Development of an in vitro keloid scar model for identifying and testing new anti-scar therapies.

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Of note, the objectives and end points described below all refer to in vitro assessments. The only patient intervention is the removal of peripheral blood in order to provide samples for the in vitro tests. First Objective: Development of a human,...

Ethical review	Approved WMO
Status	Recruitment stopped
Health condition type	Cutaneous neoplasms benign
Study type	Observational invasive

Summary

ID

NL-OMON39107

Source
ToetsingOnline

Brief title
Development of an in vitro keloid scar model.

Condition

- Cutaneous neoplasms benign

Synonym
cheloid, keloid, keloidal scar

Research involving
Human

Sponsors and support

Primary sponsor: Vrije Universiteit Medisch Centrum
Source(s) of monetary or material Support: AgentschapNL

Intervention

Keyword: keloid, model, scar, therapy

Outcome measures

Primary outcome

Development of a human, in vitro, immunocompetent full-thickness tissue-engineered keloid scar model and identification of robust and relevant parameters to assess scar formation in vitro

Keloid scar parameters:

• epidermal and dermal thickness (histological analysis)

• contraction

• myofibroblast α-SMA expression (immunohistochemical staining)

• wound healing mediator expression (ELISA)

Immune cell parameters:

• changes in monocyte and T cell phenotype after co-culture with keloid tissue compared to normal tissue. Flow cytometric analysis to identify transition into e.g.: macrophages, Th1, Th2, fibrocytes, endothelial cells etc.)

Secondary outcome

Validation of the in vitro keloid scar model with the aid of parameters identified above

• Positive controls: therapeutics currently used for treating scars (e.g. 5FU, corticosteroids). Normalization (partial) of scar parameters is expected.

• Negative controls: therapeutic known to be ineffective in scar treatment

(e.g. vitamin D3). No normalization effect of scar parameters is expected.

Study description

Background summary

Keloid formation is a result of adverse wound healing, in which a surplus of scar tissue forms that exceeds the boundaries of the original wound. Currently no adequate therapies exist and recurrences after treatment are common. In order to develop better treatment strategies, superior scar models need to be developed in order to test novel therapeutics, combinations of therapeutics and to identify new drug targets. Since animals do not form keloids, relevant human in vitro models need to be developed. Therefore we have developed a tissue engineered basic keloid scar model from rest material isolated from excised keloid scars. This model consisted of a reconstructed epidermis on a fibroblast populated dermal matrix. Although this basic model did show abnormalities indicative of a keloid scar developing, the strong keloid phenotype was lacking. Recent literature suggests that an immune component (monocytes) is essential for the development of keloids in addition to an intrinsic property of the keratinocytes and fibroblasts. This finding therefore strongly indicates that it is essential to include monocytes derived from keloid patients in the keloid scar model. It stands to reason that other immune cells might also demonstrate intrinsic abnormalities in keloid patients, therefore we aim also to isolate T-cells in addition to PBMCs and bring these into co-culture with the keloid model.

Study objective

Of note, the objectives and end points described below all refer to in vitro assessments. The only patient intervention is the removal of peripheral blood in order to provide samples for the in vitro tests.

First Objective:
Development of a human, in vitro, immunocompetent full-thickness tissue-engineered keloid scar model and identify robust and relevant parameters for the assessment of the degree of keloid scar formation in vitro. The keloid model will be compared with the healthy scar counterpart in order to identify the parameters.
- In vitro keloid scar parameters: e.g.: increased epidermal and dermal thickness; contraction, myofibroblasts (α-SMA expression), cytokine/ chemokine secretion (ELISA).
- In vitro immune cell parameters: e.g.: changes in monocyte and T cell phenotype after co-culture with keloid tissue compared to normal tissue. Flow cytometric analysis to identify transition into e.g.: macrophages, Th1, Th2,

fibrocytes, endothelial cells etc.)

Second Objective(s):
Validate the in vitro keloid model with known therapeutics currently used in the scar clinic of the Dept. of Plastic Surgery (e.g. 5FU, corticosteroids can serve as positive controls) and therapeutics known to be ineffective in scar treatment (e.g. vitamin D3 can serve as a negative control). The therapeutics will be applied to the keloids during culture and at the time of harvesting, the parameters identified in the first objective will be analysed. It is to be expected that currently used scar therapeutics will (partially) normalize the scar parameters whereas ineffective therapeutics will not. The model will then be ready for testing new therapeutics and combinations of therapeutics in preparation to clinical studies in the future.

Study design

Peripheral blood will be removed from keloid patients at the time when they are scheduled for standard treatment in the Dept. Plastic Surgery. The blood will be transported to the dermatology lab where PBMCs (e.g. monocytes and T-cells) will be isolated for co-culture with skin equivalents. In parallel, peripheral blood will be isolated from healthy volunteers with a known history of developing normal scars.

Keloid skin equivalents and normal healthy skin equivalents will be constructed from rest material obtained from standard surgical procedures. These will be co-cultured with immune cells (monocytes, macrophages, T-lymphocytes, PBMCs) isolated from peripheral blood with the aid of a transwell 2 compartmental chamber. Hereafter, cultures will be harvested and scar forming parameters assessed.

The total duration of the project is expected to take three years (Agentschap NL financed project already underway). This includes the development of the in vitro keloid scar model and its validation with therapeutics currently used to treat scars and with therapeutics known to be ineffective in correcting scars. Each independent experiment will require four independent runs each with different donor material in order perform statistical analysis. Over a period of three years, 140ml of peripheral blood will be drawn from a maximum of n=30 keloid patients and n=30 healthy volunteers in order to develop the immunocompetent in vitro human keloid model and to validate it with known therapeutics.

Patients with a history of keloid scar formation will be selected and approached by plastic surgeons of the VUmc plastic surgery department during their outpatient clinic sessions to participate in this study. Upon their informed and signed consent, withdrawal of peripheral blood samples will be done during regular clinic appointments.

Healthy controls known to form normal scars after trauma will also be selected and approached by the plastic surgeons and researchers.

Intervention

140 ml of peripheral blood will be withdrawn via standard procedures used in venapuncture. This is not part of the medical treatment for the patient and therefore is extra for this study. This blood will only be used for the isolation of immune cells, no further laboratory tests (clinical/diagnostic or otherwise) will be performed.

A questionnaire composed of eight questions is required.

Study burden and risks

Venapuncture itself is associated with slight risks such as excessive bleeding, fainting or feeling light-headed, hematoma formation and infection (minimal risk occurring whenever there is a break in the skin). However, venapuncture is used on such a large scale without any problems worth mentioning, that there are few people left who have not ever undergone the procedure. In addition to these risks, there is a theoretical risk of keloid formation at the site of venapuncture in keloid patients, although no such event has ever been described in literature.
While there is no direct benefit for the patient group, our study may lead to improved treatment strategies for keloid patients and could therefore certainly be of benefit to them in the future. Given the limitations of current therapies and their inability to effectively treat keloid scars, keloid patients are often very willing participants because they know research is the only way forward towards better management of their condition.

Contacts

Public
Vrije Universiteit Medisch Centrum

de boelelaan 1117
Amsterdam 1081 HV
NL
Scientific
Vrije Universiteit Medisch Centrum

de boelelaan 1117
Amsterdam 1081 HV
NL

Trial sites

Listed location countries

Netherlands

Eligibility criteria

Age
Adults (18-64 years)
Elderly (65 years and older)

Inclusion criteria

For keloid patients:
- history of keloid formation: presence of at least 1 major keloid (>0.5cm)at least 6 months old, diagnosed as such by plastic surgeon specialized in scarring
- 18 years or older and capable of giving informed consent;For healthy controls:
- previously undergone surgery of the trunk region (thorax, abdomen) resulting in a normal scar at least 5cm in length
- normal scar should be at least 6 months old
- no abnormal scarring in medical history
- 18 years or older and capable of giving informed consent

Exclusion criteria

- systemic illness
- chronic use of systemic medication (e.g. corticosteroids, anti-inflammatory drugs such as aspirin)

Study design

Design

Study type:	Observational invasive
Intervention model:	Other
Allocation:	Non-randomized controlled trial

Masking:	Open (masking not used)
Control:	Active
Primary purpose:	Basic science

Recruitment

NL

Recruitment status:	Recruitment stopped
Start date (anticipated):	23-06-2014
Enrollment:	60
Type:	Actual

Ethics review

Approved WMO

Date:	20-03-2013
Application type:	First submission
Review commission:	METC Amsterdam UMC

Study registrations

Followed up by the following (possibly more current) registration

No registrations found.

Other (possibly less up-to-date) registrations in this register

No registrations found.

In other registers

Register	ID
CCMO	NL40595.029.12