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Type 1 ribotoxin-curcin conjugated biogenic gold nanoparticles for a multimodal therapeutic approach towards brain cancer

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ABSTRACT

Background: Gliomas have been termed recurrent cancers due to their highly aggressive nature. Their tendency to infiltrate and metastasize has posed significant roadblocks to in attaining fool proof treatment solutions. An initiative to curb such a scenario was successfully demonstrated in vitro, utilizing a multi-conceptual gold nanoparticle based photo-thermal and drug combination therapy.
Methods: Gold nanoparticles (Au NPs) were synthesized with a highly environmentally benign process. The Au NPs were PEGylated and conjugated with folate and transferrin antibody to achieve a dual targeted nano-formulation directed towards gliomas. Curcin, a type 1 ribosome inactivating protein, was attached to the Au NPs as the drug candidate, and its multifarious toxic aspects analyzed in vitro. NIR photo-thermal properties of the Au nano-conjugates were studied to selectively ablate the glioma cancer colonies.
Results: Highly cyto-compatible, 10–15 nm Au NP conjugates were synthesized with pronounced specificity towards gliomas. Curcin was successfully conjugated to the Au NPs with pH responsive drug release. Prominent toxic aspects of curcin, such as ROS generation, mitochondrial and cytoskeletal destabilization were witnessed. Excellent photo-thermal ablation properties of gold nanoparticles were utilized to completely disrupt the cancer colonies with significant precision.
Conclusion: The multifunctional nanoconjugate projects its competence in imparting complete arrest of the future proliferation or migration of the cancer mass.
General significance: With multifunctionality the essence of nanomedicine in recent years, the present nanoconjugate highlights itself as a viable option for a multimodal treatment option for brain cancers and the like.

1. Introduction

Treatments of cancers related to the brain have posed extreme complications due to their location and the complexity in reaching them. The most common and chronic type of brain cancers are the gliomas [1–3]. These originate from glial cells and are highly vascularized. They possess the tendency to aggressively infiltrate and are associated with cases of extensive necrosis and hypoxia in addition to severe disruption of the blood brain barrier (BBB), thereby compromising the integrity of the brain. Gliomas are commonly referred to as recurrent cancers, as they intrusively grow back, even after comprehensive surgical excisions [4,5]. Also, the surgical procedures often add to the risk of metastasis, increasing the chances of possible future malignancies at multiple locations. Chemotherapeutics rarely reach these tumors, mainly due to the selective trafficking monitored by the BBB, and are also accompanied by the non-specific targeting and accumulation risk to normal cells [6]. Other options such as radiotherapy, accompany imminent short and long-term side effects as normal cells and organs are unintentionally exposed to the radiation [7]. Cumulatively, gliomas have been considered as rarely curable and the demand for alternative promising solutions has taken the forefront.

The nanomedicine platform offers exciting options for treatment of numerous life consuming ailments [8–11]. The focal core of this technology employing diverse nano-vehicles, lies in the claim to achieve maximum specificity with minimal side effects by the incorporation of single or multiple targeting ligands [12,13]. The meager quantity of drug requirements compared to conventional chemotherapy approaches, with the possibility of monitoring their fate by the conjugation of various imaging agents, proves a highly supportive factor. The majority of tumors express fenestrated neo-vasculature along with inferior lymphatic drainage, which presents the nano-drug delivery systems (NDDS) to preferentially accumulate at the tumor site over time [14]. Owing to their intriguing features as size, shape, high biocompatibility, etc., gold nanoparticles (Au NPs) have been receiving increased attention in the area of NDDS, as a variety of drugs, proteins, antibodies, peptides, etc., can be conjugated with them [15–17]. Apart from their ease of synthesis, control over shape/size and the relative

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facile modes of surface modifications, Au NPs have been widely acclaimed for their thermal characteristics, which could be efficiently harnessed for photo-thermal ablation applications [18–22].

In this study, we present a multifunctional, PEGylated, biogenic Au NP based nanocarrier, capable of delivering a potent ribosome inactivating protein (RIP), curcin, which has been previously attributed with protein synthesis inhibition [23,24], cellular organelle disruption, cytoskeletal damage, etc., (unpublished) specifically targeting glioma cells. A dual targeted approach was successfully attempted employing folate and anti-transferrin antibody, to achieve maximum specificity towards glioma. Curcin was conjugated with the Au NPs via pH-sensitive bonds to effectuate pH-controlled release in the acidic environments of tumor cells, thereby rendering it benign to normal cells. Curcin exhibited superior therapeutic efficacy by curbing the migratory and proliferative properties of glioma cells in mono-layered as well as in 3D glioma spheroids. The 3D spheroids mimic, to an extent, the in vivo scenario, where the cancer cells proliferate as a solid mass, therefore the effects of curcin on such an in vitro mass may, in principle, be advantageous to suppress the propagation of in vivo tumor masses as well. The Au NPs, apart from their role as efficient drug carriers, were tested for their excellent thermal properties under NIR influence, to ablate the cancer cells and 3D cancer colonies in a controlled fashion. It is proposed

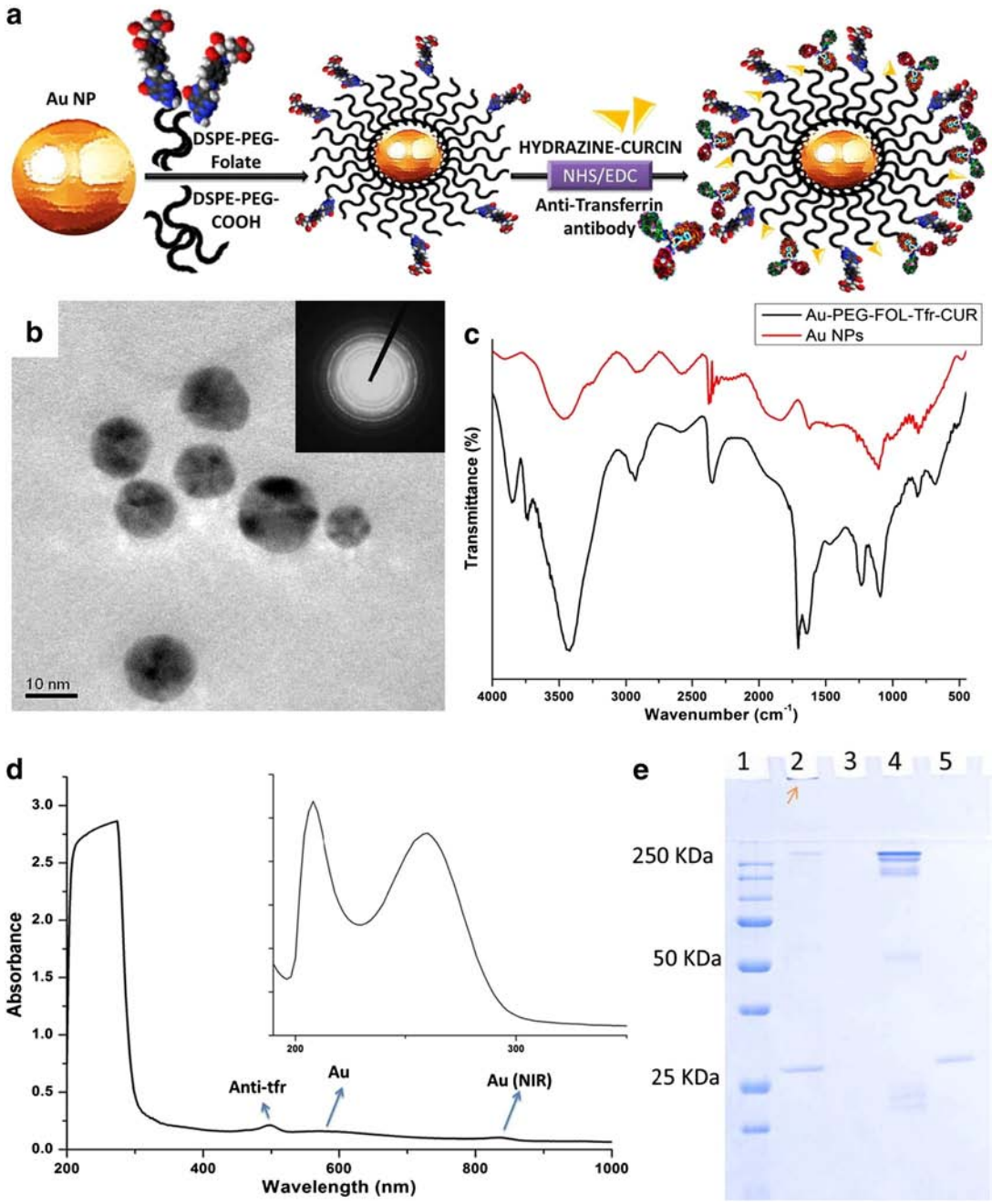

Fig. 1. (a) Preparation scheme of Au-PEG FOL-Tfr-CUR conjugate. (b) HRTEM image of bio-functionalized Au NPs (inset: SAED pattern). (c) FT-IR spectrum of Au NPs synthesized as such and after bio-functionalization. (d) UV–vis spectra of Au-PEG FOL-Tfr-CUR conjugate exhibiting the absorption of curcin at 220 and 272 nm (inset), the absorption of transferrin centered at 490 nm, broad SPR of Au NPs centered at 520 nm and NIR absorption of Au NPs around 800 nm. (e) CBB stained SDS PAGE of Au-PEG FOL-CUR-Tfr conjugate. Lane 1: protein marker, Lane 2: Au-PEG FOL-CUR-Tfr, Lane 3: filtrate post 50 KDa ultra-centrifugal filtration, Lane 4: anti-transferrin antibody and Lane 5: curcin (28.2 KDa). The conjugation of Tfr antibody and curcin to the Au NPs is confirmed by the presence of corresponding bands. The arrow indicates the Au NPs which failed to electrophorese and settled in the loading wells itself.