ABSTRACT

Dermal fibroblasts participate actively in the regulation of wound healing process. They migrate and proliferate during the proliferative phase into the wound site and respond to signals from damaged tissue by secretion of a various pro-inflammatory molecules (e.g. interleukins IL6 and IL8) and extracellular matrix components (e.g. collagen type I, hyaluronan, fibronectin). The disruption of the balance and timing of synthesis and degradation of these molecules can lead to the transition of normal to abnormal non-healing process. Several stress factors act simultaneously in this type of wounds – impaired nutrition supply, inflammation, bacterial contamination, oxidative stress etc. However, the latest *in vitro* research of dermal fibroblasts in wound healing apply most often only one stress factor. This thesis deals with the response of dermal fibroblasts to the wound conditions. For the first time, a comprehensive study of the functional response of the cells to the two key wound stress factors is described in 2D culture and 3D self-made collagen hydrogel culture.

In the first part of the thesis response of dermal fibroblasts to the wound conditions applying two stress factors - low nutrition (2% FBS) and inflammation simulated by bacterial lipopolysaccharide (LPS) - is characterized. The functional response of the cells includes metabolic activity, proliferation, changes in morphology, migration, production of IL6 and IL8, synthesis of collagen type I and production of matrix metalloproteinases (MMPs) 2 and 9. It was shown that the low nutrition and the LPS promote metabolic activity of the cells; however, this promotion was not followed by increased proliferation. Further, the LPS potentiated the migration of the cells. The pro-inflammatory phenotype of the cells evidenced by increased production of IL6 and IL8 was potentiated by LPS. Moreover, the change of fibroblasts to myofibroblasts decreased by the low nutrition and even more by the LPS. The remodeling capability of the cells was influenced only by low nutrition when documented by increased MMP2 and unchanged MMP9 activities. Finally, the production of collagen type I was not affected by neither factors.

In the second part the model of wound contamination is improved by soluble factors secreted by bacterial species present in wounds. The responses of dermal fibroblasts to soluble factors from *Staphylococcus aureus* and *Pseudomonas aeruginosa* are characterized. Further, the effects of the three types of conditioned media originated in different steps of *S. aureus* and *P. aeruginosa* media preparation (polybacterial conditions) on dermal fibroblasts are characterized. The functional response of the cells includes proliferation, changes in morphology, migration, production of IL6 and IL8 and synthesis of collagen type I.

Proliferation of the cells was suppressed by conditioned medium from *P. aeruginosa* and polybacterial mixture of the both bacterial species. The cell morphology was affected by together cultivated polybacterial conditions. Further, only the polybacterial mixture of the both bacterial species suppressed the migration of the cells. The pro-inflammatory phenotype of the cells was potentiated only by *P. aeruginosa* conditioned medium. The production of collagen type I was not affected by neither factors. Finally, soluble factors from *S. aureus* did not affect the functions of dermal fibroblasts at all.

In the third part of the thesis the responses of dermal fibroblasts cultivated in 3D culture are characterized and compared with their responses in 2D culture. Moreover, the functional response of the cells in wound conditions applying the two stress factors in 3D culture was examined. The functional responses of the cells examined in these parts include contraction ability, metabolic activity, proliferation and changes in morphology. The metabolic activity was independent on the type of culture and was increasing in time. The proliferation of the cells was increasing in time in 2D culture and constant in 3D culture. The wound conditions did not affect the metabolic activity and the cell proliferation in both types of culture. The contraction ability of the cells was disrupted by both low nutrition alone and the low nutrition in combination with LPS. The spindle-shaped morphology of the cells was flattened in 2D culture, while in 3D culture the shape was more elongated and stellate.

In the last part the methods for the absolute cell number determination in both 2D and 3D cultures as the quantitative approach how to analyze cell proliferation is developed since suitable method for absolute cell number determination in 2D and 3D cultures has not been optimized. In this thesis, the method for absolute cell number determination in 2D was used for monitoring of cell proliferation in time and for IL6, IL8, MMPs and collagen type I relation to cell number at each time point.

Taken together, results of this thesis helped to describe behavior of dermal fibroblasts in 2D and 3D *in vitro* model under wound conditions. Our results can serve as a valuable tool in the early stages of therapeutics development.