ABSTRACT

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Title of the diploma thesis: Automated monitoring of *Metridia* luciferase released from genetically modified cell line.

The diploma thesis reports automatic real-time monitoring of the release of *Metridia* luciferase from genetically modified cells. A previously optimized method for monitoring the natural release of luciferase from cells was used during the experiment. At the same time, the toxic effect of bile acids (chenodeoxycholic and deoxycholic acid) on changes in cell membrane permeability was tested. Liver epithelial cell line modified with the *Metridia* luciferase plasmid were used during the experiment. After this genetic modification, the cells were able to synthesize the active *Metridia* luciferase enzyme. This enzyme generates luminescence upon reaction with substrate coelenterazine. The emitted light was detected in the flow cell of a spectrofluorometer at a wavelength of 485 nm. The measurement was taken in a sequential injection system connected to a 3D printed module containing an insert with the tested cell monolayer. This automated system allowed real-time monitoring of the luciferase release on both sides of the cell monolayer. Samples were taken alternately from the apical and basal compartments every 30 minutes for 3 to 10 hours. The influence of the permeability of the cell membrane was manifested by an increase in the chemiluminescence signal, which was caused by the increased release of luciferase from the cells. The effect of chenodeoxycholic and deoxycholic acids with a concentration of 0.01 mg/ml was noticeable on average after 1.5 hours to 5 hours.