Abstract

The skin is the largest organ of a human body with a crucial role in the maintenance of homeostasis; therefore any extensive skin injury leads to severe complications. Since the application of auto-, allo- and xeno-grafts is accompanied by severe problems like the source limitation and the graft rejection, a bioengineered skin substitute seems to be one of the promising healing approach. This work is focused mainly on the construction of a pre-vascularized skin substitute consisting of a collagen hydrogel reinforced by a biodegradable nanofibrous membrane. Another strategy described in this work is the development of temporary cellulose-based wound dressings. For both research strategies, various cell types were utilized, i.e. normal human dermal fibroblasts (NHDFs), human keratinocytes (hKs), adipose tissue-derived stem cells (ADSCs) and human umbilical vein endothelial cells (HUVECs).

In order to enhance the cell adhesion and growth, the synthetic nanofibrous membranes were improved by protein nanocoatings. It was found out that NHDFs and ADSCs preferred fibrin nanocoatings, mainly thin fibrin homogeneous mesh on the surface of the membrane. Keratinocytes rather adhered and stratified on collagen substrates. These observations further motivated the construction of the bi-layered construct, where the NHDFs migrated from the fibrin-coated nanofibrous membrane into the collagen hydrogel, and the epidermal hKs were cultivated on the surface of collagen. Unlike to the direct cell embedding into the gel, the gradual degradation and synthesis of the collagen by the migrating cells allowed them to colonize the entire volume of the collagen hydrogel without any considerable shrinkage. This novel approach was also utilized for pre-vascularization of the bi-layered construct in which the migrating ADSCs supported the formation of a tubular structures from the HUVECs embedded in the collagen.

Another research area of this work is focused on a cellulose-based material as a promising nature-derived wound dressing. The cellulose meshes were coated with negatively and positively charged cellulose nanofibrils (CNFs) or with two types of fibrin nanocoatings to increase the cell attachment and proliferation. The results showed that the negatively charged CNFs, in contrast to the positively charged CNFs, enhanced the growth of NHDFs and ADSCs. However, it depended on a particular cell type and on the composition of the cell adhesion-mediating proteins. It has been also detected that the fibrin mesh on the surface of the cellulose mesh supported the adhesion and growth of NHDFs better than just the fibrin coating.