

**ANTIBODY-FUNCTIONALIZED AUSHINS ENABLE ENHANCED
PHOTODYNAMIC–PHOTOTHERMAL EFFECTS IN MELANOMA CELLS**

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Skin cancer accounts for a substantial proportion of global cancer cases. Minimally invasive approaches such as photodynamic therapy (PDT) and photothermal therapy (PTT) have attracted increasing attention due to their therapeutic potential. Photosensitizers (PS) generate reactive oxygen species (ROS) upon light activation, inducing oxidative stress-mediated cell death, while metallic nanoparticles act as photothermal agents, promoting hyperthermia-induced cytotoxicity. The combination of PDT and PTT may enhance therapeutic outcomes by leveraging complementary mechanisms. In this study, silica-coated gold nanoparticles (AuSHINs) were conjugated with the photosensitizer Methylene Violet 3RAX (MV), followed by the deposition of a secondary silica layer (AuSHINs@MV@) and subsequent functionalization with anti-CD146 antibodies (AuSHINs@MV@Ab), targeting melanoma-derived cells to improve therapeutic specificity. Physicochemical characterization by UV–Vis

spectroscopy, transmission electron microscopy (TEM), dynamic light scattering (DLS), and zeta potential analysis confirmed spherical morphology, colloidal stability, monodispersity, and progressive size increase after coating and conjugation steps. Melanoma-derived A375 cells were incubated with AuSHINs@MV@ and AuSHINs@MV@Ab and compared with non-tumor keratinocyte-derived HaCaT cells. Cytotoxicity and phototoxicity were evaluated following irradiation at 525 nm. Cell viability and death mechanisms were assessed by flow cytometry. The nanostructures exhibited negligible dark toxicity (97% viability in A375 and 95.5% in HaCaT at 0.5×10^{12} NP/mL). After irradiation (525 nm, 30 min, 24 h recovery), A375 cells treated with AuSHINs@MV@ maintained high viability (93.8%), indicating therapeutic resistance, whereas AuSHINs@MV@Ab significantly reduced viability to 28.7%, with approximately 43% of cells in late apoptosis. In HaCaT cells, viability decreased to 36.6% with AuSHINs@MV@ (50% early apoptosis) and to 21.5% with AuSHINs@MV@Ab (42% late apoptosis). These findings demonstrate successful synthesis and physicochemical stability of the nanostructures, as well as the biocompatibility of gold nanoparticles under dark conditions. Functionalized nanoparticles exerted enhanced cytotoxic effects on tumor cells compared to non-functionalized systems. However, additional studies are required to clarify the underlying mechanisms, as non-tumor cells lacking the target antigen were also affected.

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Palavras-chave: melanoma; photothermal therapy (ptt); photodynamic therapy (pdt); gold nanoparticle; methylene violet 3 rax.