RADIOLUMINESCENT NANOPARTICLE FORMULATIONS FOR ENHANCING RADIATION-INDUCED CANCER TREATMENTS

ABSTRACT

Radiation therapy (radiotherapy, RT) is a standard-of-care cancer treatment, but its effectiveness is often constrained by collateral damage to healthy tissues and the inherent radioresistance of many tumors. This dissertation investigates calcium tungstate ($CaWO_4$) radioluminescent nanoparticle formulations as a strategy to enhance radiation-induced cancer treatments through chemo-radiotherapy and radiation-induced photodynamic therapy. In these radiation-induced combination therapies, calcium tungstate nanoparticles (CWO NPs) were employed both as radiosensitizers to tumor cells and as energy transducers that emit UV/blue light under X-ray irradiation. Multiple CWO NP formulations were developed and characterized for chemo-radiotherapy (CRT) and radiation-induced photodynamic therapy (RT-PDT) with the gola of improving therapeutic outcomes.

In the first part of this work, I investigated how variations of formulation characteristics and radiation parameters influence the radiation-induced drug-release behavior of drug-loaded nanoparticles. Specifically, I synthesized five amphiphilic block copolymers—three PEG-PLA materials with different molecular weights and two PEG-PLGA materials with gradient and uniform comonomer-sequence distributions. Paclitaxel-loaded PEG-PL(G)A-encapsulated $CaWO_4$ nanoparticles (PEG-PL(G)A/CWO/PTX NPs) were prepared using the emulsion-evaporation method. The different polymers exhibited distinct physical properties arising from their chemical composition and sequence-distribution characteristics. *In vitro* PTX-release studies revealed polymer-dependent release kinetics. *In vitro* clonogenic survival assays with 4T1 cells further demonstrated that formulations exhibiting faster PTX release produced stronger radiosensitizing effects These results highlight that rational tuning of polymer molecular characteristics enables simultaneous control over PTX release kinetics and CRT efficacy.

In the second part of this work, I extended the application of previously developed PEG-PLA-coated calcium tungstate nanoparticles (PEG-PLA/CWO NPs) as radioluminescent energy transducers for RT-PDT. Upon X-ray irradiation, these NPs emit blue light that activates intracellular PPIX—produced from ALA—to generate ROS, thereby mimicking conventional PDT with an ALA prodrug while enabling treatment of deep-seated tumors *in vivo*. However, the

efficacy of NP-based therapies is often limited by the extracellular matrix (ECM), which is densely populated with stromal cell-derived matrix components and restricts intratumoral transport. To address this barrier, I functionalized the NPs with collagenase to enzymatically degrade collagen, a major ECM component, thereby enhancing NP penetration into solid tumors. *In vivo* micro-CT imaging showed a sevenfold increase in intratumoral NP-distribution volume. In a mouse tumor model, collagenase-functionalized NPs combined with ALA and X-ray irradiation produced significant tumor-growth inhibition and improved survival relative to X-rays alone. However, this benefit was not substantially greater than that achieved with RT-PDT using non-functionalized CWO NPs. Mechanistic studies revealed that maleimide linkers and surface-bound collagenase scavenged a portion of the ROS generated during RT-PDT, thereby diminishing therapeutic efficacy. This finding highlights a critical trade-off; while collagenase functionalization can effectively overcome stromal transport barriers in collagen-rich tumors, it can also introduce unintended ROS quenching. Maximizing therapeutic impact will therefore require careful optimization of NP surface chemistry to minimize ROS scavenging while retaining enhanced tumor penetration.

As an additional strategy to improve intratumoral spreading of CWO NPs, I investigated a block-copolymer-templated approach to synthesize small (< 60 nm), monodisperse PEGylated $CaWO_4$ nanoparticles. Two templating strategies were explored: (i) micelles formed from a linear PEG-PAA-PS block copolymer (BCP), and (ii) macroinitator-derived star-shaped polymers. In the first approach, linear PEG-PAA-PS micelles served as nanoscale reactors for producing PEGylated $CaWO_4$ nanoparticles. Although this BCP-templated synthesis successfully yielded CWO nanoparticles, the resulting particles displayed poor colloidal stability, likely due to insufficient PEG grafting density. To address this limitation, I pursued a second strategy employing star-shaped polymer templates, using β-cyclodextrin (β-CD) as a core macroinitiator to synthesize (β-CD)-PAA-PPEGMA star-shaped polymers through sequential RAFT polymerizations. Future work will focus on refining the synthetic route for these star-polymer templates, which represent a promising approach for improving nanoparticle stability and enhancing intratumoral transport.

In summary, this dissertation investigates the formulation and characterization of radioluminescent CWO NPs to enhance the efficacy of radiation therapy in cancer through chemo-radiotherapy and radiation-induced photodynamic therapy.