Size-Shrinking Nanogels with Tumor-Responsive Degradation

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Introduction: While the use of size-shrinking nanoparticles to achieve deep tumor penetration has only recently begun to be exploited, the potential for the size-shrinking strategy to enhance localized extravasation remains largely overlooked. Silk-elastinlike protein (SELP) polymer nanogels (composed of repeat silk and elastin peptide motifs) exhibit functional size-shrinking properties based upon lower critical solution temperature (LCST) transition. As these polymers are synthesized via recombinant methods, genetic modification has allowed the insertion of peptide sequences into the SELP backbone that are degradable to matrix-metalloproteinases, highly upregulated in many cancers. This research focuses on the addition of SELP nanogels as excipients in anticancer drug formulations to encapsulate drug compounds, enhance extravasation at externally-heated tumors, degrade at tumor sites, and selectively release the pharmaceutical compound in the local environment.

Aims: We aim to develop a pharmaceutical encapsulation excipient that will render anticancer drugs the ability to locally extravasate via thermal stimulation.

Methods: A controlled, self-assembly process was utilized to synthesize nanogels from recombinant SELPs. SELP nanogel LCST behavior was visualized *in situ* using aqueous-phase transmission electron microscopy with a Poseidon Liquid Protochip. Passage of the nanogels across a 9 nm-pore regenerated cellulose membrane at temperatures above and below nanogel LCST was tested and quantified using bicinchoninic acid assays. Degradation of the nanogel backbone was evaluated in MMP concentrations relevant to healthy and cancerous tissues. A model hydrophobic drug, doxorubicin hydrochloride, was incorporated into SELP nanogels utilizing a combination of diisopropylethylamine (DIPEA) precipitation, incubation, and tangential flow filtration. Drug release was assessed from various SELP nanogels at defined MMP concentrations.

Results: SELP nanogels showed temperature-dependent size-shrinkage and aggregation. The nanogels significantly increased their membrane passage at 43°C compared to 37°C, while non-thermoresponsive proteins did not. Incorporation of MMP-degradable peptides significantly enhanced SELP nanogel degradation at cancer-relevant concentrations of MMP. Drug loading was maximized when DIPEA was used to enhance hydrophobicity of doxorubicin, with subsequent drug release dependent upon MMP concentration.

Conclusions: Membrane passage results indicate that size-shrinking nanoparticles may be used to enhance extravasation. Upon enhanced localization within tumor tissue, the high MMP concentrations can degrade the SELP nanocarrier, releasing its drug contents selectively within the tumor environment.

Keywords
Silk-elastinlike protein, nanogel, thermoresponsive, cancer, matrix-metalloproteinases