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A Gold-Based Nanoformulation for Delivery of the CRISPR/Cas9 Ribonucleoprotein for Genome Editing

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Abstract : CRISPR/Cas9 technology has gained the interest of researchers in the field of biotechnology for genome editing. Since its discovery as a microbial adaptive immune defense, this system has been widely adopted and is acknowledged for having a variety of applications. However, critical barriers related to safety and delivery are persisting. Here, we propose a new concept of genome engineering, which is based on a nano-formulation of Cas9. The Cas9 enzyme was conjugated to a gold nanoparticle (AuNP-Cas9). The AuNP-Cas9 maintained its cleavage efficiency in vitro, to the same extent as the ribonucleoprotein, including non-conjugated Cas9 enzyme, and showed high gene editing efficiency in vivo in zebrafish embryos. Since CRISPR/Cas9 technology is extensively used in cancer research, melanoma was selected as a validation target. Cell studies were performed in A375 human melanoma cells. Particles per se had no impact on cell metabolism and proliferation. Intriguingly, the AuNP-Cas9 internalized spontaneously in cells and localized as a single particle in the cytoplasm and organelles. More importantly, the AuNP-Cas9 showed a high nuclear localization signal. The AuNP-Cas9, overcoming the delivery difficulties of Cas9, could be used in cellular biology and localization studies. Taking advantage of the plasmonic properties of gold nanoparticles, this technology could potentially be a bio-tool for combining gene editing and photothermal therapy in cancer cells. Further work will be focused on intracellular interactions of the nano-formulation and characterization of the optical properties.

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