

Photothermal therapy (PTT) is a minimally invasive and precise thermo-ablative treatment that exploits photothermal agents to convert optical energy into heat, inducing localized temperature increases capable of destroying cancer cells. Its clinical application against infiltrating malignancies like glioblastoma demands therapeutic selectivity. We addressed this challenge by functionalizing gold nanorods (AuNRs) with an anti-EGFR antibody (AuNRs/Ab) that specifically targets the overexpressed epidermal growth factor receptors on U87-MG glioblastoma cells. Bioconjugation was confirmed by UV-Vis absorption spectroscopy, photoluminescence, dynamic light scattering (DLS), and transmission electron microscopy (TEM). A customized thermo-optical setup operating in the first biological window (700-900 nm) was employed to evaluate the 3D distribution and localization of PTT heating. Cells targeted with AuNRs/Ab were embedded in a PDMS/polystyrene phantom mimicking a tumor at a depth of approximately 1cm and illuminated from below with a CW 808 nm NIR laser. Three synchronized thermal cameras enabled real-time 4D heat mapping and its relation to therapeutic outcomes. The efficacy of AuNRs/Ab-mediated PTT was quantified by fluorescence-activated cell sorting (FACS). After 10 minutes of PTT illumination, AuNRs/Ab-treated samples showed a mean temperature increase of about 9 °C, inducing a cell death of approximately 40%, while bare AuNRs induced only a 2 °C temperature increase and negligible cell mortality. Cellular uptake of AuNRs/Ab and ultrastructural changes were confirmed using TEM analysis, highlighting a significant enhancement of AuNRs/Ab compared with bare AuNRs. These results demonstrate the robustness of AuNRs/Ab-mediated PTT in treating aggressive brain tumors.