ABSTRACT

Charles University

Faculty of Pharmacy in Hradec Králové

Department of Biochemical Sciences

Candidate: Bc. Lenka Brieslingerová

Supervisor: RNDr. Miloslav Macháček, Ph.D.

Title od diploma thesis: Development of 3D spheroid cell culture derived from tumor cell lines suitable for photodynamic therapy research

Growing number of tumorous diseases worldwide is urging great effort to make anticancer treatment more effective. Conventional testing of newly developed drugs is performed on tumor cell cultures growing in a monolayer. However, the use of 3D spheroid models provides a more appropriate method of testing, as these models are able to better mimic the in vivo properties of tumors and thus provide a more realistic response to administered drugs. In addition to testing new cytostatics, 3D spheroid models can also be used as tumor models to study the effect of photodynamic therapy (PDT), although this model is still not widely utilized in the field of PDT research. PDT is an alternative and minimally invasive treatment method used not only to treat tumors. It utilises a photosensitizer (PS), molecular oxygen (3O2) and light to form reactive oxygen species (ROS), consequently damaging tumor tissue.

This diploma thesis deals with the production of 3D spheroid cell cultures from the HeLa cell line, which were consequently used for further experiments, including PDT. Spheroids were formed by the hanging drop method and ultra-low adhesion (ULA) enviroment method. For the formation of optimal and uniform spheroids, the method using ULA plates was evaluated as more suitable ULA plates has also enabled long-term cultivation and easier preparation of more uniform spheroids. To obtain more compact spheroids formed by a hanging drop method, the utilization of extracellular matrix proteins in the form of collagen was examined, but its positive effect was not demonstrated in our experimental setup with HeLa cells.

A biochemical viability assay using resazurin was used to evaluate the efficacy of PDT. To utilise this assay, it was necessary to optimize the incubation time with resazurin. 4 hour incubation time was chosen as the ideal period. Newly synthesized amphiphilic compounds from the group of (aza)phthalocyanines were used as PSs to study the efficacy of PDT on spheroids. The positively charged compond P40 showed a higher photodynamic activity than the negatively charged compound P44. The work also dealt with the optimalization of the process of staining spheroids for their imaging by the laser scanning confocal microscopy. 3D spheroid invasion assay (spheroid sprouting assay) and migration assay (spreading assay) were also introduced.