 500 μ l medium in triplicate/condition in 24-well plates and stimulated for 6 or 24 h with nicotine and/or LPS. Supernatants were analyzed by ELISA. Since significant levels of growth factors are present in bovine serum used in tissue culture medium, for quantification of VEGF, PDGF-AB and TGF- β 1 in macrophage supernatants, medium was changed to Optimem-1 (Gibco-BRL) overnight and cells were stimulated the following morning with nicotine for 6 or 24 h. Experiments were performed in triplicate and data represent mean $\pm$ SEM of 3 independent experiments.

Statistical analysis

Statistical analyses were performed using Graphpad Prism (Graphpad Software) or SigmaPlot statistical tests. Data are expressed as means $\pm$ SEM. A $p < 0.05$ is considered statistically significant.

Results

Nicotine down-regulates growth factor expression in skin wounds

The initial response upon a cutaneous injury is characterized by a strong inflammatory reaction with induction of different inflammatory mediators. Since nicotine was previously shown to negatively regulate inflammation[6-9], and to promote wound healing in normal[14] and genetically diabetic mice[15], we aimed to examine the effect of nicotine administration during the inflammatory phase of cutaneous wound healing in mice. Full thickness excisional wounds were generated on the dorsum of C57BL/6 mice and mice were divided in groups that received either saline or nicotine solutions. Saline or nicotine solutions at two different concentrations (10^{-4} M or 10^{-8} M) were injected around the wounds

Fig. 1. Nicotine administration in wounds. (A) Macroscopic appearance of representative saline, 10-8 M and 10-4 M nicotine injected wounds. Day 0 pictures were taken immediately after wounding. (B) Quantification of wound closure rate. At the indicated days, wound areas were determined using image analysis and expressed as percentage of wound area immediately post-injury as described in methods ($n = 6-8$ wounds/group). (C) mRNA expression analysis by real-time PCR of CD-68, IL-12, TNF, IL-6, IL-10 and (D) VEGF, PDGF, TGF- β 1, TGF- β 2 in either unwounded (NMS, normal mouse skin) or wounded skin, after three days from wounding. Unwounded skin expression levels for each gene were set to one and for simplicity only one control is shown. $n = 3$ mice/group. (E) mRNA expression analysis by real-time PCR of CD-68, IL-12, TNF, IL-6, IL-10 and (F) VEGF, PDGF, TGF- β 1, TGF- β 2 in wounded skin after three days from wounding, injected with either saline or nicotine solutions (10⁻⁴ or 10⁻⁸ M). Expression levels for each gene in unwounded (NMS, normal mouse skin) were set to one. $n = 3-4$ mice/group. Statistical significance was evaluated for each nicotine concentration compared to saline control or between wounded and nonwounded skin, by t-test. * $p < 0.05$, ** $p < 0.01$.

daily for three days, similar to other studies[14] and as described in the methods section. Quantification of wound area indicated a not significant delay in wound closure in the nicotine injected wounds compared to saline controls (Fig. 1 A and B). After three days, real time PCR analysis of RNA isolated from either wounded mice or non-wounded controls (normal mouse skin, NMS) was performed. As shown in Fig. 1 C, wounded skin exhibits a strong inflammatory response which is absent in non-wounded controls. CD-68 was over five-fold increased indicating extensive macrophage infiltration and expression of different inflammatory mediators (TNF, IL-6, IL-10) was also found several fold increased (Fig. 1C). A milder effect of wounding on expression of growth factors was observed (Fig. 1D).

Nicotine injections resulted in significant mild down-regulation in the expression of IL-10 in the wounds of 10⁻⁸ M nicotine injected wounds compared to saline controls (Fig. 1E). Interestingly, nicotine administration indicated a clear down-regulation in them RNA expression of growth factors, with PDGF, TGF- β 1 and TGF- β 2 showing significant differences while VEGF expression had a similar trend but differences were borderline not significant (Fig. 1F).

Nicotine down-regulates TNF and growth factor expression in mouse macrophages

Macrophages play a crucial role during cutaneous wound healing and represent a major source of cytokines and growth factors in the wound.[3] Previous studies have shown that nicotine attenuates macrophage activation and inhibits the production of a number of pro-inflammatory cytokines.[7, 9] To evaluate the effect of nicotine in our setting, we used primary mouse macrophages like bone marrow derived (BMDM) and resident peritoneal macrophages (RPM) and the mouse macrophage cell line RAW 264.7. Cells were stimulated with LPS in order to induce cytokine production, and with or without addition of nicotine in the culture medium (nicotine added 30 min. before LPS) for either 6 or 24 h. Nicotine concentrations used were comparable to those in similar studies[7, 9] and in the range of nicotine concentrations in blood during smoking.[19] No major effect of

nicotine on the production of TNF, IL-6, IL-12 and IL-10 was observed after 6 h, as measured by ELISA in culture supernatants (data not shown). However after 24 h, we could confirm previous studies showing that nicotine induces a significant dose-dependent down-regulation in the production of TNF in all macrophage cells tested (Fig. 2A). No significant effect of nicotine on the production of IL-6, IL-10 or IL-12 was observed (Fig. 2B, C, D).

Fig. 2. Nicotine down-regulates TNF production by mouse macrophages. ELISA for (A) TNF, (B) IL-6, (C) IL-10 and (D) IL-12 in culture supernatants of either mouse bone marrow derived macrophages (BMDM), resident peritoneal macrophages (RPM) or RAW 264.7 macrophages stimulated with 100 ng/ml in the presence or absence of different doses of nicotine (10^{-4} M, 10^{-6} M, 10^{-8} M), after 24h. Left Y axis shows protein levels for BMDM and either RPM or RAW 264.7 cells. Right axis shows protein levels for RAW 264.7 or RPM cells. * $p < 0.05$, ** $p < 0.01$. Statistical significance was evaluated for each nicotine concentration compared to no nicotine by t-test.

Since macrophages are major sources of growth factors and nicotine administration in wounds indicated a down-regulation in growth factor expression, we analyzed macrophage growth factor gene expression in the presence of nicotine. Similar

to the in-vivo findings, nicotine was found to down-regulate mRNA levels of VEGF, PDGF and TGF- β 1 in BMDM and RAW 264.7 macrophages (Fig. 3A and B respectively) (TGF- β 2 levels were undetectable). A comparable down-regulation in the protein levels of VEGF, PDGF and TGF- β 1 was measured in BMDM culture supernatants after 24 h of incubation with nicotine (Fig. 3C). Expression levels were below detection limit in supernatants of RAW 264.7 cells. Cell viability measurement by tetrazolium salt MTT assay showed that concentrations of nicotine used did not result in significant differences in cell survival and therefore the observed effects were not due to cytotoxicity (Fig. 3D).

Nicotine does not significantly affect NF- κ B transcriptional activity in mouse macrophages

Nicotine has been proposed to inhibit pro-inflammatory gene expression through a nicotinic anti-inflammatory pathway which results in the inhibition of NF- κ B.[8, 20] Moreover, NF- κ B regulates the expression of a number of growth factors as VEGF and PDGF.[21] To determine the effect of nicotine on NF- κ B transcriptional activity we used a macrophage RAW 264.7 cell line stably transfected with an NF- κ B luciferase reporter and that expresses luciferase under the control of NF- κ B. This cell line maintains the inflammatory characteristics of the parental line used in the previous experiments.[18] As shown in Fig. 4A, nicotine alone (without LPS induction), does not activate NF- κ B in any of the concentrations or time points examined (1, 6 or 24 h; for simplicity only the one hour time point is shown). Activation of RAW 264.7 macrophages with LPS induces NF- κ B activation already after one hour, as measured by luciferase activity. NF- κ B transcriptional activity peaked at six hours and returned to baseline after 24 h. However, nicotine showed only a very mild and statistically non-significant reduction in NF- κ B activity at one and six hours indicating that NF- κ B inhibition by nicotine is only minimal.

Fig. 3. Nicotine inhibits growth factor expression in mouse macrophages, mRNA expression analysis by real-time PCR of VEGF, PDGF, TGF-β1 and TGF-β2 in either (A) bone marrow derived macrophages or (B) RAW 264.7 macrophages stimulated with nicotine (10^{-4} M, 10^{-6} M, 10^{-8} M), after 24h. Expression levels in cells not stimulated with nicotine (no nic.) for each gene were set to one and for simplicity only one control is shown. (C) ELISA for VEGF, PDGF and TGF-β1 in culture supernatants of mouse bone marrow derived macrophages not stimulated or stimulated with nicotine (10^{-4} M, 10^{-8} M) for 24h. (D) MTT assay performed in bone marrow derived macrophages (BMDM), resident peritoneal macrophages (RPM) or RAW 264.7 macrophages in the presence of different doses of nicotine (10^{-4} M, 10^{-6} M, 10^{-8} M), after 24h. * $p < 0.05$, ** $p < 0.01$. Statistical significance was evaluated for each nicotine concentration compared to no nicotine control by t-test.

Fig. 4. Minimal inhibition of NF-κB transcriptional activity by nicotine. (A) Luciferase assay performed in RAW 264.7 macrophages stably transfected an NF- κB luciferase reporter, stimulated for 1, 6 or 24 h with 100 ng/ml LPS in the presence or absence of different doses of nicotine (10⁻⁴ M, 10⁻⁶ M, 10⁻⁸ M). In the absence of LPS, no luciferase activity was detected (for simplicity only the 1 h time point is shown). Statistical significance was evaluated for each nicotine concentration compared to no nicotine control by t-test. (B) RT-PCR showing transcript expression of nAChRα7 and β2 subunits in RNA derived from mouse brain, bone marrow derived macrophages (BMDM), resident peritoneal macrophages (RPM) or RAW 264.7 macrophages not stimulated or stimulated for 24 h with 100ng/ml LPS, and in unwounded or day 3 wounded mouse skin. RNA derived from mouse brain was used as a positive control for nicotinic acetylcholine receptor expression while GAPDH was used as loading control.

Since nicotine dependent NF-κB inhibition in human macrophages was reported to be mediated by the α7 nicotinic acetylcholine receptor (α7 nAChR)[8, 20], we evaluated the expression of this receptor in the different macrophage cell types used in our study. RNA was isolated from BMDM, RPM and RAW 264.7 cells and PCR

was performed on cDNA for the identification of nAChR transcripts. As shown by previous studies[22], while expression of another nicotinic receptor like $\beta 2$ nAChR was detected in mouse macrophages, $\alpha 7$ nAChR transcript was not detected in any of cell types analyzed (Fig. 4B). Use of two additional primer pairs for the $\alpha 7$ nAChR failed as well to detect transcripts for this receptor (data not shown; primer sequences listed in Table 1). Similar analysis of RNA isolated from either unwounded or wounded mouse skin confirmed the presence of $\beta 2$ nAChR and the absence of $\alpha 7$ nAChR transcripts in mouse skin.

Fig. 5. Analysis of nicotine action on SVEC4-10 murine endothelial cells. (A) ELISA for IL-6 in supernatants of SVEC4-10 cells stimulated with 100ng/ml LPS in the presence or not of different doses of nicotine (10⁻⁴ M, 10⁻⁶ M, 10⁻⁸ M), after 24h. (B) MIT assay performed on SVEC4-10 cells in the presence or absence of different doses of nicotine (10⁻⁴ M, 10⁻⁶ M, 10⁻⁸ M) and not stimulated with 100ng/ml LPS after 34h. (C) RT-PCR showing transcript expression of nAChR $\alpha 7$ and $\beta 2$ subunits in RNA derived from mouse brain and SVEC4-10 cells stimulated or not for 24h with 100ng/ml LPS. (D) mRNA expression analysis by real-time PCR of VEGF, PDGF, TGF- $\beta 1$ and TGF- $\beta 2$ in SVEC4-10 cells in the presence of different doses of nicotine (10⁻⁴ M, 10⁻⁶ M, 10⁻⁸ M), after 24 h. Statistical significance was evaluated for each nicotine concentration compared to no nicotine control by t-test.

Fig. 6. Analysis of nicotine action on 3 T3-L1 murine fibroblasts. (A) ELISA for IL-6 in supernatants of 3 T3-L1 cells stimulated with 100ng/ml LPS in the presence or absence of different doses of nicotine (10⁻⁴ M, 10⁻⁶ M, 10⁻⁸ M), after 24 h. (B) MTT assay performed in 3 T3-L1 cells in the presence or absence of different doses of nicotine (10⁻⁴ M, 10⁻⁶ M, 10⁻⁸ M) and stimulated or not for 24 h with 100 ng/ml LPS. (D) mRNA expression analysis by real-time PCR of VEGF, PDGF, TGF-β1 and TGF-β2 in 3T3-L1 cells in the presence of different doses of nicotine (10⁻⁴ M, 10⁻⁶ M, 10⁻⁸ M), after 24h * p < 0.05. Statistical significance was evaluated for each nicotine concentration compared to no nicotine control by t-test.

Nicotine effect on endothelial and fibroblast cells

In addition to macrophages, fibroblasts and endothelial cells are abundantly present in the skin and have pivotal functions in the process of cutaneous wound healing. To evaluate the effect of nicotine on these cell types, we analyzed cytokine responses in the murine endothelial cell-line SVEC4-10 and in 3 T3-L1 murine fibroblasts after LPS activation in the presence or absence of nicotine (nicotine

added to the medium 30 min before LPS). In both cell types no detectable amounts of TNF, IL-12 or IL-10 were measured by ELISA after 24 h of either LPS or LPS and nicotine incubation (not shown). On the contrary, IL-6 was measured in the culture supernatants of both SVEC4-10 cells (Fig. 5A) and 3 T3-L1 fibroblasts (Fig. 6A), but no differences were seen in the presence of different nicotine concentrations. MTT toxicity assay showed that the concentrations of nicotine used (10⁻⁴ M, 10⁻⁶ M, 10⁻⁸ M) were not toxic to either cell line since toxicity effects were only seen from concentrations higher than 10⁻³ M (Figs. 5B and 6B). Contrarily, MTT assay showed that nicotine actually promoted proliferation of 3 T3-L1 fibroblasts. As shown in Fig. 6B, in the absence of LPS stimulation, nicotine induces an approximately 10–20% increase in cell proliferation compared to non-treated cells. Addition of LPS to 3 T3-L1 cells induces a similar increase in cell proliferation but there is no further increase if nicotine is added in the presence of LPS.

To evaluate $\alpha 7$ nAChR expression in 3 T3-L1 and SVEC4-10 cells, RNA was isolated from either non-stimulated cells or cells stimulated with LPS for 24 h and cDNA was synthesized. Neither cell type was found to express $\alpha 7$ nAChR mRNA (Figs. 5C and 6C). SVEC4-10 cells expressed the $\beta 2$ nAChR transcripts while this nicotinic receptor subunit was absent from 3 T3-L1 fibroblasts. Moreover, real-time PCR analysis of RNA isolated from nicotine stimulated SVEC4-10 or 3 T3-L1 cells indicated that contrary to macrophages, nicotine does not induce a significant down-regulation of growth factors in these cell-types and actually shows a trend towards increased PDGF expression in 3 T3-L1 cells (Figs. 5D and 6D respectively).

Discussion

The discovery approximately a decade ago of a nicotinic anti-inflammatory pathway mediated by the $\alpha 7$ nAChR has raised interest in the use of nicotine or nicotinic agonists as potential therapeutic agents in different chronic inflammatory conditions. The therapeutic use of nicotine has been suggested for the treatment of inflammatory disorders such as Crohn's disease, ulcerative colitis and obesity.

[5, 23] However, the potential of a therapeutic role of nicotine in the skin is unclear and controversies exist as to whether the overall effect must be regarded as positive or negative.[13]

Effect of nicotine on wound healing

Previous studies regarding the effect of nicotine in cutaneous wound healing responses in mice have shown contradictory results. In one study, day eight wounds treated with 10^{-3} M nicotine solution resulted in delayed wound healing with a larger wound area compared to saline controls, while wounds treated with a nicotine concentration of 10^{-4} M showed improved healing and a smaller wound area. Treatment with nicotine concentrations of 10^{-7} M or 10^{-10} M showed no significant differences.[14] In a different study, nicotine concentrations of 10^{-8} M and 10^{-9} M accelerated wound healing only in genetically diabetic (Leprdb) mice but not in control mice (heterozygous for the diabetes allele) compared to saline treated controls.[15] In both studies, improved healing was associated with improved wound angiogenesis. In our wound healing experiment we could not observe an improvement in wound healing induced by nicotine treatment in wild type mice compared to controls. Differences with previous studies regard both the background of the mice but also the time-point in which wound area is measured (the first three days in our setting versus days eight or fourteen in other studies). Although no significant dysregulation in wound inflammatory parameters like TNF, IL-6 and IL-12 was detected, nicotine induced a down-regulation in the expression of anti-inflammatory IL-10 and growth factors like VEGF, PDGF, TGF- β 1 and TGF- β 2. This latter effect was also confirmed in-vitro in both primary mouse bone marrow derived macrophages and RAW 264.7 cells, at both the RNA and protein levels, while a similar down-regulation was not observed in either fibroblasts or endothelial cells. Therefore, it is likely that the inhibition of growth factor production by wound macrophages accounts for the down-regulation of growth factors observed in nicotine-injected wounds.

Nicotine action on growth factor expression

Previous studies have suggested that the effect of nicotine on growth factor expression is likely to be cell-type specific. Chronic exposure to nicotine was found to reduce plasma VEGF levels in mice and to impair cholinergic angiogenesis.[24] In other studies, while nicotine was shown to induce VEGF and FGF in vascular smooth muscle cells and endothelial cells,[25-27] it inhibited VEGF, PDGF and TGF- β 1 in rabbit osteoblasts[28] and VEGF in porcine retinal pigment epithelium.[29] Moreover, nicotine was shown to upregulate the expression of VEGF but suppress the expression of PDGF in nasopharyngeal carcinoma (NPC) cells.[30] Our data show that in macrophages, a major source of growth factors like VEGF and TGF- β 1 in wounds[31], the effect of nicotine is inhibitory. In support of a cell-type specific nicotine action, we observed a trend towards increased PDGF production by nicotine in 3 T3-L1 fibroblasts.

Nicotine action on monocytes/macrophages

Nicotine was found to inhibit the production of pro-inflammatory cytokines in human and mouse monocytes or macrophages through activation of a Jak2-STAT3 pathway and inhibition of the NF- κ B transcriptional activity.[6, 9, 20] However, discrepancies from these findings in studies where nicotine was shown to augment TNF and proinflammatory synthesis in macrophages exist.[32] Using either primary mouse cells like bone marrow derived macrophages and resident peritoneal macrophages, or the mouse macrophage cell line RAW 264.7, we could confirm previous findings of an inhibitory action of nicotine on TNF production. Using an NF- κ B luciferase reporter system, we also show that, although nicotine induces down-regulation of TNF expression in RAW264.7 macrophages, this is only minimally dependent on inhibition of NF- κ B transcriptional activity. Discrepancies from other studies[8, 20] that show inhibition by nicotine of NF- κ B activity in luciferase assays may be explained by the differences in transiently transfected cells used in those studies compared to the stable transfected line we used in our experiments. Therefore, additional mechanisms to NF- κ B suppression seem

to be responsible for the nicotine induced down-regulation of TNF expression in macrophages. In line with this, a different mechanism of nicotine mediated suppression of TNF release in human macrophages was recently reported, through induction of tristetraprolin (TTP), an ARE-binding protein that binds and promotes degradation of the TNF transcript.[33]

Nicotinic acetylcholine receptor expression

The anti-inflammatory effect of nicotine is considered to be mediated by the $\alpha 7$ nAChR expressed by many different cell types ranging from neurons to immune cells. Regarding the skin, the $\alpha 7$ nAChR has been detected in the upper spinous and granular layers of human scalp epidermis[34] and in skin of BALB/c mice, where $\alpha 7$ nAChR positive staining was observed in epidermis, hair follicles, sebaceous glands, endothelial cells, resident dermal fibroblasts, but also in inflammatory cells like macrophages and PMNs during skin wound healing.[35] However, concerns have been raised regarding the specificity of anti- $\alpha 7$ nAChR antibodies due to the discrepancies in the results between immunodetection data and mRNA or genotyping results in $\alpha 7$ nAChR deficient mice.[22, 36, 37] Using three different primer pairs, we were unable to detect mRNA of $\alpha 7$ nAChR in either unwounded or wounded skin in C57BL/6 mice or in primary mouse cells or cell-lines. Our results are supported by similar studies that show presence of other nAChR transcripts like the $\beta 2$ nAChR but absence of the $\alpha 7$ nAChR mRNA in mouse alveolar[38, 39], intestinal, splenic or peritoneal macrophages.[22] Since $\alpha 7$ nAChR has been detected in human monocytes, macrophages, endothelial cells[6, 10, 12] but not in the corresponding mouse cells, it is likely that expression patterns of nicotinic acetylcholine receptors may differ between human and mouse tissues or between different mouse strains, and comparison of different studies or translation of mouse studies to humans should be done with caution.

Finally, to evaluate the effect of nicotine on additional cell types present in the skin, we have used the mouse vascular endothelial cell line SVEC4-10 and the 3

T3-L1 murine fibroblast cell line. In both cell types nicotine was found to have no effect on IL-6 secretion, the only cytokine among TNF, IL-12 and IL-10 that was produced after LPS stimulation. However, we observed that nicotine induced a mild increase in cell proliferation in 3 T3-L1 fibroblasts under non-LPS stimulated conditions. This effect is potentially mediated by nicotinic acetylcholine receptor subunits expressed by fibroblasts that are different from $\alpha 7$ nAChR or $\beta 2$ nAChR since expression analysis indicated absence of these subunits in mouse 3 T3-L1 cells. Similar nicotine-induced stimulation in cell proliferation was noted by others in endothelial cells[40, 41], bone cells[42], epithelial cells[43], and chondrocytes. [44]

Taken together, our results show that during the inflammatory phase of murine cutaneous wound healing, the main effect of nicotine administration was a negative regulation of growth factor expression, an effect which is likely to be due to reduced growth factor expression by wound macrophages. The inhibitory effect of nicotine on growth factor production may reflect, to a certain degree, the damaging effects of smoking on the skin vasculature and oxygenation and may provide critical insight into the overall detrimental effects of tobacco use in wound healing and general skin diseases.

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Chapter 4

***The effect of triclosan-coated sutures
in wound healing: a double blind
randomized prospective pilot study***

A.E.K. Deliaert
E. van den Kerckhove
S. Tuinder
S. Fieuids
J.H. Sawor
M.A. Caberg-Meesters
R.R.W.J. van der Hulst

Abstract

Background: Wound infection and dehiscence are both major contributors to post-operative morbidity. One potential cause or co-factor is the use of suture material. A recently introduced subcutaneous suture is coated with triclosan (TC), an antiseptic drug. It is suggested to reduce wound complications.

Methods: To investigate the effect of TC on wound healing a double blind prospective pilot study in women undergoing a breast reduction was performed. Each patient was her own control. After randomisation the TC-coated sutures were used either on the left or right side. The contralateral side was used as the control. The incidence of dehiscence was studied.

Results: Twenty-six patients were included. In the TC breasts there was a wound dehiscence in 16 cases, whereas in the control breasts in seven cases a dehiscence was observed ($p < 0.023$).

Conclusion: These results suggest that TC-coated sutures should be used with caution. These sutures have already been introduced on to the market without good clinical studies and might have potential adverse effects as shown by these data.

Introduction

Wound infection and dehiscence are two of the most important factors in postoperative morbidity in surgical patients. In addition to delayed healing and increased costs, they are also related to impaired scar quality and cosmetic outcome. The cause of wound infections is multifactorial. Co-morbidity, malnutrition, and nicotine, for example, are important risk factors.[1] However, suture material might also play a significant role. Excessive inflammatory responses can be provoked by suture material. To reduce the effect of suture material on the risk of developing an infection a polygalactin 910 suture coated with triclosan (Vicryl plus, Ethicon Inc., Sommerville, NJ, USA) was recently introduced. Triclosan (TC) is an antiseptic drug mainly used in dentistry and in several antiseptic soaps. The safety of TC has already been investigated in several experimental studies.[2] The clinical efficacy however has not been studied yet. In this pilot study we investigate the effect of TC-coated suture material on wound healing.

Materials and methods

A pilot study of 26 patients was performed at the Viecuri Medical Centre in Venlo, the Netherlands, during the second half of 2006. Patients who were operated on for breast hypertrophy were asked to participate in the study. A double blind randomised design in which each patient was their own control was used to evaluate the effect of TC-coated subcutaneous sutures. Inclusion criteria were women between 16 and 65 years of age with bilateral breast size higher than cup DD and clinical complaints such as intertrigo, head neck and/or shoulder complaints. Patients with diabetes, skin diseases, history of keloid formation, use of corticosteroids and other immunosuppressive medication, metabolic and/or degenerative diseases were excluded. Patients were informed about the study and written informed consent was obtained. Breast reduction was performed using the Strombeck technique modified with a mediocranial pedicle, which is

standard in our clinic. Three different surgeons with comparable experience were involved in the study. In each patient both breasts were operated on by the same surgeon. There was no prophylactic use of antibiotics. Patients were randomly allocated to be treated with the TC-coated suture on the left or right side. The control suture was polygalactin 910 (Vicryl, Ethicon Inc., Sommerville, NJ, USA) which cannot be distinguished from the TC-coated polygalactin 910 suture. The circulating nurse obtained an envelope in which the side that would receive the TC-coated sutures was written. Subsequently she prepared the sutures for the control and experimental side on the table of the scrub nurse. Thus, scrub nurse and surgeon were blinded. A suction drain was left in both breasts and skin was sutured in both breasts with an intradermal soluble suture (Monocryl Ethicon Inc., Sommerville, NJ, USA). Distance between jugulum and nipple as an indicator for preoperative breast anatomy, data on the condition of the patient (body mass index, smoking, age) and surgical data (reduction weight, number of sutures) were collected. After being discharged 1 day after surgery, patients were seen at the outpatient clinic at fixed postoperative days (1 week, 2 weeks, 4 weeks) and more frequently if necessary. The clinical outcome was evaluated. Dehiscence was defined as a spontaneous disruption of the wound with or without infection occurring during 3 weeks postoperatively. Each wound dehiscence, independent of size, was registered. In case of clinical signs of infection (secretion, fever, erythema) antibiotics were given. There were no routine cultures taken. Drain production and day of removal of the drain was also noted. Using the statistical software SAS (version 9.1), an exact McNemar test has been used to compare the proportion dehiscence between both groups. The reported P-value is two-sided and considered significant if smaller than 0.05.

Results

Twenty-nine patients were seen during the study period. Three patients were excluded because of diabetes, or use of corticosteroids. Twenty-six patients were thus included. Clinical follow up was obtained in these 26 patients. Mean age was

36 (range 17–65 median: 35) years. Body mass index was between 21 and 31 (median 25.9). There were nine smokers included. The data on resection, sutures used and anatomy of the breast are presented in Table 1.

Table 1. Distance between jugulum and nipple (in cm), reduction weight (in grams), the number of different sutures sizes used, and postoperative suction (in ml) in breasts treated with standard pol galactin and those with triclosan. Ranges (min-max) and medians are given.

	Control		TC coated	
	Range	Median	Range	Median
Jugulum nipple distance (cm)	22–39	27	22–37	28
Resection weight (g)	76–720	460	190–970	435
Sutures 2.0	1–16	1	1–10	1
Sutures 3.0	4–24	11	8–25	11
Sutures 4.0	0–7	3	0–9	3
Suction production (ml)	5–85	20	5–100	25
Removal of suction (postop day)	1–3	1	1–3	1

There was a wound dehiscence in seven breasts treated with standard suture material. In the TC-treated breasts there was a dehiscence in 16 cases ($p = 0.023$), Figure 1. Of all patients there was bilateral dehiscence in five cases. There was no difference in the incidence of wound dehiscence between the different surgeons. Wounds were locally treated with dry gauzes and/or povidone iodine solution and generally healed within 4 weeks. Antibiotic therapy was started in two bilateral dehiscent cases, and in four unilateral dehiscent cases in the TC group. In one case there was no dehiscence, but antibiotics were started because of redness of the breast. In the smokers group (nine patients), there was a bilateral dehiscence in three cases and a unilateral dehiscence in four cases. The unilateral dehiscent cases were all in the TC-coated site. Wound dehiscence occurred in both cases at day 21 in the control breasts. In the TC group dehiscence was seen at day 7 until day 21 (median 11).

Fig. 1. Incidence of dehiscence in the two groups including bilateral cases ($p = 0.023$).

Discussion

This pilot study shows that there is no evidence for any effectiveness of TC coating. Even more, TC coating seems to have adverse effects on wound healing.

TC is an antiseptic drug that has been used since the 1970s mainly in disinfecting soaps and dentistry. Safety studies have been performed in the past, mainly focusing on systemic toxicity and local adverse reactions. Overall, these studies concluded that TC has no carcinogenic, genotoxic, pyrogenic, or teratogenic effects. In addition, systemic levels of TC are extremely low due to the use in these products.[2] Recently, however, concern ensued after reports of the formation of toxic byproducts of TC- containing soaps in household conditions.[3] Laboratory and modelling studies indicate that the formation of chloroform and other chlorinated daughter products can occur when triclosan-containing antimicrobial products react with free chlorine and that these reactions can potentially lead to

enhanced chloroform exposures.[3] The use in suture material has been tested in animal and in vitro experiments. Handling and tension force of the sutures was found to be comparable to standard suture material. The antimicrobial effect was shown to be significant without any negative effect on wound healing.[4-6] In the only clinical study TC-coated sutures had a positive effect on post-operative pain. No effect on wound healing was observed.[6]

Although our group is small we think this study is of value because each patient is her own control. This excludes the effect of confounding factors such as smoking. The difference with our study and previous, experimental results can be due to several reasons. First, the wounds tested until now were linear wounds, which have a tendency to heal well. This is different in our situation where flaps were sutured with tension and, consequently, the blood supply due to the extreme undermining is decreased. This is demonstrated in the control site where a high incidence of wound dehiscence was found, in agreement with reported data in the literature.[7] As this incidence is high, the breast reduction model is very suitable to find positive but also negative effects of new suture material. In addition to the decreased blood supply and tension, we used subcutaneous knots instead of a continuous subcutaneous suture as in other studies. Although TC is relatively non-toxic in classical toxicological terms, negative effects such as dermatitis, skin irritation, and allergic reactions have been described.[8] Potential formation of toxic products due to chemical reactions has to be elucidated.[3]

In conclusion, our observation, together with the lack of evidence illustrating the efficacy of triclosan-containing suture material, suggests that more research is needed before this product is generally introduced to the market.

The findings in this study also contribute to the discussion on whether the introduction of new suture materials, but also new wound-mediating products, should be evaluated and tested in the same way as new medication. As TC-

containing sutures have already been introduced and are used on a large scale, marketing seems to be more important than clinical evidence and may potentially put our patients at an unnecessary risk.

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Chapter 5

***A comparison between laser-doppler
imaging and colorimetry in the
assessment of scarring: “a pilot study”***

W. Peeters

M. Anthonissen

A.E.K. Deliaert

R.R.W.J. van der Hulst

E. van den Kerckhove

Abstract

Background: This cross-sectional pilot-study investigated the reproducibility of the LDI (Moor Instruments, Moor-LDI-B2) and the chromameter (Minolta chromameter CR-300) when used in scar assessment.

Methods: Twenty-seven scars in 14 subjects were included between January and June 2003. One observer performed two times both measurements with 10 minutes apart. The intra-observer agreement is quantified by means of the intra-class correlations (ICC) and the standard errors of measurement (SEM) for both the LDI and the chromameter.

Results: Ignoring one outlier, the ICC of the LDI = 0.856 and the SEM = 34.56. The chromameter shows a better reproducibility with an ICC of 0.93 and a SEM of 0.79.

Conclusion: This pilot-study with a limited number of measurements shows a moderate reproducibility of the LDI compared to the chromameter measurements, in the assessment of respectively flux and redness in scars.

Introduction

Objective assessment of scar formation after skin injury is crucial to set up a valuable scar treatment plan. Colour, thickness and pliability are frequently assessed parameters in the follow-up of scars.[1-6] In this regard, different instruments have been introduced and were tested for validity and reliability.

Van den Kerckhove et al. elucidated that erythema is a predictor for hypertrophic scarring.[7] In this study, two elements of erythema are examined: the underlying blood flow is studied with the LDI (Moor-LDI-B2; Moor Instruments, Devon, UK) and the redness itself is measured by the chromameter (Minolta chromameter CR-300; Konica Minolta Holdings, Inc., Tokyo, Japan).

The laser Doppler imaging instrument is a combination of the laser Doppler principle and a scanning technique. A two dimensional image of the microcirculation together with a digital photograph of the examined area is performed. The laser Doppler principle is based on a laser which sends out photons, that reflect with a different frequency when they hit a stationary or a moving object, the red blood cells in this case. That gives an idea of the blood flow in the scar tissue. Due to the scanning technique, there is no need for a probe that has to make contact with the skin and the measurements can be done over a larger area.[8]

Pape et al. investigated the accuracy of LDI in the evaluation of the depth of burn wounds, and found it to be a very useful and reliable measurement tool.[9]

In an attempt to judge the added value of the LDI in a test battery for scar assessment, its reproducibility was tested and compared with the Minolta chromameter CR-300. The last is considered a reference, as it is valid and reliable in the evaluation of healthy skin [10] as well as scar tissue (Van den Kerckhoven et al., unpublished data)

Material and methods

Patients and scars

Fourteen patients (12 women and two men) aged between 9 and 70 years (average 37.6 ± 20.9) with scars in the active maturation phase were included between January and June 2003. Twenty-seven scars were measured; nine burn scars and 18 surgical scars.

Technical examination

The Moor Laser Doppler Imaging device (Moor LDI2-BI) measures blood flow, often called 'flux'. Flux is a quantity proportional to the product of the average speed of the blood cells and their number concentration. This is expressed in 'perfusion units' (PU) and is calculated using the first moment of the power spectral density. The device has a range of 0–5000 PU, with a reported precision of $\pm 3\%$ of measurement.

The LDI was switched on 30 min before the first measurement, as described in the manual. The device was calibrated following the manufacturer's guidelines. The device consists of an infrared laser beam (near infra-red 785) that scans site back and forth across the tissue surface in a raster pattern using a moving mirror. The head is adjustable in height and mounted on a substantial mobile base. The maximum scanning area is 50 by 50 cm. The highest resolution ($x = y = 256$) was chosen, with a velocity of 4 ms/pixel. The scanner is connected with a PC that includes specific software, transforming the reflected light in a two-dimensional colour image. A six-colour palette is used to determine the velocity of the blood flow, with the colour blue representing low velocity and the red representing high velocity. A digital photograph (colour photo image) was made, which closely matched the blood flow image. Data were analyzed statistically by the software, and recorded on the PC.

The Minolta chromameter CR-300 was calibrated to a standard white plate before measurements were done and was then gently placed vertically on the skin. This colorimeter is a reference instrument for colour assessment and uses the Commission Internationale de l'Éclairage system. A three dimensional measurement (L^* , a^* , b^*) of reflected light was done in threefold. Luminescence [1] expresses the relative brightness from total black to pure white. The range from green to red is represented by parameter a^* , with -60 being green only and +60 being red only. The third dimension [4] is the measurement of the colour spectrum from blue (-60) to yellow (+60).[11]

Assessment

The first assessment took place with the colorimeter and subsequently a scan was taken with the LDI. One observer was performing two sets of colorimeter and LDI-measurements.

Silicone bandages and pressure garments were removed 15 min before the start of the measurements, and the patients were asked to rest during the interval. The patients were in a sitting position for scars on the upper limb and they were lying for scars on the trunk or lower limb. The room temperature was registered to assess possible influences. The plastic template with the anatomical reference points was used to know where the measurements took place for both the scar and the reference site. This gave a standardisation for the first and second measurement.

Statistical analysis

Intra-class correlations (ICC) and standard errors of measurement (SEM) have been used to quantify the intra-observer agreement, for Laser Doppler Imaging as well as for the colorimeter. The ICC is the ratio of the between-unit variance and the total variance (the sum of the within-unit and the between-unit variance), with unit pertaining to a scar in the current context. Note that the SEM is the

square root of the within-unit variance. Bland–Altman plots were used to visualize the agreement between the two replications.

Results

Scars

Twelve women and two men, aged between 9 and 70 years (average 37.6 ± 20.9) were included with 27 scars: nine burn scars and 18 surgical scars. The burn scars were situated at the medial site of the left and right knee, left elbow, left forearm and hand, right hand and right dorsum of the hand. The surgical scars were situated on the belly, upper arm, lower leg and upper leg. The healing time of the wounds ranged from 3 weeks until 11 months (average 4.2 ± 3.0 weeks).

Table 1. Intra-class correlation (ICC) and the standard error of measurments (SEM) with the LDI and colorimeter

Reproducibility of measurements			
Reproducibility	Ignoring patient clustering	ICC	SEM
LDI, all observations		0.84784	38.2527
LDI, without one outlier		0.85628	34.5608
Colorimeter		0.93427	0.78885

The LDI

The results for the intra-observer reproducibility with the LDI are presented for all observations and without one outlier ($LDI > 400$) in Table 1, both ignoring the patient clustering. Results showed that the $SEM = 38.25$ on all observations and 34.56 after exclusion of one outlying observation, mentioned above. The $ICC = 0.848$ and 0.856 respectively. Note that the result for the SEM implies that 95% of the repeated measurements on a scar are expected to fluctuate in the range of $\pm 1.96 \times 38.25 = -75$ to 75 around the true LDI level. The Bland-Altman plot (Fig. 1) visualises the agreement between the two replications.

Fig. 1. Bland-Altman plot presents the difference between two replications for all observations, measured with the LDI.

The chromameter

The results for the intra-observer reproducibility with the colorimeter are presented for all observations, ignoring patient clustering, in Table 1. The SEM for the redness values was 0.79, with an ICC = 0.93. The ICC value showed a good to excellent intra-observer reproducibility and a low SEM value. The same information for the SEM value is presented in the Bland-Altman plot in Fig. 2. This plot gives a 95% range of the difference between two repeated measurements.

Fig. 2. Bland-Altman plot presents the difference between two replications, measured with the colorimeter.

Discussion

Objective assessment of scar formation after skin injury is important to create a valuable scar treatment plan, and thereby intra-observer reproducibility is crucial. This pilot study is the first to compare the reproducibility of the Laser Doppler Imaging with that of the chromameter in the assessment of scarring.

The ICC coefficient was chosen to verify the reproducibility of results, because it's the current standard for the investigation of agreement between measurements. The SEM gives an idea of clinical relevant changes. The ICC is the ratio of the between-subjects variance and the total variance, the latter being the sum of the between-subjects and the within-subjects variance. When there is a large heterogeneity between the scars, the ICC will be good, but this can be a false positive interpretation.

In this study, we noticed for the intra-observer reproducibility that the ICC values were good, but in the setting described, the SEM values were very high for the measurements with the LDI. This means that the reliability dropped back from good to moderate.

For the colorimeter, excellent ICC and SEM values were found. This was already shown in previous studies.[7] The subjects included showed a large heterogeneity in type of scars, location of scars and age of scars. Since this heterogeneity is directly reflected in the between-unit variance, the actual level of the ICC was highly influenced by the specific sample (as opposed to the level of the SEM). Therefore, the SEM is more informative in the current study.

We believe future studies are necessary to find out whether we can lower the SEM by measuring in homogenous patient groups, using a fixed scanning height and background, and by changing settings of the LDI device other than the number of pixels and the scanning velocity that were mentioned above.

Conclusion

We conclude from this pilot-study that the colorimeter has a better reproducibility compared to the LDI, when the current scanning settings are used. Well designed studies are needed to check whether reproducibility can be enhanced by changing scanning software and hardware settings and the use of the device in homogenous patient groups. We conclude from this pilot-study that the colorimeter has a better reproducibility compared to the LDI, when the current scanning settings are used. Well designed studies are needed to check whether reproducibility can be enhanced by changing scanning software and hardware settings and the use of the device in homogenous patient groups.

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Chapter 6

Smoking and its effect on scar healing

A.E.K. Deliaert

E. van den Kerckhove

S.Tuinder

S.M.J.S. Noordzij

T.S. Dormaar

R.R.W.J. van der Hulst

Abstract

Scar formation is influenced by several factors such as wound infection, tension, wound depth and anatomical localization. Hypertrophic scarring is often the result of an imbalance in the wound and scar healing process. The exact underlying pathophysiological mechanism remains unclear. Smoking has a higher risk of postoperative complications probably due to a diminished macrophage induction. Following our clinical impression that smokers without postoperative wound infections show esthetically better scars, we evaluated the scars after a reduction mammoplasty in smoking and non-smoking patients in a prospective clinical trial. Between July 2006 and September 2007, 13 smokers and 30 non smokers with a reduction mammoplasty were included. They were recruited from Viecuri Medical Centre and Atrium Medical Centre in the Netherlands after written consent. Surgical data and data of the patients' condition were collected. Follow-up for erythema values of the scars was done with a colorimeter (The Minolta CR-300, Minolta Camera Co., Ltd., Osaka Japan) at 1, 3, 6 and 9 months postoperatively on four standardized postsurgical sites. ANOVA and Chi-square test were used for statistical analysis. In the smoking group, the scars were significantly less red compared to the nonsmoking group. No significant differences were found in BMI, resection weight and drain production between both groups.

Although smoking is certainly not recommended as a preventive therapy to influence scar healing, this study confirms our assumption that smokers tend to have faster and less erythematous scar healing to nonsmokers. Further research is needed to understand the mechanism of the effect of smoking on scars.

Introduction

Wound healing and the subsequent scar formation require an accurate balance of inflammation.[1] Smoking is known to result in more wound related postoperative complications, probably by disturbing the normal inflammatory physiology. Cooperating cellular responses have to be appropriate in several phases of wound healing. Diminished function of the neutrophils and macrophages results in chronic nonhealing wounds, e.g., in diabetes.[2] Hypertrophic scarring, on the other hand, can be the result of an exaggerated inflammatory response. The production of certain subspecies of TGF- β by the macrophages seems to be important in inducing hypertrophic scarring.[3] Local discomfort such as pruritus, pain, restricted mobility and aesthetic impairment result in a reduced quality of life.[4] Hypertrophic scars often arise after burn wounds and appear 1 to 3 months after trauma. Our clinical impression is that smokers, although having complicated or delayed wound healing, almost never present with hypertrophic scars. Therefore, we hypothesize that smoking has a positive influence on postoperative scarring due to its effect on the inflammatory response. To evaluate this clinical impression, we performed a prospective patient-controlled study comparing postoperative scar activity in smoking and nonsmoking women undergoing a reduction mammoplasty.

Material and methods

A double center, prospective study was performed in female patients undergoing reduction mammoplasty. All patients between the ages of 17 and 70 years with at least a DD-cup were included in the study. Inclusion was done between July 2006 and September 2007 at Viecuri Medical Centre in Venlo and Atrium Medical Centre in Heerlen, both situated in the Netherlands. The study was approved by the ethical committees of both hospitals and written informed consent was obtained from each patient. Patients were referred to the Department of Plastic Surgery by the general practitioner because of complaints of breast hypertrophy

such as intertrigo and head, neck and/or shoulder complaints. The indication to perform a reduction mammoplasty was made by the plastic surgeon. Exclusion criteria were the presence of diabetes, skin disorders, use of corticosteroids and immunosuppressive medication, history of keloids or hypertrophic scarring and degenerative or metabolic disorders.

Surgery was performed by a plastic surgeon or a senior resident using a mediocranial pedicle technique. There was no prophylactic use of antibiotics. Data on the condition of the patient (body mass index, age) and surgical data (reduction weight) were collected. One day after surgery, the patients were dismissed from the hospital. One month postoperatively, the patient started to use a hydrating crème on the scar. Follow-up took place at 1, 3, 6 and 9 months after reduction mammoplasty. Dehiscence was defined as every spontaneous disruption of the wound occurring during the first 3 months postoperatively. Each wound dehiscence independent of size was registered. Coloric measurement was not performed in case of dehiscence at this particular area. In case of clinical signs of infection (secretion, fever, erythema), antibiotics were given. Colorimetric measurement was performed under standard conditions at the medial and lateral parts of the scar, the scar at the center of the inframammary fold and at the nipple (at the topmost point). Normal skin was measured at each time point in each patient and subtracted on the values of the different sides of the scars. Statistical analysis was performed using ANOVA with time and smoking as independent variables. Chi-square testing was used for statistical analysis of wound dehiscence between both groups. SPSS for Mac version 16 UK was used for analysis. Values are presented as means $\pm$ STD.

Results

Forty-nine patients presented for reduction mammoplasty at the two centers. Three patients were excluded because of diabetes and/or corticosteroid use. Three other patients were excluded because no follow-up took place during scheduled data at the outpatient clinic. The study sample size was 43 patients.

There were 13 smokers and 30 nonsmokers in this group. The mean age of the smokers and nonsmokers was 32.6 years ($\pm$ STD12.7) and 37.4 years ($\pm$ STD 13.2). There were no differences in mean body mass index (BMI) or resection weight between the two groups (Table 1).

Table 1. Patient characteristics

	Nonsmokers (n=30)	Smokers (n=13)
Age (years)	37.4 (STD 13.2)	32.6 (STD 12.7)
Body mass index (kg/m ²)	26.1 (STD 3.1)	26.3 (STD 3.1)
Resection weight (g)	436.8 (STD 197.3)	438.5 (STD 180.4)
Wound dehiscence (%)	36.6%*	69.2%*
Drain production (ml)	26,3 (STD 19.2)	22.6 (STD 20.9)

* $p < 0.05$ (statistical significance)

Antibiotics were given postoperatively in four patients from the smoking group and in seven patients from the nonsmokers because of signs of inflammation (fever, redness, pain and edema of the breast). Silicone dressings were used in two smoking patients and in eight nonsmokers because hydration was not sufficient and signs of hypertrophy arose. The incidence of any postoperative dehiscence was higher ($p < 0.05$) in the smoking group compared to the nonsmoking patients (69.2% versus 36.6 %, Table 1). The redness of scars in both groups decreased over time, mainly in the center of the inframammary fold and at the nipple. In addition, the scars of the smokers were significantly less red compared to the nonsmokers. This was seen at the lateral site after 1 month ($p < 0.01$) and 9 months ($p < 0.05$),

at the medial site after 3 months ($p < 0.005$) and at the nipple after 1 month ($p = 0.01$) and 9 months ($p < 0.05$) (Figs. 1–3). At the center of the mammary fold, no significant differences were found during the period of follow-up (Fig. 4).

Fig. 1. Redness measured postoperatively with the colorimeter at the lateral part of the scar. Significant difference in redness after 1 month ($p < 0.01$) and after 9 months ($p < 0.05$).

Fig. 2. Redness measured postoperatively with the colorimeter at the medial part of the scar. Significant difference after 3 months ($p < 0.005$). After 6 months, there was a slight difference in redness ($p = 0.066$).

Fig. 3. Redness measured postoperatively with the colorimeter at the topmost point of the nipple. Significant difference after 1 month ($p < 0.01$) and after 9 months ($p < 0.05$).

Fig. 4. Redness measured postoperatively with the colorimeter at the center of the inframammary fold. No significant differences were found in the scar of the nipple. The differences in redness are $p = 0.116$ after 1 month, $p = 0.209$ after 3 months, $p = 0.107$ after 6 months and $p = 0.575$ after 9 months.

Discussion

This study was conducted to evaluate the effects of smoking on wound healing and scar formation assessed by erythema measurements. The clinical impression that smokers, although having increased risk of developing wound complications, have better cosmetical appearing scars and a diminished risk of hypertrophic scar formation was confirmed by demonstrating decreased vascularity reflected by the diminished redness of the scar. Although the number of patients in the smoking group is small, the diminished redness in this group was statistically clearly demonstrated. It has been shown that the presence of scar redness is correlated with the occurrence of hypertrophy. Moreover, it has been considered to be associated with the degree of elevation, hardening and retraction of the scar. [5] Smoking, therefore, might influence the risk of hypertrophy. Our hypothesis is that the mechanism by which this happens is the same as the one responsible for the increased wound complication rate in smokers: a deterioration of proliferative and/or immunological response.

Smoking is one of the most important causes of perioperative complications. [6] Postoperative complication rates among smokers varies from 3.1 to 19%. [7-10] Most of these wound complications consist of the development of a postoperative infection and hematoma formation.[6] In a well-performed recent study in breast reduction surgery, the effect of smoking on wound complications was demonstrated. An incidence of 40% in postoperative complications was seen among smokers proven by cotinine levels in urine compared to 12% in nonsmokers.[11] In our study, the overall incidence of postoperative complications was relatively high. This is explained by the definition of wound complications, which was defined quite broadly in our study. Any wound defect until 3 months after surgery, independent on size including, e.g., spitting of suture material was defined as a wound complication. It is obvious that measurement of redness was not performed at the sites where there was or had been a wound dehiscence.

The pathological mechanism of an increased risk for postoperative wound complications due to smoking can be explained in several ways. One possible mechanism could be through the changed availability of nitric oxide as is hypothesized in the association between smoking and vascular damage. Another possibility could be an altered inflammatory response. Sorensen et al studied the effect of smoking on wound healing.[12] The influx of macrophages is shown to be less in smokers. Macrophages produce proinflammatory cytokines that control adhesion and migration of inflammatory cells. Their subsequent proliferation is triggered by multiple growth factors such as PDGF, EGF, TGF- β , and cytokines IL-1 and TNF. A diminished number of macrophages result in a disturbed cascade of this inflammatory response.

Interestingly, the consequences of smoking on cytokines and growth factors resulting in an increased risk of wound complications may have a beneficial effect on scar formation, resulting in cosmetically superior, less active scars. It has been well established that in a normal situation TGF- β causes fibroblast migration and proliferation, increased synthesis of collagen and a decreased degradation of extra cellular matrix (ECM) by metalloproteinases needed for normal wound healing. In adults, the subclasses TGF- β 1 and TGF- β 2 are mainly responsible for this effect. In fetal healing, there is a low expression of TGF- β 1 and TGF- β 2 and increased expression of TGF- β 3 associated with less visible scars.[3, 13] Therefore, the relative concentrations of the several subclasses of TGF- β seem to play an essential role in the appearance of scars. Smoking has been shown to have an inhibiting effect on the influx of macrophages, the initiators of the inflammatory cascade and the subsequent production of TGF- β . The hypothesis is that the relative contribution of TGF- β 1 is less in wounds of smokers, resulting in increased risk of complicated wound healing but also resulting in cosmetically better looking scars. In this line, it has been shown that serum levels of TGF- β 1 in nicotine-treated rats versus saline-treated rats showed a significantly reduced serum level of TGF- β 1.[14]

In conclusion, we hypothesize that smoking alters the inflammatory response resulting in more wound complications but also less active scarring. Without doubt, smoking has severe adverse effects and creates a strong addiction. Therefore, it is absolutely not recommended as treatment. However, our observations give rise to further exploration of the pathophysiological effect that smoking and its specific components may have on wound healing and scar formation. This may eventually result in new ways to treat scars and prevent wound complications.

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Chapter 7

***The effect of smoking on sternal scar
healing: a prospective cohort study***

A.E.K. Deliaert

J.F. Mermans

T.S. Dormaar

E.M. Heerdt

S. Xanthoulea

J.G. Maessen

E. van den Kerckhove

R.R.W.J. van der Hulst

Submitted

Abstract

Background: Median sternotomy is an electing factor for the development of a hypertrophic scar. Smoking, due to its negative effect on vascularisation, could have an effect on scar healing. We conducted a prospective study to evaluate the effect of smoking on scar healing after cardiac surgery with a median sternotomy.

Methods: One hundred patients who underwent cardiac surgery with a median sternotomy were included. Patients were divided into three groups: smokers, ex-smokers and non-smokers. Erythema values of the scar were measured with a colorimeter on three standardized parts of the scar. Scar evaluation was done 6 weeks, 3 months, 6 months and 1 year after surgery.

Results: During one year, 90 patients were followed after a median sternotomy. Ten patients were lost to follow up. There were 23 smokers, 52 ex-smokers and 15 non-smokers with a mean age of 61.5 years (SD 8.87). No significant difference in redness as parameter for hypertrophic scarring was seen between the three groups. However, a trend in favour of the smokers was seen, resulting in less hyperaemic scars.

The caudal part of the scar was showing a significantly greater incidence of hypertrophy than the middle and cranial part of the scar ($p < 0.001$) at all time points.

Conclusions: In patients undergoing cardiac surgery, no significant difference in hypertrophic scarring of sternal wounds was found between smokers, non-smokers and ex-smokers. However, the subxiphoidal part of the wound is prone to hypertrophic scarring and should be avoided in planning the incision.

Introduction

Presternal scarring after cardiac surgery is known for its aberrant scar formation that could lead to hypertrophic or keloidal scar formation. These scars are notorious because of their functional and aesthetic problems accompanied with a reduced quality of life.[1-3] Hypertrophic scars evolve frequently after full thickness burn injuries and after surgery with a tendency to develop at locations where skin tension is largest.[4, 5] The border of a hypertrophic scar remains typically within the confines of the original lesion in contrast to keloids, which are characterised by expansive growth and a genetic predisposition. The thorax is one of the preferential locations for hypertrophic scar formation. These scars are seen in 10-57% of the Caucasian patients after cardiothoracic surgery. [2, 6, 7]

The exact aetiology for aberrant scar formation remains unclear. One of the predicting factors is an increased vascular density compared to normotrophic scars.[3]

Smoking may have a negative effect on vascularisation of the wound, due to endothelial dysfunction and is thought to be responsible for a significant increase in postoperative wound complications. [8, 9] However, as a side effect, due to its effect on vessels it may also diminish the risk of development of hypertrophic scarring.

Therefore, we conducted a prospective study to evaluate redness as parameter of hypertrophy and the incidence of scar hypertrophy among smokers, ex-smokers and non-smokers after a median sternotomy.

Materials and methods

From September 2010 until September 2011, 100 patients after cardiothoracic surgery were included at Maastricht University Medical Center. A power analysis

was calculated using a previous pilot study where the redness of the scar after reduction mammoplasty was measured.[10] The medical ethical committee of Maastricht University Center approved the study (project number METC 10-4-009) and patients were included after written informed consent.

Cardiothoracic patients were contacted on the fifth postoperative day, before leaving the hospital. Inclusion criteria were age above eighteen years and cardiac surgery through a median sternotomy incision. Patients with a history of hypertrophic scarring or keloid formation, diabetes mellitus, degenerative or metabolic diseases, pre-existing skin disorders and patients using corticosteroid or immunosuppressive medication were excluded from the study. Ex-smokers were defined as having quit smoking more than one month prior to the operation, whereas smokers had to be smoking at time of surgery or when they quit within one month prior to surgery. Objective measurement of the scar was performed by measuring the redness of the scar at six weeks and at three, six and twelve months post surgery. The exact day post surgery was documented at every time point. A Minolta CR-300 (Minolta Camera Co., Ltd, Osaka Japan) was used for measurements. These measurements were performed under standardised conditions at three locations on the scar: two centimetres from the cranial end of the scar, at nipple height (or in women with ptotic breasts, a height corresponding with half the length of the humerus was used as normal nipple height). The final measurement point was situated 5 cm from the caudal end of the scar.

Coloric measurement was not performed in case of incomplete wound healing of the wound in the particular area. Normal skin colour was measured near the scar at each time point and subtracted from the measured values of the different measurement points of the scar. The Patient and Observer Scar Assessment Scale (POSAS) was used to evaluate the scar at each time point. Patients started to use a hydrating cream (Alhydran®, BAP Medical) twice a day on the scar at six weeks after surgery. In case of hypertrophic scar formation, treatment was not postponed

and an alternative treatment such as silicone dressings (Scarban Elastic®, Gent Belgium) was proposed. Hypertrophic scarring was defined as a situation in which the scar, or a part of the scar, is raised above the level of the surrounding skin. The size of the scar had to remain within the original border of the incision. If this last condition was not fulfilled, a scar was deemed as having more keloidal instead of hypertrophic characteristics.

Statistical analysis

The analysis was based on patients with known smoking status (smoker, ex-smoker, non-smoker). Four response variables were analyzed: 'base redness' which was calculated as measured redness of the scar minus the redness of the normal skin measured at the corresponding location (cranial, nipple, caudal) and three POSAS Patient scale components (color, thickness and irregularity). Time (6 weeks, 3 months, 6 months, 12 months) was scaled to months for computational reasons and the evolution of scar redness (with normal skin measurement subtracted) and the 3 components of the POSAS patient scale by smoking status over time was analyzed using a linear model correcting for age of the patient, location (only for base redness), length and width of the scar. Time was modeled continuously in the mean evolution. The assumption that time was linear was tested by adding a squared time effect including the interactions with smoking status and location (only for base redness) in the models and jointly testing the null hypothesis that time was linear. An unstructured variance-covariance matrix was assumed using a categorical version of time point (6 weeks, 3 months, 6 months, 12 months). The Patient and Observer Scar Assessment Scale (POSAS) was summarized descriptively by means of mean, standard deviation, median, quartiles and range of numbers and percentage, whichever was appropriate. A Fisher exact test was used to analyze the difference between the location (cranial versus middle together with the caudal part) within every patient with a hypertrophic scar.

Results

One hundred patients with a median sternotomy were recruited and gave their informed consent for participation in the study. During the study, a total of 10 patients were lost to follow up. Therefore, the study sample consisted of 90 patients. Of the enrolled patients, the distribution of men versus women was respectively 81.1% versus 18.9%. There were 23 smokers, 52 ex-smokers and 15 non-smokers with a mean age of 61.5 years (SD 8.87). Not single wound infection occurred in all of the population. Patients' baseline characteristics are shown in Table 1.

Table 1. Baseline characteristics.

Variable	Statics	Total
Age (years)	N	90
	Mean	61.5
	Std	8.83
	Median	61.5
	(Q1, Q3)	(56.0; 68.0)
	Range	(39.0; 81.0)
Gender		
Male	n/N (%)	73/90 (81.11%)
Female	n/N (%)	17/90 (18.89%)
Smoking status		
Smoker	n/N (%)	23/90 (25.56%)
Ex-smoker	n/N (%)	52/90 (57.78%)
Non-smoker	n/N (%)	15/90 (16.67%)
Number of cigarettes per day	N	60
	Mean	15.8
	Std	9.04
	Median	17.5
	(Q1, Q3)	(10.0; 20.0)
	Range	(2.0; 40.0)

No significant difference was found in regression of redness over time as an indicator for hypertrophic scarring among the groups (figure 1).

Figure 1. Regression of redness of scar tissue on covariates. Evolution of the predicted average profiles of smoking status over time.

Location=Cranial

Location=Nipple

Location=Caudal

There was no significant difference in incidence of hypertrophic scars between the three groups (Figure 2). The caudal part of the scar was showing a significantly greater incidence of hypertrophy than the middle and cranial part of the scar ($p < 0.001$) at all time points (Figure 3). There was no significant difference between the groups for the subjective and objective scar assessment scale (POSAS).

Figure 2. Incidence of hypertrophic scarring between groups. No significant difference was found between these groups ($p > 0.05$).

Figure 3. Location of hypertrophic scarring ($p < 0.001$).

Discussion

In this prospective study, we studied the difference in scar healing between smokers, non-smokers and ex-smokers after median sternotomy. No significant difference in redness, as indicator for hypertrophic scarring, was seen among the groups.[11] There are several factors, which may have had an effect on the outcome of this study.

We observed a trend towards hyperaemic scars in non-smoking patients at six months. The possible positive influence of smoking on reducing hypertrophic scarring can be explained; smoking reduces the systemic inflammatory response as nicotine, a vasoconstrictor, reduces proliferation and migration of macrophages and fibroblasts and subsequently a reduction in collagen deposition occurs.[12] Van der Veer et al. investigated the time course of the angiogenic response in wound healing in both hypertrophic and normotrophic scars of human median sternotomy incisions.[3] They established that hypertrophic scars were associated with increased neovascularization compared to normotrophic scars. Microvessel density in the deeper part of the reticular dermis was significantly increased in the hypertrophic group at 12 weeks and 52 weeks. Unfortunately, in that study, no measurements were done at 6 months. Nevertheless and in accordance with our study, it shows that there is an increased vascular density after 3 months in hypertrophic scarring compared to normotrophic scarring in median sternotomy incisions. In addition, we have recently shown that nicotine, a major constituent of tobacco smoke, has inhibitory effects on wound growth factor expression that can result in reduced scar neovascularization and thus reduced scar hypertrophy. [13]

Although we did not find statistical evidence that smokers are less susceptible to hypertrophic scars after a median sternotomy, a consistent trend indicates that it should be possible to confirm our hypothesis with a larger sample of patients. One of the reasons for lack of power might be that our power analysis was based on women between 18 and 55 years old. [10] In this study the mean age of women was 37.4 years (STD 13.2) for smokers and 32.6 years (STD 12.7) for non-smokers. This patient category is more sensitive to develop hypertrophic scars because this form of scarring has the highest incidence in the second decade of life.[14] This is in contrast with our population with a mean age of 61.5 years.

Although it was not a separate item in this study, we found that the caudal part of the sternotomy scars showed twice as much incidence of hypertrophy ($p < 0.001$) compared with the cranial and central parts. This is in accordance with the literature, where a tendency towards hypertrophic scar formation is seen in the lower half of the incision.[2, 15] Nevertheless, skin tension is supposed to be less over the caudal part of the sternum and therefore a better cosmetic result should be achieved in this area. However, increased mobility seems to be the predominating factor for the development of a hypertrophic scar.[16] Based on the literature and our findings we suggest to minimise the length of the incision where the caudal end has to be proximal of the xiphoid process.

Conclusion

No significant difference in redness as parameter for hypertrophic scarring was seen between the three groups. However, a trend in favour of smokers resulting in less hyperaemic scars was seen in this study and therefore a larger sample of patients is needed.

In addition, the caudal part of the scar -especially the subxiphoidal part- is prone to hypertrophic scarring and should be avoided in planning the incision.

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Chapter 8

***Summary, discussion and future
perspectives***

Summary and discussion

Inflammation plays a key role in wound healing and subsequent scar formation. These two physiologic processes are inevitably connected to each other. The aim of this thesis was to evaluate the effect of smoking and the use of an antiseptic (triclosan) coated suture, in skin and scar quality. In addition some objective parameters to evaluate appropriate therapy and follow-up were studied. This part describes the main findings of this thesis and concludes with recommendations for further research.

Chapter 2 describes the relation between smoking and patients who undergo a standard cosmetic procedure. The aim of this study was to compare smoking habits in patients undergoing a blepharoplasty and the general population to determine whether smokers undertake this procedure earlier in life. A questionnaire was sent to a large group of patients that underwent a superior upper eyelid correction. The procedure was reimbursed for all patients in the study to avoid socio-economic status bias. Analysis showed no difference in smoking habits between the blepharoplasty group and the general population. Smokers underwent the procedure 3.7 years earlier in life than ex-smokers ($p = 0.0007$) and 3.5 years earlier than never smokers ($p = 0.006$). This is the first study that describes an earlier need for upper eyelid correction in smokers. In the literature it is described that next to sun exposure, smoking results in premature aging probably due to blockage of cellular responses of the fibroblasts.[1, 2] No difference in time to surgery was seen between ex-smokers and non-smokers, which might implicate a regenerative capacity of the skin.

Chapter 3 evaluates the effect of nicotine during the inflammatory phase of cutaneous wound healing responses in mice. In this study nicotine or saline solutions were injected in the back of mice after excisional wounds were generated. No significant differences in wound closure rate between the saline

and nicotine group was observed. However, a significant difference in down-regulation of growth factor expression of PDGF, TGF- β 1 and TGF- β 2 due to nicotine (10^{-8} M) was seen. As macrophages play a crucial role during wound healing and represent a major source of cytokines and growth factors in the wound, the effect of macrophages after nicotine administration was studied in wound healing.[3] In vitro primary mouse macrophages like bone marrow derived macrophages, resident peritoneal macrophages and the mouse macrophage cell line RAW 264.7 were used. A significant down-regulation of Tissue Necrosis Factor in macrophages was observed after nicotine stimulation. Similar to the in-vivo findings, nicotine was found to down-regulate mRNA levels and protein levels of VEGF, PDGF, and TGF- β 1 in bone marrow derived macrophages. TGF- β 2 levels were undetectable. Finally, it was concluded that most likely the inhibition of growth factor production by wound macrophages accounts for the down regulation of growth factors observed in nicotine-injected wounds. In this study an inhibitory effect of nicotine on growth factors production may reflect, to a certain degree, the damaging effects of smoking on the skin vasculature and oxygenation.

Wound infection and dehiscence are important contributors to postoperative morbidity. Various therapies are aimed to reduce postoperative infections. Suture material might play a significant role in the development of wound infections because it can provoke an excessive inflammatory response. **Chapter 4** evaluates the effect of subcutaneous sutures coated with an antiseptic drug (triclosan coated (TC)) to reduce postoperative wound complications. Women undergoing breast surgery were included in this double blind randomized pilot study where each patient was her own control. No advantage of TC on postoperative healing was found. Even more, an increase in postoperative wound dehiscence three weeks after surgery was seen in the triclosan coated group ($p = 0.023$).

Wounds with prolonged epithelialization time of more than 2-3 weeks are prone for the development of hypertrophic scars.[4] Wound infection and thermal injuries

are the most frequent causes for delayed healing, especially deep partial thickness burns are susceptible for the development of hypertrophic scar formation due to prolonged healing time. Accurate assessment of burn depth is needed; especially the differentiation between a superficial second-degree and a deep second-degree burn wound, because treatment differs between conservative treatment and a surgical procedure. Laser-doppler imaging (LDI) has shown to determine burn depth, differentiating in these two levels, resulting in an adequate treatment plan.[5] In an already closed wound, objective scar assessment is important to set up and to evaluate a treatment plan. Frequent assessed parameters in the follow-up of scarring are color, thickness and pliability (elasticity). Erythema has already been shown to be an excellent predictor for hypertrophic scarring in post burn wounds and is used as the standard method in the evaluation of post burn scars.[6] In **Chapter 5** two elements of erythema are evaluated in the assessment of scarring. The underlying blood flow is studied by laser-doppler imaging (Moor-LDI-B2; Moor Instruments, Devon, UK) and the redness is measured by a colorimeter (Minolta Chromameter CR-300; Konica Minolta Holdings, Inc., Tokyo, Japan). Intra-class correlations (ICC) and standard errors of measurement [7] have been used to quantify the intra-observer agreement. The colorimeter showed a better reproducibility compared to the LDI.

As smoking is a major contributor to postoperative infections possibly due to disturbed macrophage induction, prolonged healing is expected.[8, 9] **Chapter 6** evaluates the postoperative scar quality in smoking and non-smoking patients undergoing a reduction mammoplasty. Objective scar assessment was performed by measuring erythema values. Measurements were done 1, 3, 6 and 9 months after surgery with a chromameter (Minolta Chromameter CR-300; Konica Minolta Holdings, Inc., Tokyo, Japan) on four standardized postsurgical sites. Postoperative wound dehiscence was higher in the smoking group compared to the non-smoking patients ($p < 0.05$). The redness of the scars in both groups decreased over time. A difference in favour of smokers, resulting in lower erythema values, was seen at

the several sites of the scar at different time points. In conclusion, smokers tend to have faster and less erythematous scar healing compared to non-smokers.

Tension is, besides a prolonged inflammation, another contributing factor to hypertrophic scarring.[10] An anatomical preference for the development of hypertrophic scarring is seen at the back, shoulders, chest and buttocks.[11] Therefore we investigated the effect of smoking in sternal healing in **Chapter 7**. In this prospective study, 90 patients were followed during one year after a median sternotomy. Follow-up took place during one year at 4 scheduled time points (6 weeks, 3, 6 and 12 months post surgery). Both objective and subjective measurements were performed. The colorimeter was used for the objective measurement of redness as the POSAS scale (Patient and Observer Scar Assessment Scale) was used to evaluate the scar at each time point. A total of 23 smokers, 52 ex-smokers and 15 non-smokers with a mean age of 61.5 years were followed. No significant difference in redness over time was seen in this study. However, a trend in favour of smokers was seen resulting in less hyperaemic scars. Interestingly, the caudal part of the scar, in comparison with the proximal and middle part, is prone for hypertrophic scarring ($p < 0.05$).

Future perspectives

This thesis is based on clinical research and it provides insight in factors that influence skin and scar quality. Smoking is one of these factors and it seems to affect scar healing resulting in less hyperaemic scars. No former research has studied the effect of smoking on scar healing which makes it difficult to compare this thesis with the literature. Until now, only one study described a lower incidence of hypertrophic scars among smokers versus non-smokers after surgery.[9]

Plastic and reconstructive surgery is a medical specialty concerned with the correction and restoration of form and function of the body. The restoration

of the integrity of the skin is therefore an important aim in plastic surgery. The adage “even a plastic surgeon can’t erase without a trace” is unfortunately still true. However the goal in plastic surgery is to minimize scarring by hiding scars in areas that are less visible, by optimal orientation and using a meticulous suturing technique. Despite these techniques, aberrant scar formation is still seen in post-surgical wounds. These scars develop frequently after deep burn wounds and after delayed wound healing. Hypertrophic scars develop generally 4 to 12 weeks after re-epithelialization and occur in 39-68% of the surgical patients and in 32-72% of the burn patients.[9, 12, 13] It results in a raised, red and itchy scar for up to two years before regression occurs. The redness of the scar is an important clinical predictor of hypertrophic scarring and the colorimeter has shown to be a good reference instrument used during the follow-up of this healing process. Scar free wound healing or reducing hypertrophic scarring is an important goal in plastic surgery. Until now only early fetal skin will heal scarless with complete restitution of normal skin architecture.[14, 15] Fetal scarless wound healing is characterized with minimal inflammation and with a high ratio of TGF- β 3 to TGF- β 1. In contrast is adult scarring, and in particular hypertrophic scarring, characterised with high levels of TGF- β 1. Hypoxia may be responsible for this altered ratio because when hypoxia increases, the amount of TGF β 3 increases, which is seen in fetal healing. Fetal wounds have been shown to be hypoxic compared to adult human wounds.[16] Smoking seems to result in less hyperaemic scars (this thesis) and in a lower incidence in hypertrophic scars. This last effect is mainly seen in younger patients.[9] As smoking might cause hypoxemia, future studies should focus on hypoxemia in an adult population between the 20 and 50 years of age. This thesis showed a down regulation of growth-factor expression in mouse skin and macrophages after nicotine treatment possibly due to an immunologic effect or due to hypoxemia. Future research should focus on larger studies, which study the effect of nicotine on wound and scar healing. Nicotine treatment has already been successful in the treatment of pyoderma gangrenosum and is hypothesised to be effective in the treatment of endometriosis and aphtous stomatitis.[17-

19] Therefore we hypothesize that a topical nicotine gel on the wound site may result in improved scarring with less hypertrophy and subsequent contraction formation. A prospective double-blind, randomized, placebo controlled, clinical trial investigating this hypothesis will be started in the near future.

In conclusion, wound healing and subsequent scar formation depends on several pathophysiologic mechanisms that are insufficiently understood. Therefore it is difficult to focus only on one subdomain. Future research should focus on the several interacting mechanisms to prevent excessive scar healing with the ultimate goal of (near) scarless healing.

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Chapter 9

Nederlandse samenvatting

Nederlandse samenvatting

De inflammatoire fase in de wondgenezingscascade speelt een essentiële rol in wondgenezing en littekenvorming. Deze twee processen zijn onherroepelijk met elkaar verbonden. Het doel van dit proefschrift is om onder andere het effect van roken en het gebruik van een antiseptische (triclosan) hechtdraad op wondgenezing en littekenvorming te evalueren. Daarnaast wordt er in dit proefschrift gekeken naar enkele objectieve parameters die gebruikt worden in de evaluatie en follow-up van littekenbehandeling. Deze paragraaf beschrijft de bevindingen van dit proefschrift.

In hoofdstuk 2 wordt de relatie onderzocht tussen de gewoonte van het al dan niet roken en de leeftijd waarop een cosmetische ingreep wordt ondergaan. Het doel van deze studie is om te evalueren of rokers eerder een bovenste ooglidcorrectie (bleharoplastiek superior) hebben ondergaan ten opzichte van de (gemiddelde niet rokende) bevolking. Er werd een vragenlijst gestuurd naar een groep patiënten die een bovenste ooglidcorrectie hadden ondergaan. De ingreep werd voor alle deelnemers vergoed waardoor een socio-economische bias vermeden werd. Statistische analyse toonde geen verschil in rookgedrag tussen de algemene bevolking en de blepharoplastiek groep. Rokers ondergingen de operatie 3.7 jaar eerder ($p = 0.0007$) dan ex-rokers en ex-rokers ondergingen de operatie 3.5 jaar eerder dan nooit-rokers ($p = 0.006$). Dit is de eerste studie die beschrijft dat rokers eerder een bovenste ooglidcorrectie hebben ondergaan. In de literatuur wordt reeds beschreven dat roken, naast zonlicht, een van de belangrijkste factoren is die leidt tot vroegtijdige veroudering. Dit effect wordt op cellulair niveau toegeschreven aan een veranderde reactie van de fibroblast.[1, 2] Er werd geen verschil gezien in tijdstip van chirurgie tussen ex-rokers en nooit-rokers. Dit laatst genoemde effect zou mogelijk impliceren dat de huid over een regeneratief vermogen beschikt waardoor er geen verschil in tijdstip van opereren is tussen ex- en nooit-rokers.

Hoofdstuk 3 beschrijft het effect van nicotine gedurende de inflammatoire fase van cutane wondgenezing bij muizen. In deze studie werd, nadat eerst een excisie van de huid van de rug plaatsvond, een nicotine of een natriumchloride (NaCl)

oplossing geïnjecteerd in de wondranden. Er werd geen significant verschil in de snelheid van wondgenezing gezien tussen beide groepen. Een significant verschil in downregulatie van de expressie van groeifactoren waaronder PDGF, TGF- β 1 en TGF- β 2 werd geobserveerd na injectie met nicotine (10^{-8} M). Ook werd het effect van nicotine toediening op de macrofagen bestudeerd vermits deze cellen van cruciaal belang zijn vanwege hun effect op de release van groeifactoren in het wondbed.[3] Hiervoor werden in vitro macrofagen van muizen gebruikt (bone marrow derived macrophages, resident peritoneal macrophages en de muis macrofaag cell-lijn RAW 264.7). Na nicotine stimulatie werd een significante downregulatie van Tissue Necrosis Factor door macrofagen gezien. Dit resultaat is vergelijkbaar met in vivo resultaten waarbij toediening van nicotine resulteerde in een downregulatie op mRNA niveau en op eiwitniveau van VEGF, PDGF en TGF- β 1 in bone marrow derived macrophages. TGF- β 2 was niet detecteerbaar. Concluderend mogen we aannemen dat door nicotine toediening de productie van groeifactoren, gemedieerd door macrofagen, geremd wordt. Deze studie laat een remmend effect van groeifactor productie zien die het schadelijk effect van roken op de vascularisatie en huid weerspiegelt.

Wondinfectie en dehiscentie zijn beide belangrijke factoren die bijdragen aan de postoperatieve morbiditeit. Er zijn dan ook verschillende therapieën gericht op het reduceren van postoperatieve infecties. Een belangrijke rol is weggelegd voor hechtmateriaal gezien het een excessieve inflammatoire respons kan induceren. Hoofdstuk 4 evalueert of subcutane hechtingen, gecoat met een antiseptisch middel triclosan (TC), postoperatieve wondcomplicaties verminderen. In deze dubbel blinde gerandomiseerde studie werden vrouwen die borstchirurgie ondergingen geïnccludeerd waarbij iedere patiënt haar eigen controle was. Er werd geen voordeel van TC op postoperatieve wondgenezing geobserveerd. Daarentegen werd er drie weken postoperatief een stijging in wonddehiscenties gezien ($p = 0.023$).

Wonden waarbij epithelialisatie meer dan twee tot drie weken duurt, zijn gevoelig voor de ontwikkeling van hypertrofische littekens.[4] Een wondinfectie of een ernstige huidbeschadiging veroorzaken een uitgesteld genezingsproces resulterend in een versterkte inflammatoire fase. Hierdoor hebben deze

wonden een verhoogd risico op het ontstaan van hypertrofische littekens. Diepe brandwonden (vanaf diep tweedegraads) bevinden zich in een dergelijke situatie waarbij de genezingsduur deze termijn van twee tot drie weken overschrijdt. Een adequate beoordeling van de diepte van de brandwond is van cruciaal belang gezien de diepte een onderscheid maakt tussen een conservatieve dan wel een chirurgische procedure. Een oppervlakkige brandwond geneest meestal zonder aanvullende chirurgie in tegenstelling tot een diep tweedegraads brandwond waarbij chirurgie in de meeste gevallen noodzakelijk is. Laser-Doppler imaging (LDI) heeft reeds aangetoond om effectief te differentiëren tussen deze twee zones van beschadiging, resulterend in een adequaat behandelplan.[5] Na wondsluiting is een objectief meetinstrument belangrijk om het litteken en het huidige behandelplan te evalueren. Frequent onderzochte parameters in de follow-up van littekens zijn kleur, dikte en elasticiteit. Erytheem heeft reeds aangetoond om een waardevolle voorspeller te zijn in het mogelijk ontstaan van hypertrofische littekens na brandwonden.[6] De evaluatie van kleur en met name roodheid wordt veelal gebruikt als een standaard parameter voor littekenevaluatie. In hoofdstuk 5 worden twee elementen van erytheem geëvalueerd in de evaluatie van littekens. De bloeddoorstroming werd geëvalueerd middels de laser-doppler (Moor-LDI-B2; Moor Instruments, Devon, UK) en de roodheid werd geëvalueerd met behulp van een kleurenmeter (Minolta Chromameter CR-300; Konica Minolta Holdings, Inc., Tokyo, Japan). De intraclass correlatie coëfficiënt (ICC) en de standaard meetfout (standard errors of measurement, SEM) werden gebruikt om de intra-observator betrouwbaarheid van beide toestellen te kwantificeren.[7] De kleurenmeter heeft in deze studie aangetoond beter reproduceerbare resultaten te geven in vergelijking met de LDI bij de evaluatie van postchirurgische littekens en littekens na brandwonden.

Roken draagt bij tot een verhoogde kans op postoperatieve infecties en de hierbij horende verlengde wondgenezingsduur, mogelijk ten gevolge van een verstoorde macrofaag inductie.[8, 9] In hoofdstuk 6 worden de littekens tussen rokers en niet-rokers na een mammareductie (borstverkleining) geëvalueerd. Objectieve littekenevaluatie vond plaats middels de bepaling van erytheem met de kleurenmeter (Minolta Chromameter CR-300; Konica Minolta Holdings, Inc., Tokyo, Japan). Deze metingen vonden plaats op vier gestandaardiseerde

locaties van het litteken en op vier verschillende postoperatieve tijdstippen (1, 3, 6 en 9 maanden) postoperatief. In deze studie hadden rokers significant meer postoperatieve wonddehiscenties in vergelijking met niet-rokers ($p < 0.05$). In beide groepen nam de roodheid van de littekens met de tijd af. Er was echter een verschil ten voordele van de rokers zichtbaar, resulterend in minder rode littekens op verschillende locaties in het litteken en op de verschillende meetmomenten. Concluderend kunnen we stellen dat de littekens van rokers minder erytheem vertonen en sneller uitrijpen dan littekens bij niet-rokers.

Spanning op de wond, is naast een verlengde inflammatie een belangrijke factor die kan bijdragen aan de vorming van hypertrofische littekens.[10] De meest voorkomende locaties voor het optreden van litteken hypertrofie zijn: de rug, schouders, thorax en billen.[11] In hoofdstuk 7 werd het effect van roken op littekenvorming ter hoogte van de thorax onderzocht. In deze prospectieve studie werden 90 patiënten gevolgd die een mediane sternotomie ondergingen. De follow-up vond plaats gedurende 1 jaar op 4 verschillende tijdstippen (6 weken, 3, 6 en 12 maanden postoperatief). Zowel objectieve als subjectieve metingen werden verricht. De kleurenmeter werd gebruikt voor de objectieve meting en de POSAS schaal (Patient and Observer Scar Assessment Scale) werd gebruikt voor de subjectieve evaluatie van de littekens voor zowel de patiënt als de observator. In totaal werden 23 rokers, 52 ex-rokers en 15 nooit-rokers met een gemiddelde leeftijd van 61.5 jaar bestudeerd. Er werd geen significant verschil gezien in de afname van roodheid tussen rokers, ex-rokers en nooit-rokers. Er was wel een trend ten voordele van de rokers zichtbaar die resulteerde in minder rode littekens. Daarnaast werd geobserveerd dat het caudale litteken vaker hypertrofie vertoonde dan het proximale en middelste deel van het litteken ($p < 0.05$).

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Valorisatie addendum

Valorisatie addendum

Littekens ontstaan na beschadiging van de huid nadat er een trauma, brandwond of chirurgische ingreep heeft plaatsgevonden. Oppervlakkige wonden waarbij louter de epidermis (opperhuid) beschadigd is, genezen vaak zonder littekenvorming.

Recente studies hebben aangetoond dat patiënten vaak ontevreden zijn over hun littekens. Dit kan leiden tot significante psychologische stress en een verminderde kwaliteit van leven. Een groot aantal patiënten komt dan ook bij de plastisch chirurg terecht met een verzoek voor een littekencorrectie. De zorgvraag betreft niet enkel het esthetische aspect maar vaak hebben patiënten last van een bewegingsbeperking, jeuk of pijnklachten. Een normtroof litteken geneest in principe binnen een jaar en wordt aan het eind van het genezingsproces gekenmerkt door een vlak litteken met een normale tot bleke huidskleur. Naast het normtrofe litteken zijn er twee pathologische vormen van littekengenezing: het hypertrofisch en het keloïdaal litteken. Hypertrofische littekens zijn rode, verheven en jeukende littekens die binnen de wondranden blijven. Dit is in tegenstelling tot keloïdale littekens welke gekenmerkt worden door hun expansieve groei buiten de grenzen van de originele wond. Hypertrofische littekens ontstaan in 33-91% van de brandwond patiënten en in 39-68% van de chirurgisch behandelde patiënten. In de Verenigde Staten ondergaan jaarlijks 150.000 patiënten een chirurgische procedure om hypertrofische littekens te behandelen vanwege een bewegingsbeperking door contractuurvorming. Ook na een chirurgische ingreep is bij deze patiënten een intensieve follow-up gedurende een tot twee jaar noodzakelijk in combinatie met lokale siliconen- en druktherapie. Het exacte mechanisme voor de ontwikkeling van hypertrofische littekens is tot op heden niet duidelijk. In het merendeel van de gevallen is er sprake van een disbalans in het wond- en littekengenezingsproces. Een versterkte inflammatoire fase, excessieve proliferatie van fibroblasten en een verstoorde remodelleringsfase met een verminderde apoptose zijn een van de mechanismen welke reeds aangetoond zijn een effect te hebben op aberrante littekenvorming. Geïnfecteerde wonden of diep tweedegraads brandwonden zijn predisponerende factoren voor de ontwikkeling van hypertrofische littekens. Peroperatief wordt nagenoeg altijd gebruik gemaakt

van hechtmateriaal. Dit geeft een toegenomen lokale inflammatoire respons gezien het immers een vreemd lichaam is. Door het toevoegen van een lokaal antisepticum of antibiotica zou mogelijk de inflammatie geremd kunnen worden. Dit zou zich vertalen in een verminderd aantal postoperatieve wondinfecties en dus ook een betere littekengenezing met minder kans op hypertrofie. Naast het reduceren van infecties, is de release van groeifactoren tijdens een verwonding van essentieel belang. Met name de lokale release van TGF- β (Tissue Growth Factor- β) in de wond speelt hierin een belangrijke rol. Foetale wondgenezing wordt immers gekenmerkt door littekenloze genezing en hierbij wordt er een hoge ratio TGF- β 3/TGF- β 1 gevonden. Eenmaal geboren verandert deze ratio en overheerst TGF- β 1 als profibrotische cytokinen en is TGF- β 3 slechts in lage concentraties detecteerbaar, resulterend in een litteken.

Dit proefschrift heeft als doel om enkele factoren welke de kwaliteit van de huid en het litteken beïnvloeden te onderzoeken. Om dit doel te bereiken werden diverse wetenschappelijke studies verricht.

In **hoofdstuk 3** wordt het effect van lokaal toegediende nicotine bij muizen bestudeerd gedurende de inflammatoire fase van de cutane wondgenezing. Nicotine heeft, naast het welbekende carcinogeen effect, aangetoond anti-inflammatoire effecten te bezitten. Een beschermend effect werd reeds beschreven op inflammatoire aandoeningen zoals colitis ulcerosa en afteuze stomatitis. In hoofdstuk 3 studie werd er geen verschil in snelheid van wondgenezing gezien tussen muizen welke lokaal met nicotine geïnjecteerd werden versus de controle groep (NaCl injecties). Er werd wel een significant aangetoond in down-regulatie van PDGF, TGF- β 1 and TGF- β 2. Dit weerspiegelt, naast een down-regulatie van de profibrotische groeifactor TGF- β 1, ook het schadelijk effect van roken op de vascularisatie en de huid. In **hoofdstuk 6** wordt het effect van roken op littekengenezing beschreven na een borstverkleining. Hierbij wordt een significant verschil van afname in roodheid geobserveerd gedurende 4 verschillende tijdstipmomenten tot 9 maanden postoperatief. Daarnaast waren er bij rokers significant meer wonddehiscenties dan bij niet-rokers. In **hoofdstuk 8** werd de afname van roodheid in het litteken bestudeerd na een mediane sternotomie (open hart operatie). Hier werd geen significant verschil in afname van roodheid

geobserveerd tussen rokers, ex-rokers en niet-rokers. Dit zou mogelijk te maken hebben met de relatieve oudere patiëntenpopulatie gezien hypertrofische littekens met name voorkomen rond het 20ste tot 50ste levensjaar.

In dit proefschrift werd een mogelijk positief effect van roken aangetoond op littekenvorming. Roken wordt gezien de verhoogde kans op hart- en vaatziekten, longziekten, het verslavende aspect en het carcinogene effect niet aangeraden als preventieve therapie voor de beïnvloeding van littekengenezing. Patiënten met een verhoogde kans op hypertrofische littekens zoals brandwonden en patiënten met geïnfecteerde wonden, zouden zowel vanuit medisch, maatschappelijk als economisch standpunt gebaat zijn met een therapie welke deze vorm van littekengenezing reduceert. Verder onderzoek is noodzakelijk om het precieze mechanisme te ontdekken waarbij in de toekomst littekenvorming gereguleerd kan worden.

Dankwoord

Dankwoord

Graag wil ik iedereen bedanken die bijgedragen heeft aan dit proefschrift. In de eerste plaats dank aan alle patiënten voor hun bereidwilligheid tot deelname, hun tijd en interesse. Daarnaast wil ik nog enkele mensen in het bijzonder bedanken.

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Curriculum vitae

Curriculum Vitae

An Elisabeth Karel Deliaert was born on September the 5th, 1980 in Bilzen, Belgium. When she graduated from high school at the Provinciale Handelsschool Hasselt in 1998, she started nursing school in Maastricht. After completing nursing school (1998-2002), she worked as a nurse at the department of Urology, Dermatology and Plastic Surgery of Maastricht University Medical Centre (2002-2004). There, she discovered her interest and enthusiasm for Plastic and Reconstructive Surgery and she chose to continue her training, obtaining a Masters as a Physician Assistant at the Hogeschool Arnhem and Nijmegen (2004-2007). During her last phase of her Master degree, she immersed herself in wound- and scar healing and began researching this subject. This was the base of this thesis. During her job as a physician assistant she chose to continue her training, completing extra courses in physics and chemistry before going to medical school at the University of Maastricht (2008-2012). During medical school she continued research and her work, part-time, as a physician assistant. After obtaining her Medical Degree in 2012, she worked as a resident in Plastic Surgery at VieCuri Medical Centre in Venlo until she started her residency program in Plastic and Reconstructive Surgery at Catharina Hospital Eindhoven. In April 2015, she finished her two years of Basic General Surgery Training at the department of General Surgery, Catharina Hospital Eindhoven (dr. G.A.P. Nieuwenhuijzen). Currently, she is continuing her training at the department of Plastic and Reconstructive Surgery, Catharina Hospital Eindhoven (dr. M.M. Hoogbergen). She is living together with Pieter Broos and together they have a beautiful daughter Lola (2014) and they are expecting another child in January 2016.

List of publications

List of publications

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