Hyaluronic Acid conjugated Thermoresponsive Nanogels for Therapeutic application

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Abstract

Over recent decades, engineered small particles for targeted drug delivery systems (TDDS) have gained significant attention due to challenges like targeting incapability, nonspecific action, and systemic toxicity. Temperature-responsive polymers are particularly promising, and in this study, we developed hyaluronic acid (HA)-containing nanogels as a drug delivery platform. HA, a biopolymer found in synovial fluid and the extracellular matrix, has notable immunomodulatory properties and can target inflamed cells via CD44 interactions. We incorporated HA into a smart polymer-based nanogel system, confirmed by various techniques including DLS, FTIR, and LCST determination. Viability assays on murine macrophages demonstrated the cytocompatibility of the microgels, and a nitric oxide release assay showed their potential in modulating macrophage phenotype. The nanogels were uniform in size, stable, cytocompatible, and could reduce the pro-inflammatory response of macrophages, making them promising candidates for targeted drug delivery systems.

Introduction

Temperature is an important physiological parameter in cells, it can regulate many biochemical reactions and biological processes in biological organisms. The temperature-sensitivity polymer of poly (N-isopropylacrylamide) (PNIPAM) in water has the lower critical solution temperature (LCST). PNIPAMs has been widely applied in biotechnology, especially in drug delivery systems.

Hyaluronic acid (HA) is a non-sulfated glycosaminoglycan that exists throughout the human body. HA has the advantages such as non-toxicity, low immunogenicity and biodegradability. HA is also known to be the ligand for cluster of differentiation (CD44) . Thus, HA can be as targeting drug delivery by the donor and receptor mechanism of CD44.

Nanogels offer significant advantages in biomedical and pharmaceutical applications. Their high drug loading capacity, controlled and sustained release, and biocompatibility make them ideal for targeted drug delivery. Nanogels can be engineered to respond to various external stimuli, enhancing site-specific drug release while minimizing systemic toxicity.

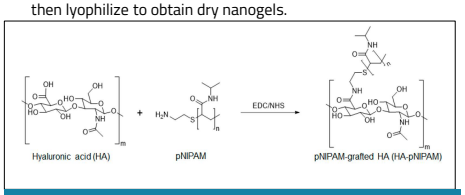
Problem Statement

Current targeted drug delivery systems (TDDS) face challenges such as poor targeting capability, nonspecific action, and systemic toxicity. This study addresses these issues by developing hyaluronic acid (HA) conjugated P(N-isoprpylacrylamide) thermoresponsive nanogels to enhance targeting precision and biocompatibility in drug delivery.

Materials and Methods

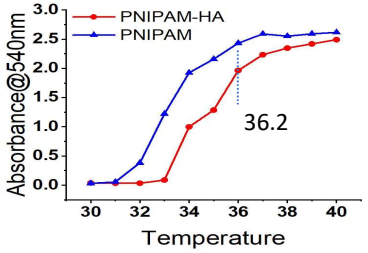
Materials :- Hyaluronic Acid (HA), Poly(N-isopropylacrylamide) (PNIPAM), N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC), N-Hydroxysuccinimide (NHS), DMSO, PBS, DI water.

- The amine-terminated poly(N-isopropylacrylamide) was synthesized by a previously established free radical polymerization method with slight modifications.
- Activation of HA: Dissolve HA in water (1% w/v), add EDC and NHS (1:1:1 molar ratio), and stir for 30 minutes.
- Conjugation: Dissolve PNIPAM in DMSO (1% w/v), add the activated HA solution, and stir for 24 hours.
- Purification: Dialyze the mixture against water for 48 hours, then lyophilize to obtain dry nanogels.

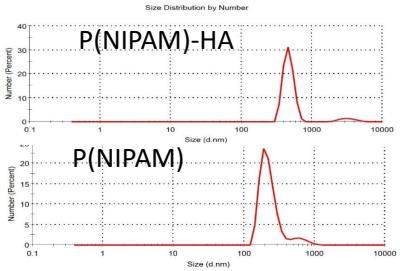


Results and Discussioin

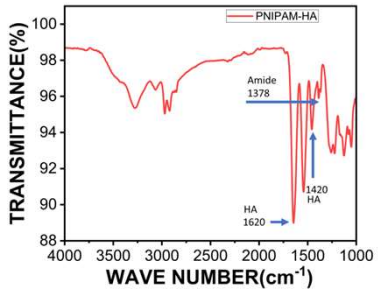
Lower Critical Solution Temperature (LCST)



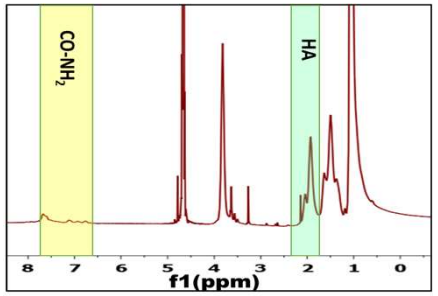
Dynamic Light Scattering for size



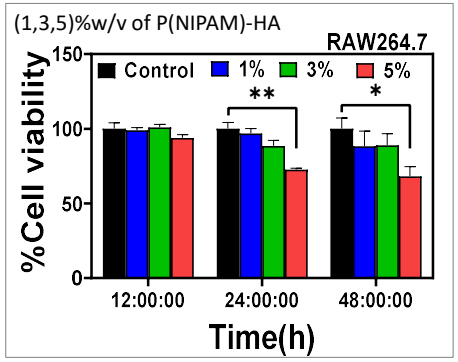
Fourier Transform Infrared Spectroscopy



Nuclear Magnetic Resonance Spectroscopy

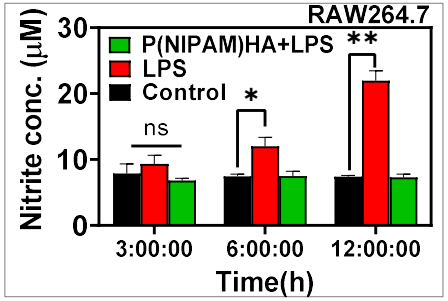


Cytocompatibility of P(NIPAM)-HA [MTT Assay]



Griess Assay

Non –immunogenic and anti inflammatory property of P(NIPAM)-HA with murine macrophages RAW 264.7 cells.



3% w/v (PNIPAM)-HA solution, 1% w/v LPS and RAW264.7 as control. It was observed that PNIPAM-HA displayed significantly higher inhibitory activity on NO production.

Conclusion and Future Perspective

1. HA-containing temperature-responsive nanogels were successfully developed as a therapeutic drug delivery platform.
2. The conjugation of HA and PNIPAM was confirmed through DLS, FTIR spectroscopy, NMR spectroscopy, and LCST determination.
3. The synthesized nanogels demonstrated uniform size, good stability, and cytocompatibility in viability assays with murine macrophages.
4. Nitric oxide release assays indicated the nanogels could modulate macrophage phenotype and reduce the pro-inflammatory response.
5. **Future research** will focus on encapsulating drug molecules within these nanogels to explore their efficacy and targeting capabilities in drug delivery applications.

References

1. Mairal, Ayushi, et al. "Hyaluronic Acid-Conjugated Thermoresponsive Polymer-Based Bioformulation Enhanced Wound Healing and Gut Barrier Repair of a TNBS-Induced Colitis Injury Ex Vivo Model in a Dynamic Perfusion Device." ACS Applied Materials & Interfaces 16.5 (2024): 5382-5400.

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