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Synthesis of Porphyrin-Functionalized Beads for Flow Cytometry

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Abstract : Porphyrins are noteworthy in biomedical science for their cancer tissue accumulation and photophysical properties. The preferential accumulation of some porphyrins in cancerous tissue has been known for many years. This, combined with their characteristic photophysical and photochemical properties, including their strong fluorescence and their ability to generate reactive oxygen species in vivo upon laser irradiation, has led to much research into the application of porphyrins as cancer diagnostic and therapeutic agents. Porphyrins have been used as dyes to detect cancer cells both in vivo and, less commonly, in vitro. In one example, human sputum samples from lung cancer patients and patients without the disease were dissociated and stained with the porphyrin TCPP (5,10,15,20-tetrakis-(4-carboxyphenyl)-porphine). Cells were analyzed by flow cytometry. Cancer samples were identified by their higher TCPP fluorescence intensity relative to the no-cancer controls. However, quantitative analysis of fluorescence in cell suspensions stained with multiple fluorophores requires particles stained with each of the individual fluorophores as controls. Fluorescent control particles must be compatible in size with flow cytometer fluidics and have favorable hydrodynamic properties in suspension. They must also display fluorescence comparable to the cells of interest and be stable upon storage amine-functionalized spherical polystyrene beads in the 5 to 20-micron diameter range that was reacted with TCPP and EDC in aqueous pH six buffer overnight to form amide bonds. Beads were isolated by centrifugation and tested by flow cytometry. The 10-micron amine-functionalized beads displayed the best combination of fluorescence intensity and hydrodynamic properties, such as lack of clumping and remaining in suspension during the experiment. These beads were further optimized by varying the stoichiometry of EDC and TCPP relative to the amine. The reaction was accompanied by the formation of a TCPP-related particulate, which was removed, after bead centrifugation, using a microfiltration process. The resultant TCPP-functionalized beads were compatible with flow cytometry conditions and displayed a fluorescence comparable to that of stained cells, which allowed their use as fluorescence standards. The beads were stable in refrigerated storage in the dark for more than eight months. This work demonstrates the first preparation of porphyrin-functionalized flow cytometry control beads.

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