Title: Zinc phthalocyanine-initiated poly-L-glutamate-based nanocarriers with theranostic properties for glioblastoma multiforme treatment

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Introduction: Glioblastoma multiforme (GBM) - the most common class of malignant primary brain tumors in adults - is known for its aggressiveness and poor survival rates [1]. To overcome inefficiencies associated with current GBM therapies, as most of the therapies in the study are ineffective due to poor pharmacokinetics (PK) and low BBB penetration, the use of nanomedicines as a non-invasive system for brain delivery of chemotherapeutics and/or imaging agents could become a good strategy for developing novel GBM therapeutics [2]. Synthetic polypeptide-based nanomedicines represent highly versatile, advanced therapeutic platforms with multiple examples currently under clinical evaluation and polypeptidic drugs achieving market approval (Vivagel® and Copaxone™) [3]. Thanks to synthetic chemistry's versatility, polypeptide-based nanomedicines can be tailored to specific molecular weights, conjugate different therapeutic or diagnostic moieties, and the possibility of including bio-responsive elements among them [4]. Biodistribution and the delivery of active moiety to target sites are crucial issues for nanomedicine, given that this property directly relates to their efficacy. To provide our polypeptides with inherent imaging properties for better evaluation, we employed Phthalocyanines, which are well-suited for optical imaging in biological tissues and have been used in a range of theragnostic applications. Zinc phthalocyanine (ZnPc) was used as a macroinitiator to synthesize a 4-armed poly-L-glutamate (PGA)-based nanocarrier that displays biocompatibility, low immunogenicity, and biodegradability [5]. Thus, employing a ZnPc core supports the development of polymers with intrinsic imaging properties. Moreover, phthalocyanines act as sensitizing agents for Photodynamic therapy (PDT) [6]. PDT represents a potential GBM treatment due to its non-invasiveness and selectivity for the infiltrative tumor area. In addition, we aimed to explore the possibility of drug conjugation to the PGA sidechain by post-polymerization modification approaches to provide a combination strategy in the same nanosystem (chemotherapy + photodynamic therapy) to establish an efficient combination GBM therapy.

Materials and Methods: PGA-modified ZnPc was synthesized via N-carboxy anhydride ring-opening polymerization and characterized using ^{1}H-NMR, UV-Vis, and DLS. Cell internalization studies used confocal microscopy and flow cytometry. Dark toxicity and PDT were assessed in A172 and U87MG GBM cell lines at 660 nm, and cell viability was measured after 24 h via MTS cell viability assay.

Results and Discussion: We synthesized 4-armed PGA nanocarriers with ZnPc-cores (ZnPc:PGA ratio 1:20, confirmed by UV-Vis and ^{1}H-NMR); given the multivalency of PGA, we also conjugated paclitaxel to the PGA sidechain using post-polymerization modification approaches to provide a combination pharmacological strategy with theranostic properties. We studied cellular trafficking by confocal microscopy and flow cytometry, demonstrating the intrinsic imaging properties of ZnPc-PGA and providing evidence for low dark toxicity and high antitumor PDT activity in human GBM cell lines. The presence of paclitaxel further enhanced the antitumor activity of ZnPc-PGA.

Conclusions: We report the synthesis and characterization of a water-soluble ZnPcPGA-based nanocarrier. Cell-based studies demonstrated the intrinsic imaging properties of ZnPc-PGAs at NIR by fluorescence confocal microscopy and their applicability as photosensitizers for PDT in GBM cells.

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