Dissertation Title	Development of Injectable Hydrogels Loaded with Small Interfering RNA (siRNA) Nanoparticles for Breast Cancer Treatment
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ABSTRACT

Breast cancer remains one of the most prevalent and serious global health challenges, with new diagnoses projected to exceed 3 million annually by 2040. Despite the widespread use of conventional treatments like surgery, chemotherapy, and radiation, these methods often lead to significant side effects and do not consistently prevent recurrence. Gene therapy, particularly using small interfering RNA (siRNA), offers a promising alternative due to its ability to specifically silence cancer-related genes while minimizing adverse effects. Furthermore, integrating gene therapy with conventional treatments could improve outcomes, reduce recurrence rates, and protect healthy tissues. This study investigated the use of siRNA nanoparticles loaded into injectable quaternized chitosan (QCS)/oxidized pectin (OxPec) hydrogels and injectable thermosensitive chitosan (CS)/silk sericin (SS) hydrogels as delivery platforms to enhance siRNA nanoparticle stability and facilitate targeted release in breast cancer treatment.

The first study focused on optimizing siRNA nanoparticle preparation using varying ratios of Trans-Booster, Prime-Fect, and siRNA. The optimal formulation (siRNA:Trans-Booster:Prime-Fect of 1:1:10) yielded stable, uniformly sized nanoparticles (~230 nm) with a moderately negative zeta potential (-6.8 to -10.2 mV). These nanoparticles demonstrated efficient cellular uptake (>98%) and significantly silenced survivin expression, resulting in reduced viability of breast cancer cells.

The second study explored injectable self-healing hydrogels composed of QCS and OxPec as a delivery platform for the optimized siRNA nanoparticles. Hydrogel

formulations were fabricated by varying QCS and OxPec concentrations. The siRNA nanoparticles formulated via electrostatic interactions using Trans-Booster and Prime-Fect were incorporated into the injectable self-healing QCS/OxPec hydrogels. The resulting siRNA nanoparticle-loaded QCS/OxPec hydrogels exhibited favorable gelation times (13-18 min) and self-healing properties (3-10 min). Importantly, media extracted from these hydrogels showed significant cytotoxicity against breast cancer cells and high cellular uptake of the nanoparticles, highlighting their potential for localized delivery.

The third study developed injectable thermosensitive hydrogels from CS and SS for localized survivin siRNA nanoparticle delivery. Optimal conditions for hydrogel preparation were determined by varying β-glycerophosphate concentrations. The siRNA nanoparticles incorporated into these injectable thermosensitive CS/SS hydrogels maintained a uniform spherical morphology (~182 nm). These hydrogels demonstrated a gelation time of 20–29 min and exhibited time-dependent swelling and degradation, which was accelerated in the presence of lysozyme. While varying β-glycerophosphate concentrations (0.8-1.0 mL) did not affect mechanical strength, the inclusion of siRNA nanoparticles slightly reduced it. Media extracted from hydrogels loaded with survivin siRNA nanoparticles significantly inhibited the growth of MDA-MB-231 and MDA-MB-436 cells after 72 h, outperforming both untreated and control siRNA groups. Additionally, high cellular uptake was observed in both cell lines.
Overall, this study successfully developed and characterized the siRNA nanoparticles-loaded hydrogel systems, each demonstrating promising properties for localized and effective siRNA delivery in breast cancer therapy.

Keywords: siRNA Nanoparticles, Injectable Hydrogel, Self-healing, Thermoresponsive Polymers, Biopolymers, Breast Cancer Cells