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Microvascular regeneration in meshed skin transplants after severe burns

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ABSTRACT

Function of the skin lymphatics as well as blood perfusion of a meshed transplant is crucial for the healing. The lymphatic regeneration and arterial perfusion of skin transplants after severe burns of the extremities had been studied in eight patients by microlymphography, laser doppler perfusion imaging and transcutaneous oxygen pressure measurements 1, 6 and 18 months after transplantation.

One month after transplantation, only fragmented as well as many giant lymphatic skin vessels were present in the transplant. After 6 months a normal lymphatic network had developed in all grafts. The extension of the dye in the lymphatics decreased from 4.5 (0–16) at 1 month to 3.0 (1–6) mm after 18 months, indicating improved lymph drainage capacity. The permeability of the lymphatics in the graft was normal. After 1 month, median laser flux in the transplant was 155.6% (105–246%) of the normal skin but it normalised within 18 months. By contrast, transcutaneous oxygen measurement ($TcPO_2$) increased from 44 (21–47) to 55 (50–76) mmHg.

In meshed transplants used to cover severely burned skin morphological and functional normal lymphatics develop within 6 months and the initially increased laser flux due to inflammatory reaction normalises. Our results provide important insights into the healing process of skin transplants after burn.

Deep burns involving the entire dermis or extending beneath the dermis into fat, muscle and bone show a poor and prolongated wound healing, taking weeks and even months, if they are managed conservatively. This long time stretch involves a high risk of systemic disease with multi-organ failure and local infection, post-burn oedema and cicatricial contracture. Therefore, the preferred approach is a combination of excision and grafting [1]. Reduction of the size of skin-graft donor sites can be accomplished by meshed grafts, allowing the skin to expand by up to six times the original area.

Different factors influence normal wound healing. Multifunctional cytokines, such as the vascular permeability factor/vascular endothelial growth factor (VPF/VEGF), induce an angiogenic as well as a strong lymphangiogenic response [2]. From day 5 of wound healing onwards, VEGF-receptor-3 (VEGFR-3)-positive vessels can be observed by immunohistochemistry in the granulation tissue. These vessels appear to sprout from pre-existing VEGFR-3-positive lymphatic vessels at the wound edge [3]. This sprouting and the formation of anastomosis between the skin microlymphatic networks around the scar in replanted fingers had been convincingly demonstrated by microlymphography [4]. On the other hand, in split-skin grafts of patients with venous ulcers, there was almost no regeneration of lymphatics even years after the transplantation [5]. The wound ground appears to play an important role in the regeneration of lymphatic vessels.

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The flux was the subject of several studies whose results suggested that laser doppler perfusion imaging (LDPI) is a suitable means of assessing tissue perfusion in healing burns. These studies demonstrated that deep-dermal- and full-thickness burns have perfusion levels less than those of normal skin and that, for this case, surgical treatment is beneficial. By contrast, superficial burns have perfusion levels elevated as much as three to five times the level of normal skin [6–9].

Transcutaneous oxygen measurement is used in experimental set-ups for monitoring of 'skin graft take' [10,11].

Only little is known about the regeneration and function of lymphatic vessels, and the early microvascular perfusion in meshed grafts on burn wounds. The aim of this study is to evaluate lymphatic regeneration and arterial perfusion in skin transplants in patients with severe burns.

1. Methods

1.1. Patients

In this prospective, non-randomised study, patients with deep dermal or full thickness burn injuries (i.e. grade II and grade III) on the extremities treated with a meshed skin graft from January 2006 to December 2008 had been included. The therapeutic management in all patients consisted of an excision of the burn eschar followed by a grafting with a meshed skin transplant. At the time of the study, all skin grafts were taken.

In all patients, the lymphatic regeneration in the skin graft was studied by fluorescence microlymphography; the blood perfusion of the grafted skin area was examined by LDPI and transcutaneous oxygen tension measurement.

The study had been approved by the local ethical committee (EK 1711) and all patients gave written informed consent.

1.2. Fluorescence microlymphography

The technique of fluorescence microlymphography has been described in detail [12]. Using a steel microcannula with a tip diameter of 0.2 mm (Arnold Bott, Zurich, Switzerland) connected to a microsyringe (Hamilton, Bonaduz, Switzerland), 10 μl of a sterilised 25% solution of FITC-(fluorescein isothiocyanate) dextran with a molecular weight of 150,000 (TdB Consultancy AB Virdings Allé 28, SE-754 50 Uppsala, Sweden) is injected into the subepidermal layer of the skin. As large molecules are exclusively drained by the lymphatic system, the fluorescent dextran molecules move into the initial lymphatics and can be visualised by a fluorescence video microscopy system. This consists of an incident-light fluorescence microscope (Leica, Heerbrugg, Switzerland), a three charge coupled device (3-CCD) video camera (model DXC-930P, Sony, Tokyo, Japan) with a camera adapter and sensitivity on automatic control (CMA-D2, Sony), a timer (VTG-22) and scale marker (IV-600; both from For-A-Company, Tokyo, Japan), a monitor (Picture Monitor model PM 171T, Ikegami Tsushinki, Tokyo, Japan) and a recorder. The microscope is equipped with 1.0/0.04, 2.5/0.08, 6.3/0.20 and 10/0.25 planar objectives (Leica), which allow a magnification of ×24, ×62, ×165 and ×240, respectively, on the monitor. The fluorescence excitation filter works at 450–490 nm and the barrier filter at 515 nm.

1.3. Morphology of lymphatic vessels in the skin transplant

The following parameters have been evaluated off-line from the videotape 10 min after dye injection: presence of visualised lymphatic meshes; fragmentation of the superficial lymphatic network (defined as network with interruptions of the meshes); cutaneous backflow (defined as abnormal retrograde flow from deep to cutaneous lymphatics away from the main network); and maximal extension of the fluorescent macromolecules into the lymphatic network in the lateral, proximal, medial and distal direction as a measure for lymphatic drainage capacity into deeper channels and diameter of single lymphatic microvessels. Giant lymphatic skin vessels are defined as lymphatic capillaries with a diameter $>56.3 \pm 9$ μm [12].

1.4. Lymphatic permeability

The lymphatic wall is permeable to water and small solutes but less to larger molecules such as FITC-dextran 150,000 MG in healthy skin. High peaks of fluorescent light intensity remain located over the capillary. Increased permeability is characterised by increased interstitial fluorescence intensity and a lower peak of light intensity over the vessel [13]. The permeability of initial lymphatics was determined by video densitometry. The recordings of the lymphatic network obtained during the microlymphography 10 min after dye injection were digitalised and colours were transformed into black and white (synedra view and image view software, synedra information technologies GmbH, Innsbruck, Austria). Single images of well-delineated lymphatic capillaries as far away as possible from the dye depot were chosen. A 10×10 pixel region of interest was localised over the brightest point in the lymphatic capillary and 0.5 mm away from the capillary wall in the interstitial tissue. The light intensity was measured three times by the image processing and analysis software ImageJ (Andreas Jahnen, Luxembourg). Light intensity was expressed in arbitrary density units (DU) and the light intensity in the vessel defined as 100%. The difference from intraluminal to interstitial given as percentage was compared with normal skin and changes over time were noted.

1.5. $TcPO_2$ measurement

The polarographic technique of $TcPO_2$ measurement has been described in detail [14]. Transcutaneous oxygen values were obtained with the electrode (Radiometer, Kopenhagen, Denmark) set at a temperature of 44 °C to produce local hyperaemia. The measurement result is indicated in mmHg and is a marker of the nutritive microcirculation of the skin.

1.6. Laser Doppler Perfusion Imaging (LDPI)

LDPI is a newer method to determine the local distribution of flux over a skin area without direct contact with the tissue surface. Laser light is emitted from the source in a box placed approximately 20 cm above the skin [15,16]. Flux is a relative measure of the microvascular flow, represents the product of speed and number of cells and is expressed in arbitrary perfusion units (PU). LDPI has an average measurement depth