EFFECTS OF HYALURONIC ACID AND GELATIN-BASED HYDROGELS LOADED WITH DOXORUBICIN ON 4T1 CANCER CELLS

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Cancer is one of the most life-threatening diseases, and conventional therapies such as chemotherapy, radiotherapy, and systemic treatments are widely used. However, these methods often cause severe side effects, highlighting the need for alternative strategies. Hydrogels, especially those based on hyaluronic acid (HA) and gelatin, are promising due to their biocompatibility, biodegradability, and drug delivery potential. They can release therapeutic agents locally, in a controlled and sustained manner. HA, a natural polysaccharide, combined with gelatin, a collagen derivative, forms hydrogels that interact efficiently with cancer cells, while their water retention and microenvironment support cell regulation. Doxorubicin (DOX), a common chemotherapeutic, inhibits proliferation and induces apoptosis but is limited by systemic toxicity. Therefore, HA–gelatin hydrogels provide an innovative carrier to enhance efficacy and reduce side effects. The 4T1 breast cancer line, known for its aggressive and metastatic behavior, is a suitable model to evaluate these systems. This study examines DOX-loaded HA–gelatin hydrogels on 4T1 cells to provide insights for effective localized cancer therapy.

In this study, we formulated doxorubicin-conjugated, gelatin-crosslinked HA injectable hydrogels (HA-G-DOX). The hydrogel preparations were carried out using varying molar ratios of HA, gelatin, and DOX (1:0.1–1:0.05–0.25 mol) across diverse solvent systems, including water, dioxane, DMSO, and DMF. Reaction parameters were optimized to achieve injectable hydrogels with stable physicochemical properties and reproducible quality. The resulting hydrogels were further purified from residual reactants and by-products through sequential washing, centrifugation, and dialysis procedures, ensuring their chemical and physical integrity.

The prepared HA–gelatin hydrogels containing 0.035% DOX equivalent were sterilized under ultraviolet light in a laminar flow hood for 12 hours and subsequently incubated in DMEM culture medium for 24, 48, and 72 hours. To maintain sterility throughout the experimental procedure, all hydrogel samples were sequentially passed through a 0.22-μm membrane filter before being applied to 96-well plates seeded with 5000 4T1 cells per well. Cell viability assays revealed inhibition rates of approximately 25% after 24 hours, 35% after 48 hours, and 45–50% after 72 hours, indicating substantial cytotoxic and antitumor activity.

These results substantiate that HA–gelatin hydrogels loaded with DOX are highly effective in suppressing the proliferation of 4T1 breast cancer cells. The integration of HA and gelatin as biocompatible scaffolds with doxorubicin as a potent chemotherapeutic agent provides a promising

approach for localized drug delivery. Importantly, these hydrogel systems demonstrate the potential to reduce systemic toxicity while maintaining or even enhancing therapeutic efficacy.

Overall, the findings from this study clearly demonstrate that HA–gelatin-based hydrogels incorporating 0.035% DOX can effectively inhibit the growth of aggressive breast cancer cells over extended incubation periods. The observed growth suppression underscores the importance of optimizing hydrogel formulations for controlled and sustained drug delivery. For future studies, the in vivo antitumor efficacy and biological activity of these HA–gelatin hydrogels are being evaluated in animal models to investigate their potential in preventing postoperative cancer recurrence, thereby confirming their therapeutic effectiveness and safety for further development as localized anticancer treatment strategies.