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Ocena *in vitro* właściwości przeciwzapalnych ramnogalakturonanu-I do zastosowania w modyfikacji powierzchni tytanowych implantów stomatologicznych

The *in vitro* evaluation of rhamnogalacturonan-I anti-inflammatory properties for application in surface modification of titanium dental implants

Rozprawa doktorska
Doctoral thesis

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Summary

The success of dental implant treatment depends on osseointegration, which is defined as direct structural and functional connection between living bone and the implant surface. One of the major factors disrupting osseointegration is peri-implant inflammation caused by local bacterial infection. It has been shown that patients with poor general health are more susceptible to peri-implantitis. Therefore, recent directions in the field of biomaterial engineering focus on the proper modification of implant surface in order to reduce the risk of peri-implant inflammation. Surface coatings of dental implant with anti-inflammatory organic molecules is one of the strategy to improve implant treatment success even among high-risk patients. Plant-derived polysaccharides, pectins, mainly represented by rhamnogalacturonan-I (RG-I) seem to be promising candidates for surface coatings due to their anti-inflammatory properties.

The general aim of this thesis, which is based on three original research articles, was to examine *in vitro* the impact of potato-derived RG-I on cells participating in initiation and regulation of inflammation as well as involved in bone rebuild. In the scope of the thesis, the response of cells stimulated with infectious agent was evaluated on polystyrene and titanium surfaces coated with unmodified and enzymatically modified RG-I (with shortened arabinian side chains).

The aim of *in vitro* study presented in the first article was to evaluate the effect of polystyrene surface coating with unmodified and enzymatically modified RG-I on cellular response of bone-forming cells (osteoblasts) infected with *Porphyromonas gingivalis* bacteria. Murine primary osteoblasts and osteoblast-like MC3T3-E1 cells were used in the experiments. The results indicate, that RG-I inhibits proinflammatory response through decreased expression level of genes encoding proinflammatory cytokines. Higher rate of proliferation, metabolic activity and mineralization as well as increased expression level of genes related to osteogenesis was detected in cells cultured on RG-I-coated surfaces. Furthermore, it has been shown, that examined pectins decrease the ability of infected osteoblasts to express gene encoding RANKL protein, which is responsible for activation of bone-resorbing cells (osteoclasts).

The ability of unmodified and enzymatically modified RG-I to inhibit the proinflammatory cellular response has been confirmed in the *in vitro* study presented in article II. Anti-inflammatory properties of examined pectins were determined in relation to human primary fibroblasts infected with *P. gingivalis* bacteria. The obtained results indicate that polystyrene surface modification with RG-I molecules regulate the activity of infected fibroblasts. In the presence of examined pectins, the expression level of genes responsible for

proinflammatory response was downregulated while the proliferation, metabolic activity and expression of genes required for extracellular matrix formation was up-regulated in infected cells. Furthermore, the surface coating with RG-I molecules inhibited the expression of gene encoding matrix metalloprotease – proteolytic enzyme responsible for bone destruction.

The *in vitro* study presented in article III evaluates the effect of unmodified and enzymatically modified RG-I coating of polystyrene and titanium surfaces with regards to human macrophages activated by infectious agent. The human macrophages were activated in two different ways: by *P. gingivalis* infection and stimulation with *Escherichia coli* lipopolysaccharide. The results obtained from *in vitro* study indicate, that polystyrene and titanium surface coating reduce macrophage activation irrespective of stimulus type. The decrease expression level of genes encoding proinflammatory cytokines was observed in cells cultured on RG-I-coated surfaces. Reduced ability of macrophages to express genes encoding inflammatory mediators could prevent excessive bone resorption following inflammation. However, surface coating with RG-I did not significantly influence anti-inflammatory interleukin-10 gene expression in activated macrophages

In summary, the results of presented *in vitro* studies indicate the ability of RG-I to inhibit proinflammatory response induced by bacterial infection agent and stimulate bone rebuild. Moreover, surface coating with enzymatically modified RG-I with shorted arabinian side chains results in higher reduction of proinflammatory cellular response when compared to unmodified RG-I coating. Thus examined pectins, in particular RG-I with lower arabinian content are potential candidate for application in modification of titanium implant surface. However, further *in vitro* study investigating the mechanism of anti-inflammatory RG-I action as well as *in vivo* studies need to be performed to verify the possibility of using RG-I in dental implantology.