The role of stromal vascular fraction in scar prevention: a model for a prospective double blind randomized placebo-controlled clinical trial

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ABSTRACT

Introduction
Results nor setup of the treatment of dermal chronic wounds or scars with the stromal vascular fraction (SVF) from adipose tissue in a proper prospective randomized double-blind placebo controlled setting has been documented to date. Therefore, the therapeutic efficacy of SVF grafting remains subject of speculations. Recently, we initiated a multi-center clinical trial, of which we present the setup as a proposal to standardize novel trials and render these comparable.

Methods & Design
This is a double-blinded multicenter randomized placebo-controlled clinical trial that compares scar treatments by injection of SVF with injection of saline. The target group comprises patients with elective surgery for mamma reduction. As primary outcome, the patient questionnaire of the Patient and Observer Scar Assessment Scale (POSAS) is used. As secondary outcomes, the observer questionnaire of the POSAS is used, as well as the histology and gene expression analysis of biopsies and a photographic panel assessment based on clinical photographs. Primary and secondary outcomes are measured six and twelve months postoperative. Histological and gene expression analyses are planned preoperative as well. A total of 38 patients will be included. This study is approved by the national Dutch ethical committee (CCMO).

Discussion
In this study, we describe a trial protocol for a placebo-controlled randomized double-blind clinical trial. The results of the clinical study may elucidate the hypothesized effect of SVF on scar formation in a clinical setting.

Trial status
Currently, patient inclusion has started. The trial is registered at the Dutch trial register with the following number: NTR5719.

INTRODUCTION

A scar – either normotrophic, hypertrophic or even keloid – is the result of the adult wound healing response. The wound healing cascade is normally initiated as a response to injury. After hemostasis, the inflammatory phase commences, followed by the proliferation and remodeling phases.\textsuperscript{1} In human skin, key players in the wound healing response are immune cells as well as resident tissue cells, including fibroblasts, keratinocytes and endothelial cells.\textsuperscript{2} Whereas inflammation and tissue generation are relatively short processes, which last respectively only days and weeks, the remodeling phase can take up to a year before being completed.\textsuperscript{1} In the ideal situation, this remodeling process results in a normotrophic scar. However, if one of the stages of the wound healing process is dysregulated, this healing process can result in either a hypertrophic or even a keloid scar.\textsuperscript{2}

Pathological scar types, such as hypertrophic and keloid scars, can lead to considerable impairments of the quality of life.\textsuperscript{1} Physically, scars located in proximity of joints can cause contractures, movement restrictions, pain and itch.\textsuperscript{4} Psychologically, the patient perceives the severity of the scar by psychosocial distress.\textsuperscript{5} Therefore, a combination of objective and subjective features determines the burden of disease for the patient. To quantify objective (i.e. color, height, pliability, relief) and/or subjective (i.e. pain, itch) features of scar severity, several validated scar scales, for example the Patient and Observer Scar Assessment Scale (POSAS), Vancouver Scar Scale (VSS) and Manchester Scar Scale (MSS) have been developed.\textsuperscript{6} Also, multiple validated devices to objectively measure different scar parameters have been developed, e.g. elastometer, colorimeter and 3D imaging methods.\textsuperscript{7}

Autologous fat grafting has emerged as a possible therapy for difficult-to-treat dermal scars. Whole adipose tissue is collected by means of liposuction, it can be processed e.g. by washing and/or centrifugation, and is reinjected into the scar area, often in combination with a percutaneous scar release. The regenerative and scar-reducing properties of adipose tissue have been attributed to adipose-derived stromal cells (ASC) that are present in large numbers in adipose tissue and therefore in fat grafts. ASC, therefore, are regarded as therapeutic cells that can be isolated by means of either enzymatic or mechanical dissociation of the adipose tissue, that result in what is called the tissue stromal vascular fraction (tSVF).\textsuperscript{8} ASC are present in this fraction along with e.g. endothelial cells, supra-adventitial cells, pericytes and lymphocytes.\textsuperscript{9} The possibility to isolate tSVF mechanically in a rather short period of time enables clinical application that is less time-consuming and safer for the patient as compared to enzymatically obtained cellular SVF (cSVF).

In our hands, a reliable, fast and validated technique to isolate tSVF mechanically during surgery opens the door to new clinical applications, such as application for improvement of wound healing, scar prevention or treatment of scars.\textsuperscript{10,11} Thus far, no clinical studies have been performed with the use of ASC, tSVF or cSVF to prevent disturbed scar healing. A large number of case reports, retrospective and prospective trials – with a lack of (placebo) control groups – have been reported.
in literature and point toward a potential improvement in pain or aesthetical outcome of scars after fat grafting. However, these case reports give not yet sufficient substantial evidence of this suggested advantage: therefore prospective, double blind randomized clinical trials are necessary to scientifically support and prove the potential advantage of fat grafting, ASC, tSVF or cSVF for better healing and better scars.

For that reason, we have set up a multi-center randomized double-blind placebo-controlled clinical trial to study the effect of tSVF on scar formation. Because the literature lacks such studies thus far, our set up might be of interest for other research groups to study all kinds of other aspects with regard to SVF and scar formation. Therefore, the aim of this report is to describe how to elaborate on patient selection, selection of outcome measures and time points for measurements and on power analysis.

METHODS & DESIGN

Trial design
The design of the study is a multicenter placebo-controlled double blind trial to study the preventive effect of tSVF on scars after a mamma reduction. The mammae of one participant are divided into two groups based on randomization. Mammae in group A receive a tSVF injection in the wounds after the mamma reduction and mammae in group B receive a physiological saline injection after the mammae reduction. Follow-up assessments are six and twelve months postoperative (Fig 1). The last follow-up assessments, twelve months postoperatively, is selected based on the period needed for scar maturation.

Patient recruitment & Consent procedure
The population in this study consists of healthy female humans (aged 18-60 years) undergoing a mamma reduction in both mammae, wise pattern technique. Mammareduction in both mammae, age 18-50 females. Inclusion criteria: Males, aged below 18 or above 50 years. Exclusion criteria: Any oncological event in the participant's history, A known psychiatric condition, A known systemic disease that will impair wound healing (e.g. diabetes mellitus, known atherosclerosis with an event that required hospitalization, collagen diseases, diseases of the skin, HIV). Use of prednisone or other immunotherapy, Smoking, Pregnancy or active child wish, Frequent exposure to known carcinogenic substances (e.g. work related), Active or previous use of hormone replacement therapy.

Table 1 | Inclusion and exclusion criteria

<table>
<thead>
<tr>
<th>Females</th>
<th>Male</th>
</tr>
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<tbody>
<tr>
<td>Age 18-50</td>
<td>Aged below 18 or above 50 years</td>
</tr>
<tr>
<td>Mamma reduction in both mammae, wise pattern technique</td>
<td>Aged between 18 and 50 and in the menopause or pre-menopause</td>
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<tr>
<td>Surgical interventions of the breasts in the year prior to the date of surgery</td>
<td>Mamma reduction of one mamma</td>
</tr>
<tr>
<td>Any oncological event in the participant’s history</td>
<td>A known psychiatric condition</td>
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<tr>
<td>A known systemic disease that will impair wound healing (e.g. diabetes mellitus, known atherosclerosis with an event that required hospitalization, collagen diseases, diseases of the skin, HIV)</td>
<td>Use of prednisone or other immunotherapy</td>
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<tr>
<td>Smiling</td>
<td>Smoking</td>
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<tr>
<td>Pregnancy or active child wish</td>
<td>Use of prednisone or other immunotherapy</td>
</tr>
<tr>
<td>Frequent exposure to known carcinogenic substances (e.g. work related)</td>
<td>Active or previous use of hormone replacement therapy</td>
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Randomization, Blinding & Treatment allocation
Randomization, blinding and coding will be done by an independent person using the randomization tool available on http://www.randomization.com. Before start of the trial, an independent person will place cards containing the words ‘SVF-L’ (nineteen times), ‘NaCl-L’ (nineteen times), in envelopes numbered one to thirty-eight following the generated list of numbers by the randomizer tool. This procedure is repeated using the same generated list, creating two identical sets of envelopes. The content of the first envelope is divided over four envelopes by the same independent person, then sealed and given to the four participating locations of this multicenter research. The second envelope is sealed and will be kept in a secure vault at the central research location (UMCG). Every enrolled participant will receive a unique number forming the range of 1 to 38 after enrolment. The participating plastic surgeons will do treatment allocation and administration of the tSVF injectable and physiological saline injectable on site, determined by the content of the envelope, corresponding with the participants’ assigned unique number. If a participant receives ‘SVF-L’, the participating plastic surgeons will inject tSVF in the wound of the left breast. If a participant receives ‘NaCl-L’, the participating surgeons will inject physiological saline in the wound of the left breast. Automatically, the wound of the other breast will receive the other treatment. The envelope will be opened before start of the operation and the participant will be kept blinded all the time. Only the independent experts and the participating surgeons...
are not blinded during the trial. The principle investigator and his staff, who perform all the data analyzes, will be blinded during the trial.

Trial interventions

Lipoaspirate is harvested from the abdominal region using very fine lipoharvesting cannulas. 40 ml of lipoaspirate is harvested to create two times one ml of SVF according to the Fractionation of Adipose Tissue (FAT) procedure. One ml of SVF is used to inject in the lateral five centimeter of the horizontal wound of the breast with the use of a sharp needle after suturing. The other one ml of SVF is used for analysis to determine the number of adipocytes (Perilipin A staining), smooth muscle cells (Alpha-Smooth Muscle Actin staining), endothelial cells (von Willebrand Factor) and extracellular matrix (Masson’s Trichrome staining). Participants will all receive under the same conditions the same volumes; 0.1 ml of SVF and 0.1 ml of physiological saline per 0.1 cm² scar (Fig 1). Suturing is performed using Monocryl 4.0 in the lateral five centimeter of the horizontal wound of the breast intracutaneously. All study procedures, including the mamma reduction, will take place under general anesthesia.

Safety concerns

tSVF is created by means of minimal mechanical manipulation without the use of animal derived or enzymatic additives. For this reason, most of the tissue-like structures in adipose tissue such as extracellular matrix and small blood vessels as well as cell types such as ASC remain intact; only most of the adipocytes will be disrupted. Therefore, the risks and types of complications, when tSVF is used in wound healing, are similar to the use of lipoaspirate in wound healing. To further minimize the risk of complications, participants with any oncological history or frequent exposure to known carcinogenic substances (e.g. work-related) are excluded, as there remains controversy about the interaction between cellular or tissue SVF, ASC and cancer. In vitro data suggest that ASC might promote progression and cell growth of cancer cell-lines, while clinical data suggests that ASC can be used safely in patients without an oncological history.

No serious adverse events are to be expected, however, they will be registered and reported to the National Dutch Ethical Committee (CCMO). The study protocol is approved by the National Dutch Ethical Committee (CCMO) and follows good clinical practice guidelines and current Dutch legislation.

Objectives

Our primary outcome is the patient questionnaire of the Patient and Observer Scar Assessment Scale (POSAS). By using the patient questionnaire as primary outcome only, the results of the primary outcome will be obtained blinded. For that reason, our clinical trial is considered to be double-blinded. The patient questionnaire of the POSAS consists of five main topics regarding pain, itchiness, color, stiffness, thickness and irregularity of the scar. One additional question regarding the overall opinion of the participant about the scar is asked. Each question asks to rate the aforementioned topics from one, best imaginable scar, to ten, worst imaginable scar.

As secondary outcomes, the observer questionnaire of the POSAS is used, histological improvement is quantified by analysis of skin biopsies and clinical photographs are taken and analyzed. The observer questionnaire of the POSAS consists of five main topics regarding
vascularity, pigmentation, thickness, relief, pliability and surface area. Again, one additional question regarding the overall opinion of the participating plastic surgeon about the scar is asked. Scores are given in a similar way as with the patient questionnaire of the POSAS. The POSAS questionnaire is a validated and commonly used assessment scale to rate the patients’ as well as the observers’ opinion about scars.\(^{18}\)

Histological observation is performed after taking skin biopsies from the resected skin preoperative and from the lateral five centimeter of the horizontal scar. Skin biopsies (maximum diameter of 4mm) will follow the border of the scar and so therefore no new scar tissue will be formed. At two locations, the biopsies will be divided into two groups: first group (25% of the biopsy) will be cryopreserved for gene expression analysis, second group (75% of the biopsy) will be used for immunohistochemistry analysis.

Evaluation of scars (e.g. scar size, color, vascularity and relief) is performed by a photographic panel evaluation assessment. Standardized photos (anterior-posterior of 1-meter distance) will be analyzed by a panel of independent experienced plastic surgeon (all blinded) of the University Medical Centre Groningen department of Plastic Surgery. The participants’ breast group and time after operation will be blinded for the plastic surgeon. Every plastic surgeon is asked to rate all the photographs twice with a minimum interval of one week in between to determine the inter- and intraobserver reliability.

All objectives are measured for each scar of one breast, six months and twelve months after surgery. Only skin biopsies for immunohistochemistry analysis and gene expression analysis are taken preoperatively as well.

Data collection

Data are collected six and twelve months postoperatively for all objectives, except for skin biopsies for histological analysis, which are collected at the baseline as well. All questionnaires are completed on paper and transferred to an online database (Openclinica).

Statistical analysis

Results from patient questionnaires will be used as continuous quantitative data. Data will be tested for normality, and groups will be compared bases on either a paired t-test or a Wilcoxon test to test the differences cross-sectionally at six and twelve months (p-value of 0.05). Uni- and multi variate regression analysis will be performed to study the effect of the treatment group, and age on the primary outcome.

Results from observer questionnaires will be analyzed in the same manner as results from patient questionnaires. Results from skin biopsies will be scored based on number of colored cells per square surface area (continuous outcome) and differences between groups will be tested using Chi square test (p-value of 0.05). Results from the photographic evaluation will be analyzed by a blinded panel of plastic surgeons and will be used as continuous quantitative data. These data will primarily be displayed in a descriptive manner. Additionally, a t-test or Wilcoxon test will be used to assess differences in assigned scores, per question and per follow-up moment between both groups. Also a paired t-test (C.I. 95%) will be used to analyze scores within each group. Consistency within one panelist, will be analyzed by calculating an Intra Class Correlation Coefficient and consistency between panelists will be analyzed by using inter observer reliability analysis (p-value of 0.05). Reasons for dropout will be registered and studied. In case of informative dropouts, we will report this in the results, and if possible, correct for it in the analysis (regression analysis).

Sample size calculation

The sample size calculation is based on differences in scores of the primary measure of effect: the total score of the patient scale of the POSAS questionnaire, between the tSVF and physiological saline group, six months postoperative. Based on the literature, the mean preoperative value of the total score of the patient scale is 35.\(^{19-22}\) The preoperative score is equal to our untreated group (physiological saline group), assuming no improvement due to physiological saline injections. Scars in those studies had a mixture of different causes (e.g. burn wounds or trauma) and therefore we anticipate a lower value of the total score of the patient scale in the placebo group of our study. To come to a representative score for the physiological saline group, we merged the scores of scars of two studies.\(^{21,22}\) Klinger et al. showed a preoperative total score of the POSAS patient questionnaire of 41.6, while Khoo et al. showed a postoperative total score of the POSAS patient questionnaire of 11.6 after treating hypertrophic scars with placebo.\(^{21,22}\) We have to remark that Khoo et al. only used six out of seven parameters of the patient scale of the POSAS patient questionnaire to measure the total score. As a result, and based on clinical experience, we assumed a total score of the patient scale of 27 in the untreated part (physiological saline group) of the scar. Based on the literature, the mean decrease in total score of the patient scale is 35% after treating scars with lipoaspirate comparing pre- and postoperative scores.\(^{19-21}\) Thus, we estimated an improvement of 25%, assuming a preoperative value of 27 of the total score of the POSAS patient questionnaire in the untreated part (physiological saline group) of the scar.\(^{21,22}\)

Giving the range of estimations in literature and differences in causes of the scars studied, a sample size calculation based on means and standard deviation is difficult. Based on the available literature, clinical experience and expert opinion, we can, however, assume a large effect size. For that reason, the sample size calculation is based on an effect size of 0.5 (t-test: mean difference between two means (matched pairs)). We assume an alpha of 0.05, a power of 0.80 and a two-sided test. This results in a required sample of 34 women. Assuming a loss of 10% in follow-up, a sample of 38 women is required.

Ethical issues

The safety of the use of tSVF and lipofilling as scar or wound healing treatment have been evaluated in several different clinical studies.\(^{21,26}\) Only a few minor complications have been reported in
these studies thus far. However, during surgical procedures, there is always a risk related to the anesthesia or the surgery. In this study, the benefit for participants is extremely low and so it is important to reduce the risk to a minimum.

Timeframe
Participants are included in this study over period of two years and followed for one year. In total, this study will cover a period of three years.

DISCUSSION
In this study, we provide a standardized protocol for a multi-center placebo-controlled randomized double-blind clinical trial to treat scar formation with tSVF injections. Publishing a trial protocol is a method to warrant unbiased and high quality, scientific data. Furthermore, this study protocol addressed difficulties regarding study designs, sample size calculation, statistical analysis and safety concerns. For that reason, this trial protocol can be used by other research groups as a guideline to set up a proper well designed randomized clinical trial.

In this study, the benefit for participants is low on short terms. However, the scientific value of the results of this study are important to the scientific community and therefore the low risk for complications in this trial is acceptable for participants. Impaired wound healing is a major problem within plastic surgery (e.g. diabetes mellitus patients). By using a model with healthy participants first, the risk of complications is even lower in comparison with patients with a pathological condition. Moreover, the origin of dermal fibrosis (scars, burn wounds) is ascribed to dysfunction of the professional stromal cell: the fibroblast.\textsuperscript{27,28} Dysfunction of fibroblasts plays a role in other kinds of fibrosis (liver, kidney, heart) as well.\textsuperscript{29-31} During the onset and progression of fibrosis, part of the local fibroblasts are activated and differentiate into myofibroblasts. Myofibroblasts not only contract the lesion, but also strongly contribute to excessive deposition of extracellular matrix, in particular collagens, in scars or fibrotic organs. In addition, during development of fibrosis, myofibroblasts proliferate vigorously which is undesirable and maintain the lesion in a fibrotic state. We therefore argued that treating dermal fibrosis with tSVF and subsequently analyze the therapeutic effect via skin biopsies will provide us valuable information about how/if tSVF can reduce fibrosis in dermal scars and burn wounds. Although the mechanisms of organ and dermal fibrosis may differ in details, the understanding of how tSVF might influence the differentiation of fibroblasts into myofibroblasts is of great value for therapy development to treat dermal and other kinds of fibrosis.

The results of the clinical study of this trial protocol will show the effect of tSVF on scar formation and might elucidate its supposed effect on scar formation in a clinical setting. To our knowledge, this will be the first placebo-controlled randomized double-blind clinical trial to use mechanical isolated tSVF as preventive treatment of scar formation.

TRIAL STATUS
This trial is currently including patients to participate in the study. This trial is registered at the Dutch trial register with the following number: NTR5719.
REFERENCES


