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Fibroblast differentiation: Relevance of the myofibroblast for tissue regeneration

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Introduction:

Fibroblasts represent a cell source having the potential of differentiating into other cell types apart from the fibroblast line. Fibroblasts are among those adult cells, which are easily to acquire and a variety of cell culture and tissue engineering methods are already available. Our research activities focus on a distinct differentiation step - from the fibroblast to the myofibroblast (MF) - due to the high clinical relevance. Because the MF is a key player during the development of tissue fibrosis (i.e. liver fibrosis, arteriosclerosis, excessive scarring after wounding) on the one hand, and is believed to support tissue regeneration (organ- and wound healing) on the other hand, it is a promising approach to develop molecular and biochemical methods, enabling the characterization and handling of MF. The detailed knowledge of the process of MF differentiation (MFD) is a prerequisite for subsequent strategies aiming at the development of tissue engineered soft tissue substitutes.

Materials and Methods:

Differentiation of fibroblasts to MFs can easily be assessed after cell culture by the detection of α-smooth-muscle-actin among fibroblasts either by FACS or fluorescence immunocytochemistry. Human wound explants were cultured and the presence of MF among other cell types was characterized and correlated to the healing status (n=10). In addition the effect of human dermal microvascular endothelial cells (HDMEC) on MFD was investigated in 2D- (n=10) and 3D-(matrigel; n=3) co-cultures. Our experiments aiming to actively control MF differentiation included the application of transforming growth factor β1 (TGFβ1) in a soluble (n=8) as well as in a covalently immobilized form. Cell culture substrates were two kinds of aldehyde- (n=5, n=6) and epoxy-functionalized (n=6) culture slides.

Results:

Enhanced MFD was observed in cultures from human wound explants compared to normal human dermal fibroblasts.

Areas, where fibroblasts were in contact with HDMEC in co-culture showed an enhanced differentiation potential, which was likely influenced by the detected secretion of endothelin-1 by HDMEC. The 3D-growth of HDMEC in matrigel - manifesting in elongated bundles- seemed to be supported by accompanying MF.
Soluble TGFβ1 was most effective in triggering MFD at a concentration of 1 ng/ml. Covalently immobilized TGFβ1 also remained biologically active leading to enhanced MFD.

**Discussion and Conclusions:**

The data show that the characterization of the MFD rate of human wounds is a promising diagnostic tool in the future. Furthermore it is possible to control MFD actively by soluble and covalently immobilized TGFβ1. Investigation of the MFD process also reveals basic differentiation phenomena and strategies transferable to differentiation strategies of stem cells from other origin.