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ROZPRAWA DOKTORSKA

Wpływ ramnogalakturonanu-I na proces osteointegracji. Badania *in vitro*.

The effect of rhamnogalacturonan-I on osseointegration. *In vitro* study.

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Gliwice 2018

SUMMARY

The challenge for tissue engineering is to receive highly biocompatible dental implants providing connection with bone during osseointegration. Significant influence on this process has the implant surface and its chemical, physical, mechanical and topographic properties. To achieve optimal mechanical and biological properties of implants wide variety of surface modifications, such as application of organic coatings is required. *In vitro* studies demonstrated that pectins, and especially ramnogalacturonan-I (RG-I) are suitable materials for surface nanocoating of dental titanium implants due to their role in the improvement of osseointegration and bone healing.

The aim of this work was to evaluate the effect of RG-I, potential candidate material for surface nanocoating of dental titanium implants, on the cells responsible for osseointegration under *in vitro* conditions.

In this study, unmodified RG-I (PU) and enzymatically modified RG-I with shorter arabinose side chains (PA) were examined. All *in vitro* studies with cell cultures were performed on tissue culture polystyrene plates (TCPS) or titanium (Ti) discs surfaces coated with unmodified and modified RG-I. The control was uncoated TCPS/ Ti discs. The effect of surface nanocoatings with unmodified and enzymatically modified RG-Is on different cells type, that play important role in tissue remodeling, especially bone tissue is described in Papers I-V.

Paper I describes results obtained for primary murine osteoblasts and mice osteoblast-like cells MC3T3-E1. The effect of RG-I on osteoblasts with regard to their proliferation, cell cycle and osteogenic response in terms of mineralization was analyzed. Furthermore, the level of expression of genes involved in osteoblast differentiation and maturation were investigated. The results showed an increase in proliferation, mineralization, and gene expression in osteoblasts cultures on surfaces coated with RG-Is. Moreover, it was found that modified RG-I with lower amount of arabinose (PA) stronger stimulates the cellular response than PU.

Paper II demonstrates impact of nanocoating with unmodified and enzymatically modified RG-Is on the cellular response of primary human gingival fibroblasts. Cells proliferation, metabolic activity and expression of genes responsible for extracellular matrix (ECM) turnover were investigated. The results showed, similarly to these obtained in Paper I, that PU and PA increased proliferation, cell metabolic activity and expression of genes reflecting the turnover of extracellular matrix in comparison to the control culture. Again, observed effects were higher for fibroblasts cultured on PA than on PU.

Moreover, in Paper II characterization of uncoated and unmodified or enzymatically modified RG-I coated polystyrene surfaces was presented. The surface properties were examined using X-ray photoelectron spectroscopy (XPS), contact angle measurements, scanning electron microscopy (SEM) and atomic force microscope (AFM). Chemical compositions, wettability and surface topography were analyzed. The results showed that nanocoating with PU and PA changed surface properties, such as chemical composition, wettability and roughness. This phenomenon influenced the cell response on tested surfaces.

In vitro study presented in Paper III evaluates the effect of unmodified and enzymatically modified RG-I on human neutrophils. The inflammatory response of neutrophils after *E. coli* LPS/*P. gingivalis* bacteria stimulation was examined on PU or PA-coated and uncoated polystyrene and titanium surfaces. The results showed that both PU and PA, with stronger effect for PA, decreased the level of proinflammatory and anti-inflammatory genes expression of human neutrophils as compared with the control.

In addition, in Paper IV and V, results of proinflammatory gene expression in mice osteoblast and human fibroblast are demonstrated. The presence of PU and PA on surface decreased the level of expression of mentioned genes.

In summary, results presented in Papers I-V indicate the influence of RG-I on the biological response of all tested cells. *In vitro* studies suggest that RG-I possess biocompatibility features. Moreover, RG-Is nanocoating might be potential for further improvement of osseointegration, increasing its stability and functionality.