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Article

Quantifying Cytosolic Cytochrome c Concentration Using Carbon Quantum Dots as a Powerful Method for Apoptosis Detection

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Abstract: Background: Cytochrome c (Cyt c) is a key biomarker for early apoptosis, and many methods were designed to detect its release from mitochondria. For a proper evaluation of these programed cell death mechanisms, fluorescent nanoparticles are excellent candidates due to their valuable optical properties. Among all classes of nanoparticles developed thus far, carbon-based quantum dots bring qualitative and efficient imaging strategies for biomedical applications as a consequence of their biocompatibility and low cytotoxicity. Methods: In this study, we synthesized carbon quantum dots smaller than 5 nm from sodium citrate and polyethylene imine. These nanoparticles were rigorously characterized, and their quenching capacity in apoptotic events was assessed in A549 cells treated with staurosporine and etoposide. For the evaluation of Cyt c release, a phenomenon directly correlated with apoptotic events, we ran a semiquantitative analysis using confocal laser scanning microscopy. Results: Carbon quantum dots were synthesized and were successfully employed for Cyt c detection by means of fluorescence microscopy. Significant drops in fluorescence intensity were observed in the case of cells treated with apoptosis-inducing therapeutic compounds compared to untreated cells, confirming Cyt c release from mitochondria to cytosol. Conclusion: Considering these results, we strongly believe this method can contribute to an indirect in vitro evaluation of apoptosis.

Keywords: carbon dots; fluorescence; cytochrome c; apoptosis; cells

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1. Introduction

The total number of cells in an organism is rigorously regulated by physiological processes, which not only include cell division, but also a controlled rate of cell death. For a normal development and homeostasis, irreversibly damaged cells that become a threat to the body will either enter a senescent phase or undergo a form of programmed cell death. Apoptosis is a type of cell death characterized by the condensation of chromatin, fragmentation of nucleus, shrinkage of the cell and plasma membrane blebbing. The process involves the controlled activation of certain caspase proteases that rapidly degrade all cellular structures. Defective apoptosis is usually detrimental, being present in various diseases, including autoimmune diseases, neurodegenerative pathologies and cancers [1]. As a consequence of apoptosis involvement in these medical conditions, highlighting this process and discriminating it from other types of cell death such as necrosis and autophagy is a key step in cell-based screening of cytotoxic compounds [2].