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The mouse dorsal skin fold chamber as a means for the analysis of tissue engineered skin

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ABSTRACT

The therapy of extensive and deep burn wounds is still a challenging task for reconstructive plastic surgery. The outcome is generally not satisfactory, neither from the functional nor from the aesthetic aspect. Several available skin substitutes are used but there is need for optimization of new skin substitutes which have to be tested *in vitro* as well as *in vivo*. Here, we show that the dorsal skin fold chamber preparation of mice is well suited for the testing of skin substitutes *in vivo*. Dermal skin constructs consisting of matriderm® covered with a collagen type I gel were inserted into full thickness skin wounds in the skin fold chambers. The skin substitutes integrated well into the adjacent skin and got epithelialized from the wound edges within 11 days. The epithelialization by keratinocytes is the prerequisite that also cell-free dermal substitutes might be used in the case of the lack of sufficient areas to gain split thickness skin grafts. Further advantage of the chambers is the lack of wound contraction, which is common but undesired in rodent wound healing. Furthermore, this model allows a sophisticated histological as well as immunohistochemical analysis. As such, we conclude that this model is well suited for the analysis of tissue engineered skin constructs. Besides epithelialization the mode and extend of neovascularization and contraction of artificial grafts may be studied under standardized conditions.

1. Introduction

The therapy of burns still displays a complex and challenging field due to the many difficulties arising from large and deep burns, *i.e.* the high risk of infections, the destruction of both epidermis and dermis and the only limited availability of autologous split-thickness skin grafts and keratinocytes for wound coverage. This leads to the need of skin substitutes for temporary or permanent wound closure. Several skin substitutes have already been used for years in the clinical application [1–3] but full success in burn regeneration has not been achieved yet. Although the available skin substitutes fulfil their very important goal of wound coverage, none is able to substitute all of the physiological functions of native skin on the functional and the aesthetic level.

Here, tissue engineered skin might be of a great benefit to these patients and many approaches are being followed to improve tissue engineered skin substitutes, *e.g.* by adding different cell types or growth factors. Nevertheless, following *in vitro* analysis, these skin constructs need to be tested *in vivo* before using them in humans. For this purpose the mouse displays one of the most ideal animal models due to several advantages, *i.e.* the easy and cheap breeding in large numbers,

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Abbreviations: SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; PBS, phosphate buffered saline; FBS, foetal bovine serum; DMEM, Dulbecco's modified Eagle's medium.

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the known genetic background and the availability of different knock-out strains. For testing of new skin substitutes, the constructs can be inserted into full thickness skin wounds, mostly on the back of mice. This animal model can be performed on a standardized manner and already showed to be valid in the analysis of cells cultured in a bioreactor in contrast to cells cultured in normal cell culture [4,5], to examine the vascularization of transplants [6,7], to test the effect of mesenchymal cells and growth factors in addition to the skin constructs [8], to analyse constructs enriched with Schwann cells to enhance nerve migration [9] or constructs containing sweat gland cells [10], to assess constructs containing bone marrow mesenchymal stem cells [11], or to establish a humanized mouse model for psoriasis [12].

A disadvantage of the above-mentioned method, however, is the contraction of the wound due to the healing mechanism of rodents [13]. A solution may be the use of the dorsal skin fold chamber. In this case, the skin is fixed in a titanium frame avoiding tissue contraction [14]. Also, in the skin fold chamber the skin constructs can be monitored continuously without potential disturbances by wound dressing changes.

Here, we adapted the dorsal skin fold chamber for analysis of a tissue engineered skin construct. Therefore, we chose to insert a construct comprising a collagen type I gel on top of a matriderm® layer into a full thickness skin wound inside the skin fold chamber and analysed it for 11 days. Macroscopic changes were easily be assessed throughout the duration of the experiments with the chambers. Additionally, the ingrowth of the constructs as well as the migration of cells and possible neovascularization are parameters which can simply be analysed after the completion of the experiments.

2. Material und methods

2.1. Production of the transplant

Small round pieces of matriderm® sheet (Dr. Suwelack Skin & Health Care AG, Billerbeck, Germany), a collagen elastin matrix, were generated by using a biopsy punch of 6 mm diameter. After disinfection with isopropanol and washing with PBS, collagen type I gel was added on top of the matriderm® which was isolated from the tails of Sprague-Dawley rats (source: local animal care facility) as follows: The tails were washed with soap, cleaned with ethanol and each time four tendon bundles were prepared. The tendons were washed with NaCl 1% (J.T. Baker, Deventer, The Netherlands) and distilled water and subsequently incubated with acetic acid 3% (v/v) (J.T. Baker) at 4 °C over night. The solution was filtered through sterile gauze and centrifuged at 10,000 × *g* at 4 °C for 2 h. The supernatant was mixed dropwise with NaCl 30% (w/v) and centrifuged at 3300 × *g* at 4 °C for 30 min. The supernatant was discarded and the obtained pellet was resuspended in 5% (w/v) NaCl plus 0.6% acetic acid and centrifuged at 3300 × *g* for 10 min. The supernatant was discarded and the pellet resuspended in NaCl 5% (w/v) plus 0.6% acetic acid (v/v). This step was repeated with a final volume of 250 ml. The solution was dialysed against 5 l hydrochloric acid (pH 3, Roth, Karlsruhe, Germany) at 4 °C for 8 h, repeating the dialysis step five times. The solution was sterilized by addition and evaporation of chloroform (Sigma–Aldrich, Steinheim, Germany). The concentration of the obtained collagen was determined with absorbance spectroscopy at 280 nm with the mass extinction coefficient $\varepsilon_m = 0.9\ \mathrm{ml\ mg^{-1}\ cm^{-1}}$. The purity of the collagen was determined by electrophoretic separation with SDS-PAGE. To produce gels for testing the skin fold chamber, a mixture consisting of the collagen (66.5% of the total volume), 10× DMEM/Ham's F12 (10% of the total volume), sodium-hydrogencarbonate ($NaHCO_3$ 7.5% for neutralization and gelification, 1% of the total volume) and 1× DMEM/Ham's F12 medium (22.5% of the total volume) was used and gelified at 37 °C for 30 min.

2.2. Skin fold chamber

The dorsal skin fold chamber was used as a model for wound healing, according to Sorg et al. [14,15]. During anaesthesia with isoflurane the dorsal skin chamber was implanted, resulting in an extended double layer of back skin between the two titanium frames. A full thickness wound was created after marking a circle of 6 mm diameter by removing the complete skin inside the circle, down to the panniculus carnosus. The opposite side remained intact, still comprising the epidermis, dermis and the panniculus carnosus (Fig. 1). The respective skin constructs of 6 mm diameter were transferred into the wound with forceps and the wound area was covered with a glass coverslip, integrated into one of the frames (Figs. 1 and 2). After 11 days the mice were sacrificed. The constructs surrounded by normal skin were removed and saved for histological analyses (Fig. 1).

2.3. Animals

All animal experiments were evaluated and approved by the standing local animal care committee and the Hannover Medical School. The animals (5 male BALB/c-Nude mice, 8 weeks) were purchased from Charles River and kept in the local animal care facility with a day-night cycle of 12 h each, according to the institution guidelines. They received standardized food and water *ad libitum*. Animals were used for the experiments when they were at least 12 weeks old and weighed at least 25 g.

2.4. Histology

After fixation of the samples in 4% paraformaldehyde and subsequent embedding in paraffin, sections of 5 μm thickness were cut and haematoxylin/eosin and Masson's trichrome stainings were carried out following standard procedures. In the Masson's trichrome staining muscles are stained in red, connective tissue (containing collagen) appears green and cell nuclei are marked in black whereas cell nuclei appear dark blue in the haematoxylin/eosin staining, accompanied by pink cytoplasm.

2.5. Immunofluorescence

In order to detect blood vessels, deparaffinised and rehydrated paraffin sections were stained with anti actin smooth muscle (1:100, Millipore, Schwalbach, Germany) as first antibody followed by Alexa Fluor 488 chicken anti mouse (1:1000, Invitrogen, Darmstadt, Germany) as secondary antibody following standard protocols. After removing the