Gold Nanorod – Affibody Conjugates for Targeted Photothermal Therapy on HER2-Positive Cancer Cells

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ABSTRACT

The need of developing less invasive and more selective tools for cancer theragnostics persists as a current world challenge. Recent targeted therapies have taken advantage of the high expression levels of the human epidermal growth factor receptor 2 (HER2) which has been correlated to poor prognosis and early relapse post-surgery in HER2 positive cancer patients. Nanomaterials have proved to be effective photothermal agents for selectively killing cancer cells by hyperthermia. However, selectivity and target effectiveness still need to be enhanced. Hence, a gold-nanorod-based system able to target the HER2 by conjugated HER2-specific affibody molecules, $Z_{HER2:2891}$, was designed.

For this, gold nanorods (Au NRs) were synthetized by the seed-growth method, PEGylated with poly(ethylene glycol) methyl ether thiol (mPEG-SH) and bioconjugated to chemically produced Cys-modified HER2-affibody molecules (Affi). A systematic study of the NR production indicated that the stirring speed during the seed preparation (800–1500 rpm) is an overlooked aspect that needs to be controlled for achieving NR synthesis repeatability. Then, the optimized conditions for PEGylation and affibody loading were found to be 0.1 M phosphate buffer (PB) pH 6, and 0.1:1 molar ratio of either mPEG-SH or Affi to either cetyltrimethylammonium bromide (CTAB) capped Au NRs (CTAB-Au NRs) or PEGylated Au NRs (PEG-Au NRs), respectively. In all cases, the starting Au NR amount was set to 500 nmol in terms of Au^0, determined spectrophotometrically by the absorbance at 400 nm. On the other hand, the strong binding affinity of the synthesized Affi for HER2 was proven by microscale thermophoresis (MST, $K_D = 29.7 \pm 3.8$ nM) and further confirmed in fluorescence flow cytometry (FFC) studies.

Internalization of the affibody loaded PEG-Au NRs (Affi-Au NRs) was proven by confocal reflectance microscopy, with the Affi-Au NRs located in the cytoplasm of HER2

positive cells. The bioconjugates did not depict cytotoxic effect in SKOV-3, MDA-MB-468, or healthy HEK293T cells. Meanwhile, the photothermal therapy — 367 J cm^{-2} light energy dose (408 mW cm^{-2}, 15 min), 100 μM Affi-Au NRs (in terms of Au^0) — showed specific cytotoxicity on SKOV-3 with 85% ($p < 0.01$) decrease in cell viability and evident altered cell morphology. Whilst the therapy did not harm HEK293T. These *in vitro* analysis constitute promising preliminary studies for further light dose and nanomaterial concentration and surface-engineering optimization. The effectivity of the therapy encourages the continuity of the research toward a plausible translation *in vivo*.