SURGE'24 REPORT

Hyaluronic Acid conjugated Thermoresponsive Nanogels for Therapeutic application

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Problem Statement

The development of targeted drug delivery systems (TDDS) has been hindered by issues such as targeting incapability, nonspecific action, and systemic toxicity. Current approaches often fall short in efficiently delivering therapeutic agents to specific sites, leading to undesirable side effects and reduced treatment efficacy. There is a need for innovative solutions that can overcome these limitations by enhancing targeting precision and reducing systemic toxicity. This study aims to address these challenges by developing hyaluronic acid (HA)-containing temperature-responsive nanogels, leveraging the unique properties of HA and poly(N-isopropylacrylamide) (PNIPAM) to create a more effective and biocompatible drug delivery platform.

Abstract

Over the past several decades, the development of engineered small particles as targeted drug delivery systems (TDDS) has garnered significant attention due to issues related to targeting incapability, nonspecific action, and consequent systemic toxicity. These small particles can be fabricated using various polymers, with temperature-responsive polymers being a class of smart polymers thoroughly explored in this domain. In the current study, we developed hyaluronic acid (HA)-containing temperature-responsive nanogels to serve as a therapeutic drug delivery platform. HA, a glycosaminoglycan biopolymer commonly found in synovial fluid and the extracellular matrix, has notable immunomodulatory properties, including regulation of macrophages, induction of antimicrobial peptides, and promotion of regulatory CD4+ T (Treg) cells. Additionally, HA can target inflamed cells via CD44 interactions, as the hyaluronan receptor CD44 is overexpressed by inflamed cells. Leveraging these therapeutic properties of HA, we incorporated it into our smart polymerbased nanogel system.

The successful conjugation of HA and PNIPAM was confirmed by dynamic light scattering (DLS), Fourier-transform infrared (FTIR) spectroscopy, UV-VIS analysis, Nuclear magnetic resonance (NMR) spectroscopy and lower critical solution temperature(LCST) determination. Furthermore, viability assays performed on murine macrophages indicated the biocompatibility of the microgels. A nitric oxide release assay on murine macrophages was conducted to evaluate the effect of these hyaluronic acid-containing nanogels on modulating the macrophage phenotype. Overall, we found that the synthesized nanogels were of uniform size, demonstrated good stability, were cytocompatible, and could mitigate the pro-inflammatory response of macrophages to some extent. These nanogels can be further utilized to encapsulate desired drug molecules, making them promising candidates for targeted and therapeutic drug delivery systems.

Keywords: p(NIPAM)-co-HA nanogels; CD44; Inflammation; Targeted drug delivery

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.

Delivery systems for macrophages are particularly attractive since these phagocytic cells play a important role in immunological and inflammatory responses, also acting as host cells for microorganisms that are involved in deadly infectious diseases, such as leishmaniasis. Hyaluronic acid (HA) is specifically recognized by macrophages that are known to express HA receptors. Therefore, in this study, we focused on HA-based nanogels as drug carriers for these cells

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.

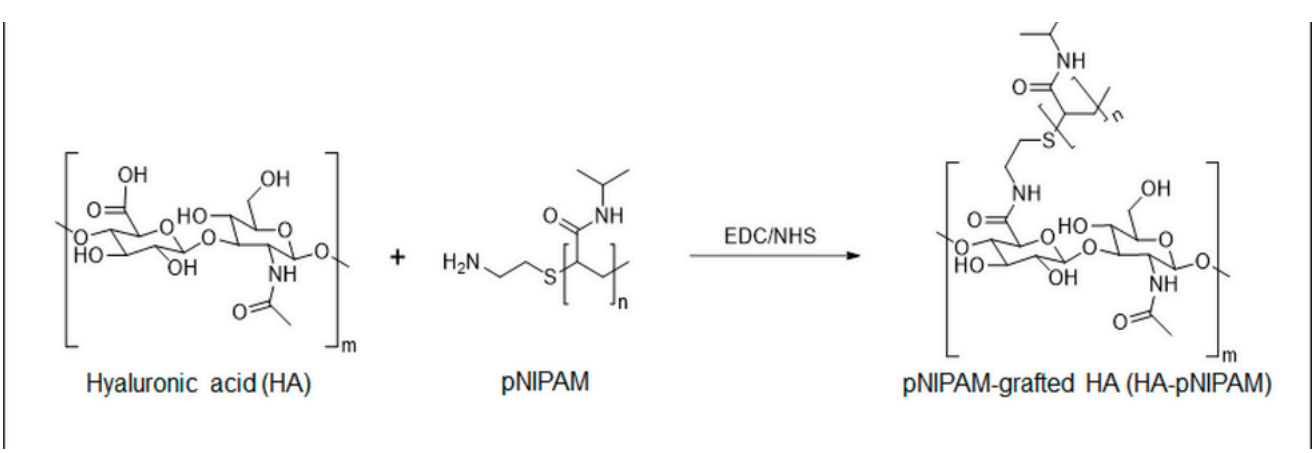
Methods:-

•The amine-terminated poly(N-isopropylacrylamide) was synthesized by a previously established free radical polymerization method with slight modifications. Particularly, for terminal amine functionalization, AET·HCl was considered to be the best candidate since it introduces an amine group to the end of the polymer chain, enabling postmodification reactions as well as providing control over the polymer chain length and molecular weight.

•Activation of HA: Dissolve HA in water (1% w/v), add EDC and NHS (1:1:1 molar ratio), and stir for 30 minutes.

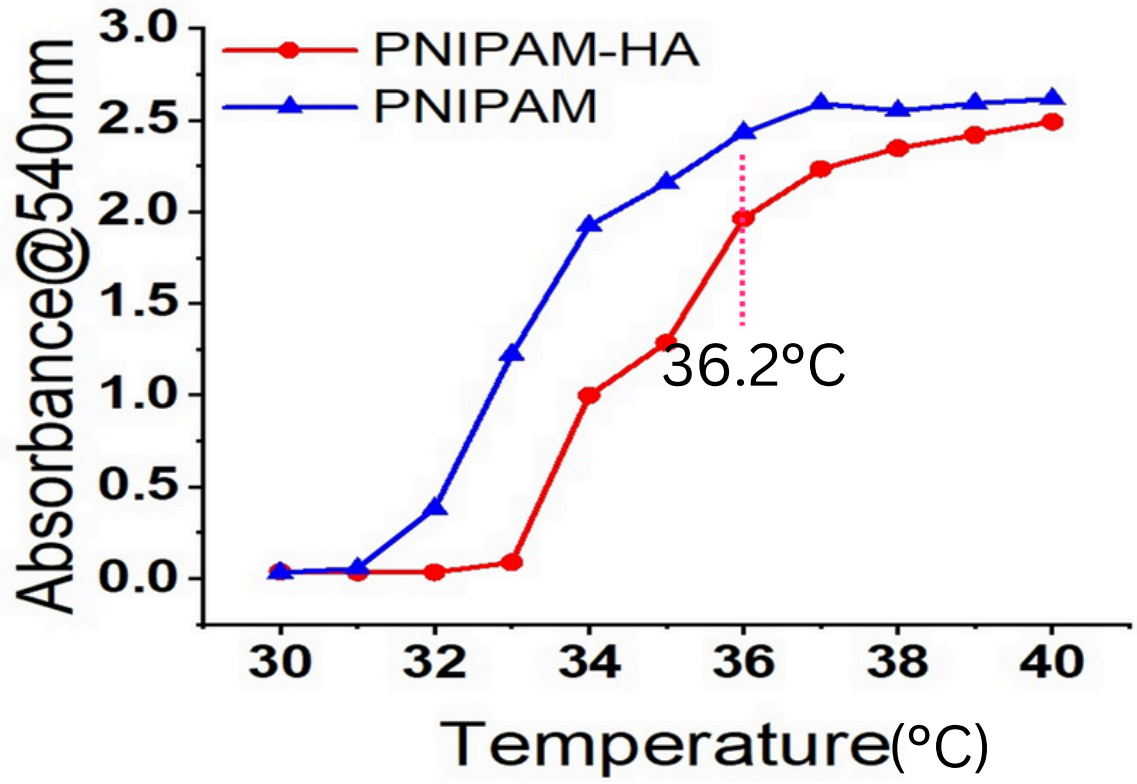
•Conjugation: Dissolve PNIPAM in DMSO (1% w/v), add the activated HA solution, and stir for 24 hours.

•Purification: Dialyze the mixture against water for 48 hours, then lyophilize to obtain dry nanogels. While NIPAM-based polymers are generally considered to be biocompatible, there may be concerns about the presence of potentially toxic monomers or byproducts. Hence, proper purification of the synthesized polymer is essential to minimize these risks.



Results and Discussion

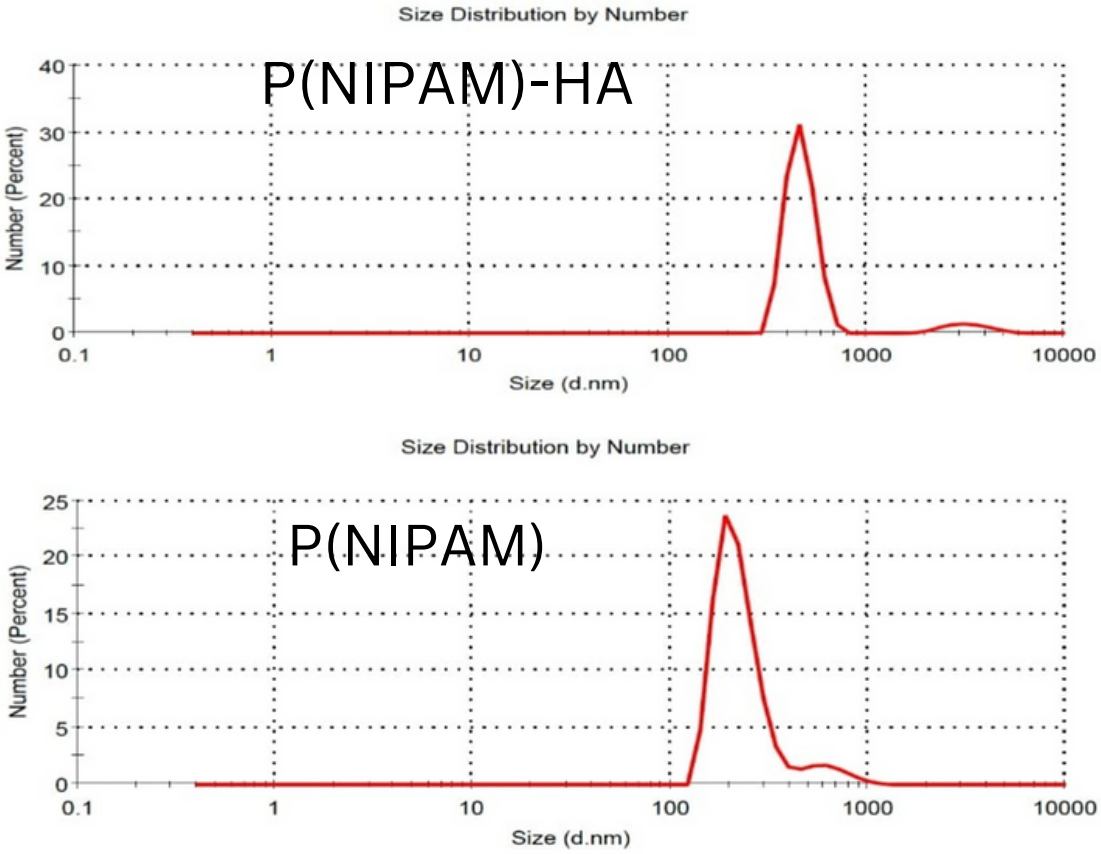
- **Lower Critical Solution Temperature (LCST)**



At this junction, it is imperative to iterate that the focus of our work was to develop a thermoresponsive therapeutic formulation, which could form a coating/barrier on the target site, thus reducing the inflammation. P(NIPAM) exhibits a well-known lower critical solution behavior in water, leading to the formation of a thermoresponsive gel at 32 °C (lower critical solution temperature, LCST).

It is important for the thermoresponsive formulation to have an LCST close to but less than the optimum body temperature (37 °C) such that gel formation could occur at 37 °C. We found out LCST of P(NIPAM)-HA to be around 36.2 which works well with our hypothesis.

- **Dynamic Light Scattering for size**



The DLS results suggest that P(NIPAM)-HA particles are significantly larger, around 1000 nm, compared to P(NIPAM) particles, which are around 100 nm. This indicates that the addition of HA (presumably hyaluronic acid) to P(NIPAM) has resulted in a significant increase in particle size.

- **Fourier Transform Infrared Spectroscopy[FTIR]**

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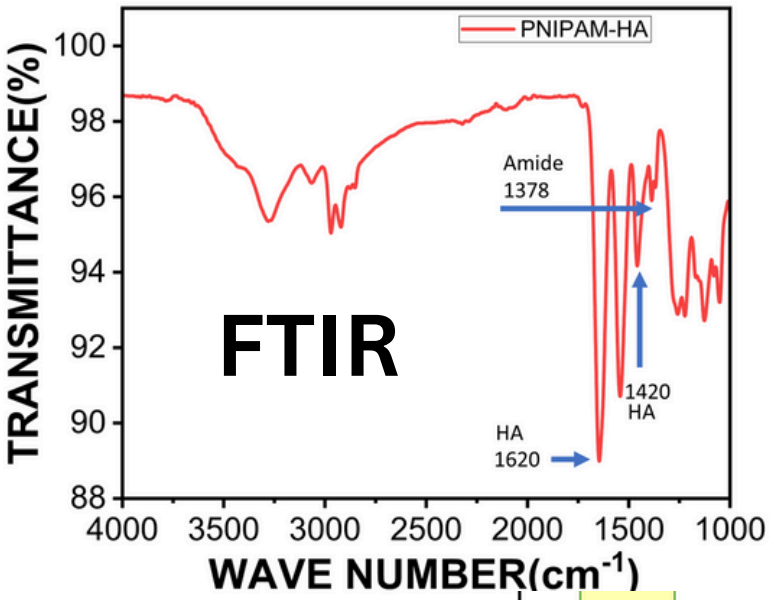
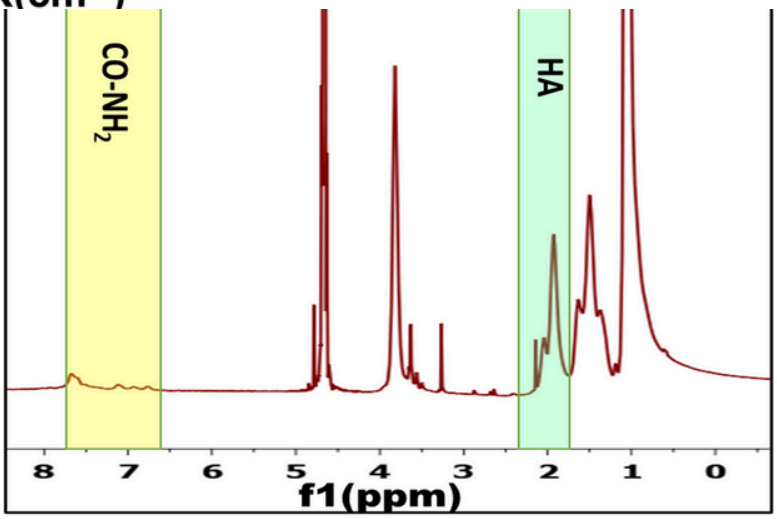
- **Nuclear Magnetic Resonance Spectroscopy**

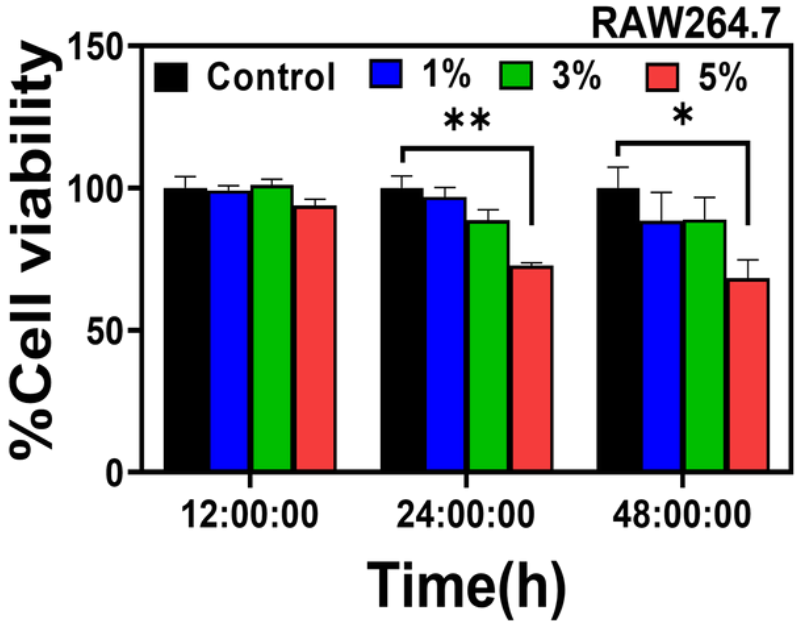


NMR



The 1H NMR spectra of P(NIPAM)-HA displayed two small peaks around 7.12 ppm that corresponded to successful amide linkage (CO-NH) formed between the carboxylic group of HA and the amine group of P(NIPAM)-NH2. Furthermore, a sharp peak observed around 2 ppm in both HA and P(NIPAM)-HA also confirmed the successful incorporation of HA onto the P(NIPAM) chains. In the FTIR spectra, characteristic peaks of HA appeared at 1620 and 1420 cm−1 for the asymmetric COO− stretching vibration and the symmetric COO− stretching vibration, respectively. Appearance of the characteristic peaks of P(NIPAM) at 1645, 1558, and 1378 cm−1 and the shifting of a carboxyl peak of HA around 1621 cm−1 caused by the formation of amide linkages in P(NIPAM)-HA confirmed the grafting of copolymer P(NIPAM)-NH2 on HA

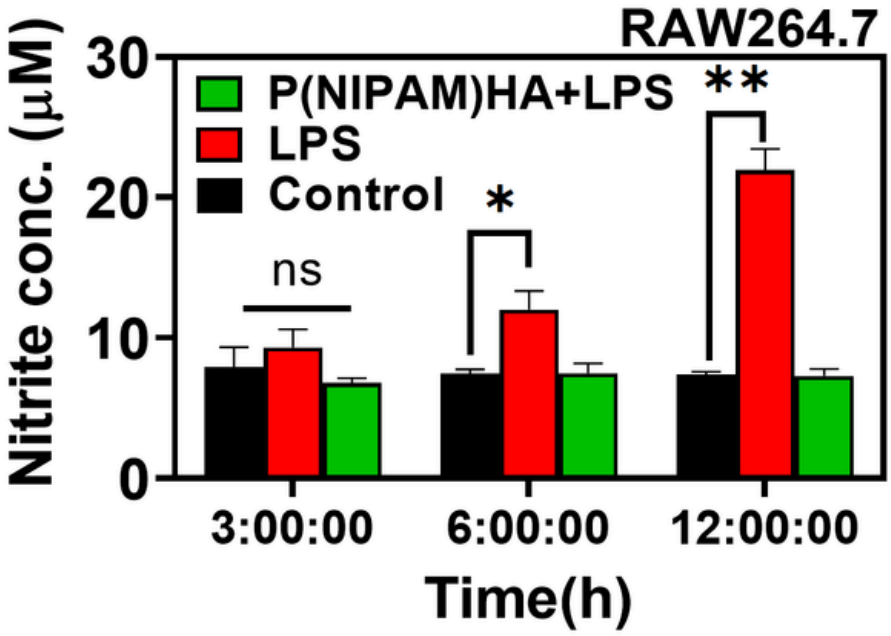
- **Cytocompatibility of P(NIPAM)-HA [MTT Assay]**



For a therapeutic formulation to be used at an interface with inflamed tissue, it is required to be both cytocompatible and immunocompatible in nature, such that it can promote healing of the injured/inflamed tissue. HA is an endogenously produced polymer of the human body that is well-known for its unique biocompatible, biodegradable, and immunosuppressive behavior.

We first purified the synthesized polymer (P(NIPAM)-H) formulation via dialysis and then analyzed its cytocompatibility with murine macrophage cell line RAW 264.7 via the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. P(NIPAM)-HA showed excellent cytocompatibility at concentrations (1–3% w/v) with no toxic effects even after 48 h of treatment. Thus, it could be said that the grafting of HA imparted better biocompatible properties to the copolymer formulation, making it suitable for such applications.

- **Anti-inflammatory Properties of P(NIPAM)-HA Griess Assay**



HA is a very well-studied glycosaminoglycan biopolymer that exhibits immunomodulatory properties, including regulation of macrophages and induction of regulatory CD4+ T (Treg) cells. Therefore, it was expected that our P(NIPAM)-HA also potentially could display such similar properties. This was assessed using a Griess assay, which estimates the release of the inflammatory marker nitric oxide (NO). NO release is crucial in a variety of inflammatory processes that occur in humans. Large amounts of NO are released from macrophages (by the action of inducible nitric oxide synthase (iNOS)) upon coming in contact with proinflammatory agents such as lipopolysaccharides (LPS) or cytokines such as IFN-γ, IL-6, and IL-1β. In our study, 3% w/v P(NIPAM)-HA solution was checked for its inhibitory effect on NO production by the LPS-stimulated murine macrophage cell line RAW 264.7. It was observed that P(NIPAM)-HA displayed significantly higher inhibitory activity on NO production.

Conclusion and Future Perspective

In this study, we successfully developed hyaluronic acid (HA)-containing temperature-responsive nanogels as a therapeutic drug delivery platform. The conjugation of HA and PNIPAM was confirmed through dynamic light scattering (DLS), Fourier-transform infrared (FTIR) spectroscopy, UV-VIS analysis, Nuclear Magnetic Resonance (NMR) spectroscopy, and lower critical solution temperature (LCST) determination. The synthesized nanogels demonstrated uniform size, good stability, and cytocompatibility in viability assays performed on murine macrophages. Additionally, the nitric oxide release assay indicated that these nanogels could modulate the macrophage phenotype and mitigate the pro-inflammatory response to some extent.

These findings highlight the potential of HA-containing nanogels as promising candidates for targeted and therapeutic drug delivery systems. Future research will focus on encapsulating desired drug molecules within these nanogels to explore further their efficacy and targeting capabilities in drug delivery applications.

Acknowledgement

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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.