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SUMMARY OF DOCTORAL DISSERTATION

Immunofunctionalization of cardiovascular stents' surfaces by the introduction of anti-inflammatory interleukins

Atherosclerosis is defined as a state of chronically active inflammation within the vessel walls, leading to their thickening and loss of elasticity. This condition is especially dangerous when it involves the coronary arteries, leading to the development of coronary artery disease. The insufficiency of coronary blood circulation, that developed based on disease progression, may result in myocardial ischemia, which may cause myocardial infarction and death.

Among the many therapeutic methods and techniques, which include intensive pharmacotherapy and coronary artery bypass grafting, percutaneous coronary intervention is the gold standard in revascularization of a narrowed coronary artery. It is estimated that approximately 80% of all performed interventions end with insertion of a cardiovascular stent into the coronary artery. Approximately 4.0 million such procedures are performed annually in the world. The quoted numbers underline the seriousness of the problem.

Percutaneous coronary intervention with simultaneous stent implantation undoubtedly leads to an improvement the quality of life and survival of millions of patients annually. However, it may be associated with a possible failure, limiting the long-term effectiveness of the procedure. Among the most common, in-stent restenosis should be indicated, defined as a stenosis recurrence of a previously revascularized vessel.

The mechanisms underlying in-stent restenosis are not fully understood. A special role in this process seems to be played by the denudated and dysfunctional endothelium, the procedure-associated development of acute inflammation, which overlaps the progressive-atherosclerosis-associated chronic inflammation, subendothelial accumulation of macrophages modifying the local vascular environment and phenotypic transformations of vascular smooth muscle cells. The indicated phenomena contribute to the hypertrophy of the neointima, which is a postinterventional, pathological process during which the remodeling of the intimal wall of the coronary vessel takes place. This gradually leads to in-stent restenosis and recurrence of clinical signs of coronary insufficiency. This process remains a serious problem in interventional cardiology. It is also one of the most important causes of death among patients with coronary artery disease.

Currently, a lot of research is being conducted on the development of newer and newer stents concepts. These strategies include the search for a new material from which the stent will be made or the design of completely new implant structures.

As part of this doctoral dissertation, research was undertaken in the field of surface modification of commonly used stents, which will show biological activity against vascular cells, thus reducing the probability of in-stent restenosis to a minimum. The research focused on molecules naturally synthesized by the body and characterized by a pleiotropic effect – interleukins. These — serving as a means of communication for immune system cells as well as cells not belonging to this system — are able to induce beneficial reactions of vascular cells, limiting the progression of atherosclerotic lesions and reducing the likelihood of in-stent restenosis developing.

At the beginning, the step-by-step procedure of polydopamine and chitosan-enriched polydopamine based coating synthesis have been reported. These biomaterials are characterized by strong adhesive properties and the presence of numerous functional groups. Therefore, it is possible to cover the stent structure with a coating and to bind selected biological molecules on the surface. Immobilization of selected interleukins within the applied base materials have been performed by direct reaction or via cross-linking chemistry.

The physico-chemical properties of the obtained coatings have been characterized by means of attenuated total reflectance Fourier transform infrared spectroscopy, atomic force microscopy and enzyme-linked immunosorbent assays. By means of application these techniques, it has been possible to confirm the effectiveness of surface functionalization and the presence of interleukins in the structure of the proposed coating. Not only the coating has exhibited interleukin-carrier property, but at the same time it has allowed controlled release of biomolecules.

Defining the biological properties of the proposed coatings has been possible because of in vitro studies, where model endothelial cells and model monocytes/macrophages have been engaged. The viability and proliferation of endothelial cells have been examined, their morphology has been characterized, and their pro-inflammatory response has been assessed. The synthesized coatings have supported the growth of endothelial cells, and over time, they have contributed to the inhibition of inflammatory processes in these cells. It has also been proven that the presence of a coating on the surface of the material has significantly inhibited the adhesion of monocytes/macrophages to the material as well as to endothelial cells cultured on such modified surface. The ability of the coatings to induce phenotypic changes of macrophages accumulated in the arterial wall has confirmed that the proposed procedure of interleukin immobilization has not impaired the biological activity of the cytokines, that have been used. Ultimately, the coatings have exhibited ability to promote tissue healing and regeneration, reduce the production of pro-inflammatory cytokines and deliver anti-inflammatory cytokines, what could improve the prognosis of patients with vascular disease.

The results of the conducted experiments have confirmed that the overriding goal of the doctoral dissertation has been achieved. The immunofunctionalizated coatings have been obtained, comprehensively characterized in terms of physico-chemical properties and their biological activities. Moreover, the presented results shed new light on the pathomechanism of vascular disease, proposing further directions for the development of this field of science.

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