

Method of Visualization and Evaluation of Proliferative Activity of K562 Cell Line with Fluorescent Carbon Nanodots

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Hematopoietic system is a complex example of the stem cell concept that provides good opportunities for proliferation and differentiation studies. True hematopoietic stem cell that is characterized by the blast-like cell morphology can only be truly identified with functional assays such as CFU. Dormant stem cells as well as dormant cancer stem cells are hard to identify due to lack of their functional activity in vitro. We hypothesized that unspecific labeling of the stem cells with non-toxic persisting fluorescent carbon nanodots can reveal proliferating and non-proliferating cells based on the decrease of the fluorescent signal with every cell division. The nature of implemented BCD and V1CD carbon nanodots was confirmed with IR and Raman spectroscopy, while their toxicity was evaluated with MTT assay. According to our hypothesis, fluorescent nanodots labeled cells will lose one half of their fluorescent signal with every division, while cells that remain outside the mitotic cycle, would retain high fluorescent signal therefore they could be easily identified with microscopy or flow cytometry for further antigenic and genetic analysis. A model approach based on the K562 erythleukaemia cell line was implemented indicating that suggested hypothesis is true for identification both proliferating and dormant cells within a complex cell population. It was also shown that fluorescence decrease strictly follows exponential decay pattern based on the number of cell divisions. Suggested methodology was used for identification of proliferating and differentiating cells in suspension culture and the CFU assay based on their residual fluorescence that was mathematically modeled in silico.