Are there difference between free and encapsulated Ga-phthalocyanine considering the photobleaching and the haemolysis?

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Introduction

The photobleaching can affect the efficacy of photodynamic therapy (PDT) if the photodegradation decreases the capacity of photosensitizer in reducing the viability of disease cells[1]. However, the photobleaching decreases the photosensitizer concentration localized in heathy tissues. This fact prevents damage to normal tissue caused by therapy. It is known phthalocyanines suffer photobleaching and that the encapsulation into polymeric nanoparticle can increase the photodynamic efficacy of these compounds[2]. Nanoparticles are internalized by simple diffusion into cancer cells, but they are also internalized into healthy cells as erythrocytes, fact that can cause the photosensibility after PDT. In view of this, this study aims to evaluate the photobleaching of free and encapsulated GaPc into PEGlated nanoparticles of polylactide-co-glycolide (PLGA-PEG), and the haemolysis caused for both.

Results and Discussion

Solutions of free or encapsulated GaPc (2-15 μmol/L) in phosphate buffer saline (PBS, pH 7.4), containing Tween® 20 (0.24 mmol/L), were irradiated using a laser diode 665 nm with a light dose of 7.5 J/cm^2 and a power of 1-104 mW. Photobleaching experiments for free GaPc were performed in the presence of 1,2-methylpirrolidone (MP, 0.03% v:v). The relative absorbance intensity (RAI) was monitored before and after irradiation. The absorbance of encapsulated GaPc was obtained after centrifugation, separation of the supernatant and dissolution of the nanoparticles with MP. Erythrocyte solution (0.5% v:v) in PBS, containing Tween® 20 (0.01 mmol/L), was incubated for 1-3h with free and encapsulated GaPc, and then irradiated with the same light source. We used pyridine (1.0 % v:v) for experiments with free GaPc because MP was toxicity to cells. The oxyhemoglobin deliveried for haemolysed cells was monitored at 540 nm. Results shown that RAI values of free GaPc (8.0 μmol/L) decreased from 1.0 to 0.07 when the power was increased from 1 to 104 mW, using MP as solvent (Figure 1). Photobleaching was observed for free GaPc in PBS solution for smaller concentrations (2.0-5.0 μmol/L) since RAI decreased from 1.0 to 0.72, and to 0.79, respectively. However, the decrease of RAI was smaller (21%) for the concentration of 5.0 μmol/L than that for 2.0 μmol/L because of the aggregation. The encapsulation prevented the GaPc photobleaching since the RAI was not reduced.

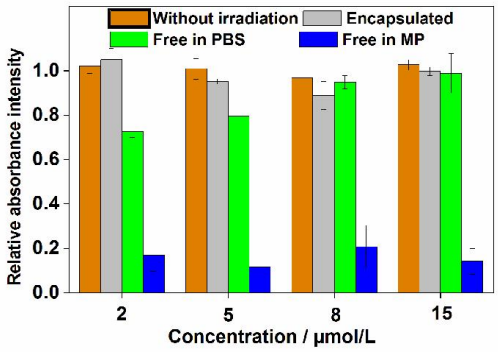

Figure 1. Photobleaching of GaPc.

Erythrocytes incubated with free or encapsulated GaPc (8 μmol/L) for 1h were completely haemolysed since the hemolysis percentage was 100% after a light dose of 7.5 J/cm^2. However, free or encapsulated GaPc did not cause haemolysis without irradiation. Aparently, the haemolysis caused for GaPc-loaded nanoparticles was due to molecules adsorbed in the surface of nanoparticle.

Conclusion

The photobleaching was decreased when the GaPc was encapsulated into PLGA-PEG nanoparticles or due to the GaPc aggregation. Both free and encapsulated GaPc caused haemolysis in erythrocytes. Therefore, encapsulation did not reduce the photosensitivity caused by the GaPc.

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