ENHANCED CULTURE TECHNIQUES AND MODEL DEVELOPMENT FOR SKIN REGENERATION

Deep, (burn) wounds in humans generally heal with the formation of a hypertrophic scar. This severe scar affects appearance and causes serious restrictions on joint mobility. The standard treatment for full-thickness burn wounds is transplantation with a meshed split skin autograft. However, this therapy has several disadvantages. Healing still results in scar formation and morbidity at the donor site may occur. In addition, insufficient donor sites may be present in patients with extensive burns, due to limited availability of healthy skin.

Transplantation with autologous keratinocytes is a promising alternative for the treatment of deep (burn) wounds. However, keratinocytes are conventionally cultured in the presence of fetal calf serum (FCS) and mouse feeder layer cells. These xenobiotic materials can be a potential risk for the patient (e.g., transfer of prions). **Chapter 2** showed that we could successfully culture keratinocytes without FCS and mouse feeder layers cells. If the keratinocytes were grown on collagen type IV, these cells were able to form a fully differentiated epidermis at least up to passage 4. This indicates that the cultured keratinocytes are still suitable for clinical application. Using our culture technique, sufficient numbers of keratinocytes can be obtained from 1 cm^2 skin to potentially cover 400 cm^2 of wound surface in 2 weeks.

For the development of new (tissue-engineered) anti-scarring therapies, it is essential to have detailed knowledge about scar formation and tissue regeneration. Many studies show that fetal skin has the ability to heal without scar formation. The composition of fetal skin itself probably plays an important role in this process. Thus, knowledge about fetal and adult skin constitution may contribute to the understanding of wound healing. In **Chapter 3**, we studied the differences between human fetal and adult skin architecture. We found that most differences between fetal and adult skin were present at the level of dermal extracellular matrix (ECM) molecular expression. The expression of fibronectin (FN) and chondroitin sulfate (CS) was higher in fetal skin than in adult skin, whereas elastin was not present in fetal skin at least up to 22 weeks of gestation. In contrast, the fetal epidermis was only slightly different from the adult epidermis in skin. These findings suggest that the presence or absence of certain ECM molecules might be beneficial in promoting adult wound healing. Therefore, a possible therapeutic intervention for improving adult healing is the use of a dermal substitute that contains certain ECM molecules (e.g., FN and CS).

To investigate the mechanisms of wound healing, wound model systems are indispensable. No ideal wound model is available at this moment. Animal models

are advantageous, because they contain all local and systemic factors involved in wound healing. However, their use is limited due to ethical considerations and human-animal differences. In vitro models involve minimal ethical considerations, but these models are only a partial representation of the in vivo situation. In **Chapter 4 and 5**, we aimed to develop fetal and adult in vitro skin models, which resemble in vivo wound healing better than the current in vitro models. The developed models consisted of ex vivo fetal, adult, and scar tissue samples that were burned before air-exposed culture. We showed that these wound models remained vital for long period of time and showed many similarities to the in vivo situation. In both the fetal and animal wound models, reepithelialization took place from the surrounding tissue and fibroblasts migrated into the wound area. As these models are based on the in vitro culture of human skin, all the matrix and cellular elements of normal skin are present. In addition, we demonstrated that the wound model can be extended with LPS. However, more contributors of wound healing (e.g., mechanical tension, growth factors, and inflammatory cells) should be added to further optimize the in vitro wound models.

These in vitro skin models can be used to investigate the process of fetal scar-free healing. In **Chapter 5**, we compared the developed fetal and adult wound models, in order to differentiate important factors involved in fetal regeneration. This may lead to the identification of potential clues for improving adult healing. We found that reepithelialization was faster in the fetal skin model than in the adult skin models. Remarkably, the fetal neo-epidermis grew over the old burned epidermis, whereas the adult neo-epidermis grew underneath it. In addition, we found that new fibroblasts were present earlier in the wound area of the fetal skin model than in the wound area of the adult skin models. These findings suggest that fast healing might play an important role in scarless healing. Possibly, adult healing can be improved by stimulating fast reepithelialization and fibroblasts migration.

In this thesis, we contributed to the further development of tissue-engineered skin by culturing keratinocytes without use of FCS and mouse fibroblasts. In addition, we developed enhanced in vitro wound models which allow detailed studies on the sequential events in wound healing. This may lead to safer transplantation of keratinocytes and to more insights into the mechanisms of scarring and regeneration. Consequently, this thesis may contribute to the development of reliable treatments preventing (hypertrophic) scar formation.