Doctoral dissertation
"The fibroblast response to the presence of biofilms formed by pathogens isolated from the chronic venous ulcers"

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Abstract

The last few decades have been a period of very intense civilization transformation leading, among others, to numerous social and lifestyle changes. The aging of society and increasing number of people suffering from such diseases as diabetes and cardiovascular diseases correlate with increasing number of chronic wound cases, such as diabetic foot syndrome, venous and venous ulcers. Therefore, chronic wounds are a distinctively arising problem for global healthcare systems. A chronic wound is defined as a wound that is inflamed and that fails to proceed through the normal phases of wound healing in an orderly and timely manner. A key reason for the transition of wounds into a chronic state is their contamination, followed by colonization by microorganisms living in a biofilm form. Biofilms due to a number of its unique features, is highly resistant to both the immune system and the use of antimicrobial agents and difficult to diagnose. Moreover, the diagnosis of bacterial infections, especially those caused by biofilm, is difficult. It is due in part by the limitations of currently available diagnostic tools. It has given rise to research that may provide a starting point for the development and implementation of an effective, non-invasive method of diagnosing chronic wound infection.

The main aim of the doctoral dissertation was metabolomic and genetic analysis of the infection caused by microbial biofilms, isolated from chronic venous leg ulcers and search for infection biomarkers based on an *in vitro* model. The research was carried out on three reference strains: *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*. The L929 normal connective tissue cell line was used as a model of chronic wound infection. In the first stage of the study, the process of biofilm formation *in vitro* by the above-mentioned strains was analyzed using the methods of classical microbiology, confocal microscopy, and scanning electron microscopy. A original method of co-culturing normal cells with microbial biofilms has been developed. The influence of microorganisms on the viability of fibroblasts

and the critical points of infection - the so-called critical colonization points were determined. Based on the obtained results, the point at which the analysis of metabolic changes induced by the host-pathogen interaction should be performed was indicated. The analysis of the biofilm formation process under conditions reflecting some of the parameters present in the chronic wound showed its high metabolic variability. The research carried out by nuclear magnetic resonance (NMR) spectroscopy allowed us to distinguish the metabolites present in increased concentration during the critical colonization of the fibroblast monolayer by the studied pathogens. The analysis of genes related to biofilm formation showed that the interaction of microbial cells with fibroblasts leads to the activation of signaling pathways responsible for the development of biofilm in wounds. The proposed, simplified wound model led to distinguishing several intracellular and extracellular metabolites characteristic of fibroblast infection with a given type of pathogen. The obtained research results contribute not only to the knowledge of the pathomechanism of biofilm formation in chronic wounds but may contribute to the implementation of an innovative method of prognosis of chronic venous leg ulcers.

Keywords: chronic wounds, bacterial biofilms, fibroblasts, metabolomics

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