THE SILESIAN UNIVERSITY OF TECHNOLOGY

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DOCTORAL DISSERTATION

The collection of published and thematically related articles

Trehalose releasing nanogels for autophagy stimulation

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Abstract

Autophagy is a lysosomal-dependent, cellular recycling process responsible for degradation of unnecessary and damaged intracellular components. Many human disorders are strongly correlated with the malfunctioning of autophagy. Trehalose is a naturally occurring disaccharide that has gained considerable attention, thanks to numerous studies, which demonstrated its ability to induce autophagy. Unfortunately, trehalose has low bioavailability due to its high hydrophilicity and susceptibility to enzymatic hydrolysis. To enhance the efficacy of trehalose, trehalose-bearing nanocarriers, in which trehalose is incorporated either by chemical conjugation or physical entrapment, have emerged as an alternative option to free trehalose.

This doctoral dissertation is a collection of published articles focused on the synthesis, characterization, and the application of trehalose-releasing nanogels as potential trehalose carriers for autophagy stimulation. First, the synthesis of nanogel *via* photoinitiated free radical polymerization in inverse miniemulsion was optimized. Trehalose was covalently conjugated to the polymer network *via* an ester bond, of which the specific location enabled its cleavage under physiological conditions resulting in trehalose release. Trehalose release appeared to be highly influenced by the composition of nanogels, the ester part through which trehalose is incorporated, network charge, and pH of environment, but it was not concentration-dependent. Trehalose-releasing nanogels were characterized by spherical shape with hydrodynamic diameter ranging from 57 to 266 nm and positive or negative zeta potential depending on the charge of the incorporated ionic moieties. The best of the developed nanogels had high content of conjugated trehalose (~50% w/w), were colloidally stable in serum-enriched media, non-cytotoxic to human umbilical vein endothelial cells, well uptaken by cells, and non-hemolytic to human red blood cells.

Two independent biological studies confirmed the autophagy stimulation effects of trehalose-containing nanogels. First, nanogels were capable to induce autophagy in transgenic zebrafish and *Drosophila* larvae. Second, they demonstrated the therapeutic effects of autophagy stimulation in promoting lipid efflux and plaque reduction in a mouse model of atherosclerosis. The developed nanogels represent a novel type of trehalose-bearing nanocarriers, and can be considered as a significant achievement in this field, because nanocarriers characterized by covalent conjugation of trehalose, which can be sustainably released at pH 7.4 have not been developed so far. Taking into account the promising results from autophagy stimulation studies, the developed nanogels may contribute to the further development of an effective trehalose delivery strategy to overcome impaired autophagy-related disorders.