

Plastic is an extremely versatile and affordable material serving people in many areas of life. The biggest demand for plastic comes from packaging production, but plastic is also an integral element of the construction, automotive and agricultural industries, as well as a material for sports goods and other everyday items. Unfortunately, global production of most types of plastic rely on non-renewable sources, with the production rising to more than 390 million tons in 2021. Poor global waste management has caused plastic to enter both terrestrial and marine environments. It is estimated that by 2050 the environment will be polluted by 12 billion tons of plastic waste of all sizes.

Plastic fragments may undergo degradation processes which lead to the formation of micro- and nanoplastics (particles with diameter of less than 5 mm). Recent studies show that micro- and nanoplastics are present in various environments: air, water, and soil affecting the lives of all organisms living on the planet. Humans are exposed to nanoplastic through direct ingestion, inhalation and skin contact. Once digested, plastic particles interact with the gastrointestinal tract. Epithelial tissue forms a barrier between the organism and external environment.

The intestinal epithelia and mucus produced by epithelial cells play a key role in preventing pathogens and pollutants from entering the internal environment of the human body. Unfortunately, our understanding of how micro- and nanoplastic affects human epithelial intestinal tissue is very limited. The toxicity of micro- and nanoplastic is still a subject of debate and the available information on their effects on living organisms at cellular and tissue level remain unclear.

The proposed study presents a novel approach to investigating the problem of plastic consumption, in particular the effect of nanoplastics on intestinal epithelium physiology. In order to achieve the goal, we plan to utilize Caco-2 cell line, Caco-2/HT29-MTX co-culture and porcine intestinal tissue. The planned experiments include the measurements of ionic currents passing through the epithelium (short-circuit current measurements in Ussing Chamber). By using different, specific ion transporting proteins modulators, we will verify their role in the response to two popular types of nanoplastic: polystyrene and polyethylene. Our preliminary studies showed that nanoplastic decreased CFTR channel activity, but increased chloride transport via other transporting protein. We also observed increased mucus secretion by the cells exposed to nanoplastic. We hypothesize that our observations of current changes and increased mucus secretion are related to TMEM16a (ANO1) channel activity. To verify our hypothesis, we are planning to perform the experiments on WT cell line, but also to knockdown the *Ano1* gene and compare the results. Additionally, we will also assess the transcript levels of different ion transporting proteins before and after exposure to nanoplastics. Since the literature data is inconsistent, we also plan to evaluate the cytotoxic properties of nanoplastic using two different approaches: LDH release assay and Trypan blue staining for increased explicitly of our findings. The proposed experiments also include the quantification of mucus produced by Caco-2 and measurements of intracellular calcium level (which activates TMEM16a ion channel) after exposure to nanoplastic.

Until today the adverse health effects on nanoplastic on different organ systems are not yet fully understood. The results of the project may contribute to better understanding of the mechanisms behind interaction of plastic with human organism. We aim to characterize the role of TMEM16a in the defense mechanism of intestinal epithelium against nanoplastic as well as asses its cytotoxicity. This study will help to evaluate the level of risks associated with nanoplastic and its impact on the intestinal epithelial tissue. Hopefully, the results will allow us to determine a promising cytoprotective strategy against homeostasis imbalances or even cell and tissue injuries induced by nanoplastic.